Poster Abstracts

XLI National Meeting of the Mexican Association of Microbiology (AMM)
VI Meeting of Biochemistry and Molecular Biology of Bacteria (BBMB)

Oaxaca, Oax. October 27 - 31, 2019.

Cell Biology

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Viability assessment and morphological changes of mycobacteria during dormancy induced by hypoxia and starvation.

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Background: One-third of the world's population is infected with dormant *Mycobacterium tuberculosis*. While trying to understand dormant mycobacteria, some morphological and physiological features, such as the presence of cytoplasmic lipid bodies, the drug-tolerant profile and the expression of metabolic pathways that promotes an intracellular redox balance have been described. However, as dormant mycobacteria populations are heterogeneous, strategies to control them, and eventually eliminate them are multifactorial. In this study, we aimed to provide new insights into the viability state and the morphological changes of dormant *M. smegmatis*, that promotes its long-term survival under starvation and hypoxia.
Materials/methods: Two *in vitro* models were performed to study dormancy based on starvation and hypoxia. *Mycobacterium smegmatis* mc^2 155 cells in exponential phase were starved by resuspension in sterile phosphate-buffered saline at 37°C/200 rpm during 120 h. For hypoxia, cells in exponential phase were centrifuged and resuspended in fresh Dubos + 10% ADC broth and incubated at 37°C during 120 h under conditions previously proposed by Wayne and Hayes in 1996. Five samples from every model were taken along 120 h of incubation, and at least two biological replicates were done. Morphological and physiological changes of every sample were followed through colony counting, OD_{640}, flow cytometry using the Live/Dead™ BacLight™ Viability and Counting Kit, transmission, and scanning electron microscopy.
Results: During starvation, we found a reduction of the mycobacteria cell length, cell permeability reduction, and the putative involvement of a phenomenon called "reductive cell division". Besides, during hypoxia, a novel population of pleomorphic mycobacteria, with a rough surface, was identified since the 36 h. Viable but not culturable mycobacteria and an increment of cell DNA supercoiling were found to be present in *M. smegmatis* growth in both *in vitro* models. These results can help to understand further the morphology and physiology of dormant mycobacteria when they have to cope and respond to different stress environments inside their host during long periods.

Cloning, expression and purification of PilW and PilV pilins of the Type IV pili of *Acidithiobacillus thiooxidans*

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The Type IV pili (TfP) of *A. thiooxidans* play important roles in the bacterial attack to surfaces of mineral such as chalcopyrite (CuFeS), to release the metal after iron and sulfur biooxidation. TfP are semiflexible polymeric filaments of pilins anchored to the cellular membrane. The TfP is comprise a structural subunit (pilin) containing the GFXXXE signal peptide, which indicates the polymerization starting site. TfP polymerization takes place within the periplasmic space and requires the energy provided by an ATPase (PilD). After a bioinformatic analysis of the genomic sequence of *A. thiooxidans* ATCC-19377 (AFOH01000001; Valdés *et al*. 2008), we identified two candidate proteins (PilW and PilV), as possible main structural proteins of the TfP of *A. thiooxidans*.

Methods

Culture conditions. Cultures of *A. thiooxidans* ATCC-19377 were aerobically incubated at 29 °C in ATCC-125 medium under orbital agitation at 120 rpm.

Cloning of *pilW* and *pilV*: The mRNA nucleotide sequences of PilW and PilV reported to the GenBank (AWP39906.1 and AWP39907.1, respectively) were used in this study. Confirmed sequences of the full mRNA-PCR products were cloned into the pGEM-T easy vector using the JM109 competent cells, followed by their subcloning into pET-32b (+) plasmid to drive their expression. Proteins were then purified using Ni-NTA superflow columns (Quiagen)

Results

The primers designed allowed to amplify the mRNA of *pilW* and *pilV*. After their purification, they were ligated and transformed into pGEM-T Easy, obtaining the clones pGEM-*pilW* and pGEM-*pilV*; using the enzyme EcoR I, *pilW* and *pilV* were restricted from pGEM, linked and transformed into the expression vector pET-32b(+) obtaining pET-*pilW* and pET-*pilV*. Finally, purified PilW and PilV proteins were obtained using Ni affinity columns.

Conclusions

For the first time, it is reported a successful method to clone and purify pilins of an acidophilic microorganism of the genus *Acidithiobacillus*.

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A New Method for the Study of Proteins that Bind Peptidoglycan.

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The bacterial cell-wall is composed of saccharide chains bound together by non-ribosomal peptides. Although the cell-wall is synthesized from a single molecule, the activity of different enzymes differentially can modify the chemical structure of the cell-wall along the cell. This diversity in the cell-wall composition, complicates the biochemical characterization of proteins that bind to the peptidoglycan cell-wall. Two *Escherichia coli* proteins have been reported to recognize differences in the peptidoglycan chemical structure, FtsN and recently YftB. FtsN, preferentially binds to peptidoglycan devoid of steam peptides (denuded peptidoglycan) via its SPOR domain, the same was suggested for YtfB, that binds peptidoglycan via its LysM domain. The main evidence for the preferentiall binding of both proteins came from cooprecipitation assays ussing as substrate cell-wall obtained from different strains. The method used to quantify the binding substrate for the assays involved a colorimetric reaction that measures the peptides present in the peptidoglycan, however, the SPOR domain and LysM domain bind to the sacharide chains. For this reason we developed a modification of the Soomogy-Nelson method to quantify reducting saccharides, and we adapted it to acid-hydrolyzed peptidoglycan.

Peptidoglycan purification is a simple but laborious tecnique and the obtained material is limited, for this reason, we needed a sensitive assay that could be performed in a low volume. Our modified method is perfomed in a final volume of 800 μl and has a sensitivity range of from 0.06 to 3 μmoles/ml of monosaccharides. We validated its sensitivity with different common monosaccharides (glucose, fructose, lactose, galactose) and with the amino-sugars present in the cell-wall (N-acetylglucasmine and N-acetylmuramic acid). Next we isoleted peptidoglycan from *Caulobacter crescentus* and we determined the acid-hydrolysis conditions that allowed a maximal reading. Using this assay we quantified the peptidoglycan isoleted from different purifications. To test this quantification method, we performed a cooprecipitation assay with a protein fragment that contains two LysM domains. The different repititions of the cooprecipitation assay using different cell-wall samples gave consistent results. In this work we adapted and tested a new method to measure the reducing sugars as an alternative way to quantify peptidoglycan, this technique will be of help in the study of proteins that bind to the peptidoglycan cell wall.

Ultrastructural damage in *Streptococcus mutans* incubated with saliva and histatin 5

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Dental caries remains a major oral health problem in most in-dustrialized countries. The World Health Organization estimated that nearly 80% of the world's population suffers from tooth decay. Colonization of the oral surface by pathogenic microorganisms, especially by *Streptococcus mutans*, is the major etiologic agent involved in human dental caries. The main cariogenic character of *S. mutans* is its ability to form biofilms in the oral cavity. Bacterial growth within biofilms confers protection from antibiotics due to the reduced penetration of several antimicrobial agents into their inner structure. Saliva contains various forms of anti-microbial polypeptides that play vital roles in combating invading foreign pathogens, promoting wound healing and supporting apoptosis. Antimicrobial peptides (AMPs), a heterogenous group of molecules produced by various tissues are promising agents in controlling microbial growth due to their concentration and selective antimicrobial activity as well as their low rates of microbial resistance induction. Histatins (HST) are a group of neutral and basic proteins rich in the amino acid histidine. Histatin 5 has been shown to be a potential novel agent against oral candidiasis, yet limited research has been conducted to assess its anticaries potential. Objective: To study the ultrastructural alterations induced in Streptococcus mutans (ATCC 25175) incubated with saliva, saliva plus histatin 5 and histatin 5. Methods: *S. mutans* incubated with saliva histatin 5 or a combination of both were morphologically analyzed and counted. The results were expressed as (CFU)ml−1. Ultrastructural damage was evaluated by transmission electron microscopy. Ultrastructural localization of histatin 5 was examined using immunogold labeling. Apoptotic cell death was determined by flow cytometry (TUNEL). Results: A decrease in the bacteria numbers was observed after incubation with saliva, saliva with histatin 5 or histatin 5 compared to the control group ($p<0.0001$). Ultrastructural damage in *S. mutans* incubated with saliva was found in the cell wall. Saliva plus histatin 5 induced a cytoplasmic granular pattern and decreased the distance between the plasma membrane bilayers, also found after incubation with histatin 5, together with pyknotic nucleoids. Histatin 5 was localized on the bacterial cell walls, plasma membranes, cytoplasm and nucleoids. Apoptosis was found in the bacteria incubated with saliva (63.9%), saliva plus histatin 5 (71.4%) and histatin 5 (29.3%). Apoptosis in the control bacteria was 0.2%. Conclusions: Antibacterial activity against S. mutans and the morphological description of damage induced by saliva and histatin 5 was demonstrated. Pyknotic nucleoids observed in *S.*

Substrate recognition by the sorting platform in the injectisome

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Many bacterial pathogens rely on a Type III Secretion System (T3SS) or injectisome for their pathogenicity, one of them is enteropathogenic *Escherichia coli (*EPEC). This bacterial pathogen belongs to the attaching and effacing (A/E) family and is one of the principal causative agents of diarrhea in developing countries, infants from 0 to 11 months are the most affected. EPEC uses a T3SS to establish an infection, this structure allows the bacteria to inject virulence proteins or effectors into the cytoplasm of enterocytes, in this way EPEC can interfere with host cell signaling pathways for its own benefit.

The injectisome is a nanomachine made up of several proteins. It is composed of extracellular components that connect the bacterium with the host membrane; a basal body built by inner and outer membrane rings; an export apparatus that allows secretion of substrates and a sorting platform which comprises a multiprotein complex that is proposed to engage and sort T3SS-dependent substrates. Assembly of the T3SS is highly regulated and occurs in a stepwise fashion. It is initiated with the assembly of the export apparatus, basal body and the sorting platform. Once the assembly of this structure is completed, the machine is ready to secrete T3SS-dependent substrates, which are classified in three categories, early, middle and late substrates. It is proposed that the sorting platform is responsible of recognizing substrates for their hierarchical secretion, however the mechanisms behind this are poorly understood. In the laboratory, we are interested in understanding how the sorting platform can recognize the different types of substrates in a defined order.

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Induction of the mycelial morphotype in *Candida albicans* yeast cells and activity of Glucosamine-6-phosphate synthase

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Candida albicans is one of the main etiological agents fungal infections in humans associated with the patient's immune status. It´s part of the yeasts of the genus *Candida* where only 3 or 4 species produce more than 90% of mycoses. *C. albicans* is the most frequently isolated in candidiasis. It´s present in the natural flora and It´s characterized by being a dimorphic fungus able to form true mycelium. Some of virulence factors are the cell adhesion by cell wall proteins (CW) and dimorphism, as hyphae decrease the activity of the immune system by the action of hydrolytic enzymes. There are currently few antifungals for clinical use and those have high levels of toxicity, so the search for new antifungals that block the PC have been considered as potential targets for antifungal chemotherapy. These studies have focused on the characterization of enzymes such as Glucosamine-6-phosphate synthase (GFA); which plays an important role in cell growth, CW conformation and therefore in the described dimorphism.

Therefore, in this study the optimal conditions for obtaining mycelium in *C. albicans* were established using two culture media, modified Lee medium (Lee et. al. 1975) and modified YPD medium, obtaining a higher percentage of mycelium in this last according to the growth curves. Achieving greater production at four and five hours of incubation, obtaining mycelium with a high percentage of purity (with a maximum of 10% yeasts). From the above results, the curves of the enzymatic activity, specific activity and total activity of the GFA (Morgan-Elson method) in yeast, mycelium in formation and mycelium formed were made; after to a filtration, centrifugation, ballistic rupture and cell fractionation of the samples and it was determined the amount of protein present (Lowry method, 1951).

According to the results obtained, is demonstrated the importance of the GFA enzyme in the morphological transition from yeast to mycelium, its activity being greater in mycelial morphology than in yeast cells, suggesting that it is a possible target enzyme for future antifungal drugs.

Effect of antimicrobial peptide LL-37 and KR-20 on *Trichomonas vaginalis* viability

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PRESENTACIÓN EN CARTEL, ÁREA BIOLOGÍA CELULAR

Trichomoniasis is the most common nonviral sexually transmitted disease in the world, being *Trichomonas vaginalis* the etiological agent of this disease. Trichomoniasis and other vaginal infections induce large recruitment of neutrophils, which are the first line of cellular defense in infections. The main mechanisms of neutrophils to eliminate pathogens are phagocytosis, degranulation, and the formation of extracellular traps. Reactive oxygen species, hydrolytic enzymes, and antimicrobial peptides are some molecules involved in these defense mechanisms. The antimicrobial peptides are small molecules -generally positively charged- with multiple functions, either as immunomodulators or as microbicides. In humans, three main families of antimicrobial peptides have been found, which are defensins, histatins, and cathelicidin. LL-37 is the only human cathelicidin reported; this peptide has smaller derivatives (i.e. KR-20) that may differ in their immunoregulatory and microbicidal activities, mainly against bacteria. The aim of this study was to determinate the effect of LL-37 and its derivative KR-20 against *T. vaginalis* viability. For this, the trophozoites (GT13 strain) interacted with different concentrations of the peptides (2, 5, 10, 50 µM) during 24 h under culture conditions. After the interaction, the number of mobile parasites was counted. In addition, peptides immunolocalization in *T. vaginalis* was performed by confocal microscopy. The results showed that antimicrobial peptides LL-37 and KR-20 decrease the integrity of *T. vaginalis* in a concentration-dependent manner. KR-20 possess higher antimicrobial activity on *T. vaginalis* than LL-37 itself, with a minimum inhibitory concentration-50 of 4.89 µM for KR-20 and 12.08 µM for LL-37. Additionally, KR-20 binds to *T. vaginalis* surface, as observed by confocal microscopy. Taken together, these results indicate that LL-37 and KR-20 decreased *T. vaginalis* growth being KR-20 more effective, which suggest that both peptides have a potential therapeutic utility.

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Outer membrane vesicles from *Rhodobacter sphaeroides*

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The outer membrane vesicles are produced naturally by bacteria to achieve different functions involved in cell to cell signaling, pathogenesis, etc. (reviewed in (M. Florencia Haurat, 2015).

In addition, the outer membrane vesicles are an interesting objective for biotechnology thanks to the ability to transport cargo proteins which can be useful in the development of vaccines (Mahmoud M. Shehata, 2019) and antibiotic treatments (Weiwei Huang, 2019).

In this work we observed that *Rhodobacter sphaeroides* is able to produce outer membrane vesicles after incubation with an elevated concentration of MgSO4 in the medium. We are trying to elucidate the molecular mechanisms involved in the biogenesis of these outer membrane vesicles.

We found that a cryptic bacteriophage is induced after MgSO4 addition, we are currently defining if lysis is responsible for the synthesis of the outer membrane vesicles. Proteomic analysis of samples obtained by ultracentrifugation of culture supernatants, showed a remarkable enrichment for outer membrane proteins but the presence of bacteriophage proteins was negligible. Given that *Rhodobacter sphaeroides* is a non-pathogenic bacteria prone to be genetically manipulated, it could be and excellent platform to produce outer membrane vesicles with specific cargo proteins for biomedical and biotechnological applications.

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Weiwei Huang, Q. Z. (2019). Anti-outer Membrane Vesicle Antibodies Increase Antibiotic Sensitivity of Pan-Drug-Resistant Acinetobacter baumannii. *Frontiers in Microbiology*.

Systems Biology

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Characterization of the temporal variability of the SOS response in individual Escherichia coli cells in the presence of beta-lactam antibiotics

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Our understanding of the size of bacteria is based on stress-free growth conditions in the laboratory. However, bacteria usually face tensions that produce elongated morphologies. In this project we focus on studying an antibiotic tolerance phenomenon that results when *E. coli* survives the action of ampicillin, through filamentation. Filamentation is dependent on the SOS response, a response with precise temporal regulation (Friedman et al. 2005).

However, we conducted an experiment in which it was observed that the filamentation in an *E. coli* strain carrying a multicopy plasmid occurred at different times at the level of individual cells. So, if the SOS response has a precise temporal modulation, where could the heterogeneity that triggers the filamentation at different times and that leads to a phenotypic plasticity come from? What is the functional benefit of presenting temporal variability in the filamentation?

To answer these questions, in this project we use individual cell microfluidics and time-lapse microscopy to assess whether to carry a resistance gene (encoding TEM-1, a beta-lactamase) in a multicopy plasmid (~ 19 copies) generates variability in population susceptibility profiles and, consequently, heterogeneity in time between exposure to antibiotics and the start of filamentation. Comparing with a homogenous strain of *E. coli* that carries only one copy of the resistance gene on the chromosome.

Our results show that a heterogeneous population increases resilience to fluctuating environments. The filamentation being a remarkable survival mechanism against an ampicillin pulse close to the MIC. As expected, those bacteria with fewer copies of the plasmid were eradicated by the pulse, while the surviving bacteria had filament events in different time ranges.

In addition, once the antibiotic was removed, the bacteria were able to leave the filamentation state and return to their normal size, as well as generate viable daughter cells. Daughter cells secreted the plasmid, thus recovering the heterogeneity of the population.

Therefore, carrying a resistance gene in a multicopy plasmid if it generates temporal variability in the filamentation. Generating greater opportunities for survival in fluctuating environments.

The tragedy of the commons: A selective integration of methods as the best strategy for DNA-sequence-based inference of regulatory networks

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Transcriptional regulatory networks integrate gene regulation as directed links between transcription factors and their target genes. Despite curation efforts, the reconstruction of these networks is far for being complete. The prediction of transcription factor binding sites has been used to discover new targets for known regulators. Even though there are many tools to identify sequence motifs and binding sites, some of them has been assessed only by the similarity between their predictions and the actual sites. Thus, poor overlapping predictions must be classified as false positives. However, these predictions are considered as true positives when assessed to predict the regulatory network. We performed a non-partisan assessment of network predictions by stand-alone tools and their combinatorial integration using the most recent meta-curated strongly-supported networks of *Escherichia coli*, *Bacillus subtilis,* and *Corynebacterium glutamicum* retrieved from Abasy Atlas as gold standards. MEME performed as the best *de novo* motif discovery tool, but an all-inclusive community network, integrating all the predictions, outperformed every individual tool. In contrast to individual predictions, the community network improved when assessed with more complete versions of the gold standard and showed robustness to the training set completeness. Besides, the high density of true positives at the beginning of the score-ranked community prediction provides hypotesis for new interactions that could be experimentally validated. This biased ranked prediction allows filtering spurious interactions through the constraint to the number of interactions for the complete regulatory network. We further analyzed the contribution of each tool to the community network and found that a selective integration of only three tools outperforms the all-inclusive community. Besides, the selective integration of two predictions is enough to outperform MEME. The integration of this DNA-sequence-based network reconstruction approach to other inference and integration strategies may prove game-changing in the prediction of transcriptional regulatory networks.

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Lessons from Abasy Atlas v2.2: complexity, completeness, quality and learning of gene regulatory networks

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Abasy (**A**cross-**ba**cteria **sy**stems) Atlas v2.2 (https://abasy.ccg.unam.mx) contains the most comprehensive collection of meta-curated bacterial gene regulatory networks (GRNs) having at least enough quality to allow system-level analyses. The first version of this database was published in 2016. Since then, we have comprehensibly extended our database with up-to-date data, a completely novel estimation of GRNs completeness, and other novel functionalities.

The current version comprises 76 networks (204,282 regulatory interactions) covering 42 bacteria (64% Gram positive and 36% Gram negative) distributed in 9 species, containing 8,459 regulons and 4,335 systems (modules). This repository also contains statistical and structural properties characterizing these GRNs. Additionally, Abasy Atlas classifies each gene as a global regulator, basal machinery gene, member of a functional module, or intermodular gene. This classification is based on estimations computed by the previously published natural decomposition approach. This is a biologically motivated mathematical approach using the global structural properties of a GRN to derive its architecture and classify its genes into the four above mentioned categories of systems-level elements. Besides, the current version of Abasy Atlas provides historical snapshots of the GRNs at different curation stages for several organisms.

Our group recently leveraged the data deposited in Abasy Atlas to found that GRNs complexity is constrained by their dynamical stability according to the May-Wigner stability theorem. Based on the results obtained in that work, we developed and implemented an improved model having a higher predictive power, whose predictions were deposited in Abasy Atlas v2.2, making Abasy the first database providing estimations on the completeness of GRNs. An interaction coverage computed for the Abasy GRNs as a proxy for completeness revealed that GRN reconstructions based on high-throughput data could yield biased networks with atypically low average clustering coefficients, showing that classical targeted discovery of interactions is still needed. The estimation of the total number of regulatory interactions a GRN could have is a valuable insight that may aid in the daunting task of network curation, prediction, and validation. All this information could be leverage to study the evolution and common organizing principles of GRNs and to develop new strategies to learn GRNs.

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System-level characterization of the evolution of the gene regulatory networks of *Escherichia coli*, *Bacillus subtilis*, and *Corynebacterium glutamicum*

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The evolution of the systems (modules) comprising gene regulatory networks (GRNs) is poorly studied. In this study, we present a novel perspective by focusing on how evolution has shaped the systems integrating a GRN. Here, we leverage the gene coreicity (defined as the number of organisms in which a given gene has at least one homologous gene) as a proxy for the gene age. We then compute the age-based historical reconstruction of a GRN by cumulative removal of batch of genes (and their corresponding edges) according their ascending age from the original network. We made historical reconstructions of the latest versions of the *Escherichia coli*, *Bacillus subtilis* and *Corynebacterium glutamicum* GRNs retrieved from Abasy Atlas v2.2 (https://abasy.ccg.unam.mx). Abasy Atlas classifies each gene of the GRNs as a global regulator, basal machinery gene, member of a functional module, or intermodular gene according to estimations obtained by applying the natural decomposition approach (NDA). We made three main findings. First, there is no correlation between gene age and degree (the number of interactions with other genes present in the GRN), the same result stands even if we discriminated between out-degree (number of regulated genes) and in-degree (number of regulators). Second, certain pairs of NDA classes show a similar integration to a GRN during evolution. Third, when we examine the NDA classes that compose each of the GRNs, we observe two behaviors: some NDA classes integrate faster (hot) and some other lower (cold) than expected by chance. Finally, we asked whether genes following the same behavior share related biological functions. We then found that accessory functions like niche specific and catabolic reactions are enriched in sets of cold genes, whereas widespread functions like DNA repairing/metabolism, cell wall biosynthesis, and other biosynthetic reactions are enriched in sets of hot genes). This novel approach cast new light on the study of GRNs evolution providing evidence that genes related to basal functions tend to integrate faster to GRNs than genes with niche-specific functions.

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Ecological dynamics of auxotrophic microbial populations

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Microbial communities are complex systems mediated by the interaction between the members and between members and the environment. Living in communities has multiple benefits to the members like the capacity to interchange metabolites or nutrients and the ability to perform functions that can not be done by individual cells. In this project we use a synthetic system of two *Escherichia coli* strains with complementary auxotrophy (leucine and tyrosine) and fluorescent markers (mCherry and eCyan) to evaluate the importance of environmental conditions to the dynamic of the community in a system defined as obligate mutualism (both strains need the presence of the amino acid they can not synthesize).

In this way the environmental properties can modify the community's dynamic changing the strength of pairwise interactions, for instance in some environmental conditions (low concentration of amino acids) the profile interaction of the community can be defined as cooperation and in the opposite environmental conditions (high concentrations of amino acids) the dynamic can be defined as competence. Using different combinations of amino acid concentrations we can estimate if the interactions are positive or negative.

We hypothesize that the different types of profile interactions have different sensibility to antimicrobial molecules, being these molecules able to modulate the interactions between the members of the community. This environmental modification can modify the population composition and enhance cooperativity or exclusion depending on the concentration in the environment and the individual sensibility.

Theoretical results have suggested that ecological feedbacks can be used to control the population structure (and therefore the profile of interaction). To evaluate this we measured total optical density and fluorescence of each marker so we can estimate if there are changes in the profile interaction and the sensibility of the co-culture at different concentrations of antibiotic.

Inferring gene regulatory networks from transcriptomic data: effects of normalization and combinatorial integration on predictions

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The modeling of transcriptional regulatory events is key for the prediction of gene expression changes in response to genetic and environmental perturbations. GRNs can be reconstructed based on transcription factor (TF) binding sites identified experimentally, through methods such as DNase footprinting or high-throughput techniques as ChIP-seq. However, these techniques are very time and resource consuming. On the other hand, despite curation efforts, the reconstruction of GRNs is far for being complete. Computational inference therefore can be considered as a valuable tool for GRN construction. There are different approaches for network inference such as: regression, mutual information, correlation, or Bayesian networks, among others. However, since each inference approach is based on different assumptions, they have different strengths and limitations, and discover diverse types of interactions. We thoroughly reviewed the literature and selected the best GRN inference methods in terms of usability and predictive power. Then, we used *in silico* data to explore how noise and data incompleteness affect GRN inference. Next, we collected genome-wide expression data for three organism *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa*. Afterwards, we systematically assessed the impact that normalization and batch-effect correction have upon the predictions by using up-to-date gold-standards (GSs) available in Abasy Atlas (https://abasy.ccg.unam.mx). Most of the methods performed better with more complete data and low noise levels. Assessment with biological and *in silico* data evidences GENIE3 as the best GRN inference method, even outperforming the community prediction. We found that using a no proper ranking procedure could decreases the prediction score of some methods. The removal of batch effect over RMA normalization seems to lightly improve the predictions but not in a significant fashion. It is important to note that only applying RMA normalization rather than improve, worsens the performance of the methods. Thus, either raw data or normalized and batch effect-corrected data should be used for GRN inference.

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Structural Biology

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Analysis of the FlgT-MotF interaction in the flagellar system 1 of *Rhodobacter sphaeroides*

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The bacterial flagellum is a complex rotary motor driven by the electrochemical potential. The rotating part of the motor includes the export apparatus, the C ring, the rod, the hook, and the filament. The stator is a proton channel that couples proton flow with torque generation made by MotAB complexes.

Rhodobacter sphaeroides is an alphaproteobacterium with two full sets of flagellar genes. One of these sets (Fla1) is constitutively expressed under the common laboratory growth conditions in which the cells assemble a single subpolar flagellum.

Within the flagellar locus 1 (Fla1) there is a putative bicistronic operon composed of RSP_0067 and RSP_6092 which is between *flhB* and the gene encoding the flagellar sigma factor, *rpoN2*. Inactivation of RSP_0067 (motF) produces a Mot- phenotype (cells are able to assemble a flagellum but are unable to rotate it). It was shown that MotF is capable of interacting with the flagellar protein, FlgT, and in its absence MotF is unable to be localized to the flagellar stators.

motF encodes a 239 amino acid polypeptide. The analysis of the primary sequence of MotF predicts a transmembrane segment, from residues 54 to 74. Recent studies have shown a high degree of conservation in the C-terminus of MotF, we found that replacing tyrosine 238 for alanine completely abolishes the functionality of the protein and it is not localized at the base of the flagellum. Such inability can be restored by replacing the residue 238 with other aromatic residues, such as phenylalanine and tryptophan, suggesting this residue as a possible binding site with FlgT.

Pioneering studies in *Vibrio alginolyticus*, where the crystallographic structure of FlgT was solved, revealed the presence of a β hairpin in the C- terminus which is fixed to the N-terminus by aromatic interactions π-π and T-stacking type. These interactions seem to be absent in FlgT of *R. sphaeroides*, in which there are aromatic residues in the region, but they are spatially distant from each other to allow these interactions where distance is fundamental.

In this work, we propose a novel interaction of an aromatic trimmer between phenylalanine 32 and 315 of FlgT and tyrosine 238 of MotF. We have collected evidence indicating that F315 is necessary for stability, and that F32 and F315 are both required for recruiting MotF at the base of flagellum through Y238.

Characterization of the open reading frame *rsp_1315* present in the flagellar set 2 of *Rhodobacter sphaeroides*

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INTRODUCTION: The bacterial flagellum is a multiproteic complex whose main function is to promote swimming motility in liquid or semi-solid media. This organelle is energized by a motor anchored to the cell wall which converts the energy from the electrochemical gradient into mechanical work[1]. The extracellular flagellar filament connects to the basal body through the hook. In enteric bacteria the basal body consists of a conserved structural core formed by 3 rings and a rod; however, new components have been described to be present in other species. This could be related to adaptations in the motor depending on the ecological niche in which these bacteria thrive[2]. The flagellar cluster 2 of *R. sphaeroides* includes open reading frames (ORFs) of unknown function or poorly characterized such as rsp_1315.
RESULTS: *rsp_1315* encodes for a 21 kDa periplasmic protein. This protein shows a MotE domain previously characterized in a flagellar protein that acts as a chaperone of the MotC in *Sinorhizobium meliloti*[3]. However, MotC is absent in *R. sphaeroides*, a mutant carrying a deletion of this gene assambles a complete flagellum that is paralyzed and complementation with the wild-type gene restores motility. Epifluorescence microscopy of GFP-RSP1315 revealed that this protein colocalizes with the flagellar structure, suggesting that it could be part of the motor. Nonetheless, its localization is independent of the flagellar proteins MotA2, MotB2, and FliL2, all of them known to be required for flagellar rotation. We observed that RSP1315 is stable in *motA2*, *motB2* and *fliL2* mutant strains. In addition, RSP_1315 did not interact with the periplasmic region of MotB2 or FliL2 when tested by a double hybrid yeast assay, indicating that its incorporation to the flagellar structure is possibly mediated through an unknown protein. Alternatively, RSP1315 could bind to its interaction partner as a dimer.
CONCLUSIONS: RSP_1315 is indispensable for flagellar rotation, it is capable of forming dimers via a disulfide bridge and could be homologous to the MotE chaperone of *Sinorhizobium meliloti*. Its recruitment to the flagellar pole does not require of MotA2 MotB2, or FliL2. Yeast double hybrid assays did not reveal an interaction of RSP_1315 with the periplasmic region of MotB or FliL.

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BIOTECHNOLOGY AND INDUSTRIAL MICROBIOLOGY

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Standardization of the conditions of growth and production of the δ endotoxin of Bacillus thuringiensis to be used in the biological control of insect pests

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Introduction *Bacillus thuringiensis* a gram-positive bacteria type that lives on soil, which main characteristic is protein synthesis with crystal structure known as δ-endotoxin or Cry protein. These proteins maintain insecticides properties. Commonly used as a biological alternative of pesticide.

Methodology For the optimum growth evaluation the Luria Bertani and Glucose crop medium were tested with salts. The measurements were made with a spectrophotometer, which obtains a optical density result equal as the parallel viable account technique were it was compared of.

Results. The Luria Bertani bacteria medium were the one with the best growth results, according to the spectrophotometer absorption numeric data. The optimum temperature for it was around 30°C. In the process of the kinetic growth a graphic chart were realized representing the exponential bacteria increase.

	MEDIUM: LURIA BROTH	MEDIUM: GLUCOSE AND SALTS
HOUR	ABSORBANCE	ABSORBANCE
0	0.095	0.043
1	0.098	0.059
2	0.121	0.094
3	0.094	0.059
4	0.125	0.06
5	0.148	0.062
6	0.163	0.0745
7	0.171	0.0775
8	0.229	0.08
9	0.343	0.165

Result Chart, absorbance recorded in each medium

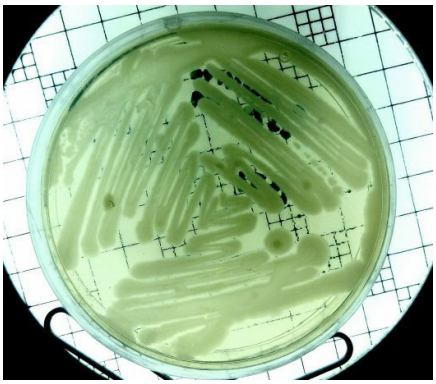

Figure. Bacillus Thuriengensis Strain

Conclusions. In base of the realized experiments at the lab it was determined that the Luria Bertani came to be the best growth medium for these bacteria, the optimum growth temperature was around the 30°C. Therefore, in experimental ways it was found the optimum growth conditions for the *Bacillus thuringiensis* and in this way, it can give continuity for the posterior δ endotoxin extraction which is the active element in the bioinsecticide production.

Characterization of *Rhodococcus ruber* MSA14: a bacterium to degrade high-molecular-weight polycyclic aromatic hydrocarbons

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are recalcitrant and persistent compounds in the environment. The US Environmental Protection Agency (EPA) has catalogued 16 PAHs as priority contaminants by their toxicity and mutagenic and/or carcinogenic effects. PAHs in marine environments are likely to increase due to anthropogenic activities in the coastal zones, such as wastewater discharges, marine traffic of ships, loading and unloading of fuels, representing a long-term environmental risk. An alternative to remove PAHs of the environment is using hydrocarbonoclastic bacteria. The *Rhodococcus* genus is a diverse bacterial group that possesses the ability to degrade a large number of organic compounds, including the PAHs. Therefore, the aim of this work was to characterize the hydrocarbonoclastic strain MSA14 of *Rhodococcus ruber*.

R. ruber MSA14 was isolated from marine sediments from the coast of Rosarito Port in Baja California, México. The isolate was previously identified using the MALDI-Biotyper system and its identity was confirmed by 16S rDNA sequence analysis. MSA14 strain was screened for growth on a variety of PAHs such as pyrene, phenanthrene and naphthalene as sole carbon and energy source, being pyrene the best substrate. Biochemical and molecular analysis have allowed to detect hydroxylating and excision dioxygenases genes, which encode enzymes that participating in PAHs degrading pathway. Furthermore, *R. ruber* MSA14 is able to survive and/or growth in high concentrations of PAHs, being more tolerant to pyrene (20 g/L) than phenanthrene and naphthalene (5 and 1 g/L, respectively). Also, this hydrocarbonoclastic bacteria was able to degrade pyrene diminishing its concentration at least 30%. Additionally, MSA14 is a biosurfactant producer and biofilm former. These results suggest that *R. ruber* MSA14 could be an excellent candidate to be applied on PAHs bioremediation processes.

Keywords: *Rhodococcus ruber,* polycyclic aromatic hydrocarbons, biorremediation.

Plant growth promoting mechanisms from heavy metal tolerant *Micrococcus* strains isolated of contaminated sites in Mexico.

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Environmental pollution by heavy metals and metalloids (HMs) is mainly caused as a result of several anthropogenic and industrial activities resulting adverse effects in diverse ecosystems and human health. The biological methods of remediation are cheap, easy to operate and they not produce secondary pollution. In order to alleviate the stress derivate of HMs exposure some bacteria have been developed several mechanisms of resistance. Also, certain plant associated bacteria can help to their host plants through direct or indirect mechanism, allowing them either to avoid or partially overcome stress generated by HMs, these bacteria are called heavy metals tolerant plant growth promoting bacteria (HMT-PGPB). The aim of the present study was evaluate Plant Growth Promotion (PGP) mechanism of eight *Micrococcus* bacteria isolated from contaminated environments in Mexico. The PGP traits such as: metallophore production, inorganic phosphate, zinc and carbonate solubilization, indol acetic acid (IAA) synthesis and ammonia production were evaluated using standard protocols previously described (Sperber 1958, Alexander and Zuberer 1991, Glickmann and Dessaux 1995, Nautiyal 1999, Mumtaz *et al*. 2017). In addition, cellulases was determined using the congo red medium (Hankin and Anagnostakis 1977). HMs resistance was evaluated in mineral medium proposed by Rhatnayake *et al*. 2013 supplemented with different amounts (0.5, 1, 2, 4, 10, 20, 30, 40 mM) of As^{3+}, As^{5+}, Cu^{2+}, Cr^{6+}, Zn^{2+}, Ni^{2+}, Co^{2}. All *Micrococcus* strains shown a metallophore production index ranged from 1 to 3, which are able to chelate Fe^{3+}, As^{3+}, As^{5+}, Cr^{3+}, Cr^{6+}. The IAA production was 0.46 µg mL^{-1} to 1.25 µg mL^{-1}. Only two *Micrococcus* strains solubilized carbonate, while, 75% of them were able to degradated cellulose. The ammonia was produced by all *Micrococcus* strains (9.21-32.51 mgN-NH_4 mL^{-1}), with exception of *M. aloeverae* AE-6^T. The strains showed a high degree of HMs resistance, especially to As^{5+}, Cr^{6+}, Ni^{2+} and Cu^{2+}. The order of the toxicity of the HMs was Cr^{3+}>As^{3+}>Co^{2+}=Zn^{2+}>Ni^{2+}>Cu^{2+}>Cr^{6+}>As^{5+}. The results obtained in this work demonstrated that several *Micrococcus* strains shown PGP characteristics and also they can tolerate high amounts of HMs, which highlights the great potential of these strains as candidates employed in phytoremediation process.

Cytotoxic activity of microalgae isolated from Cuatro Cienegas, Coah. in human cancer cell lines.

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Abstract

The biodiversity of microalgae is enormous and it has been estimated that exist until 800,000 species, but only about 50,000 are described. Cuatro Cienegas basin is located in the Chihuahua desert, north of Mexico, and has several hydrological systems which have been listed as a Wetland of International Importance within the international Ramsar Convention. This aquatic systems is characterized as an extremely oligotrophic site, with a high level of endemisms, and abundance of living organisms related to marine environments. Many types of biologically active components have been identified in microalgae and used in medicinal applications, therefore, the objective of this study was to explore the biotechnological potential of microalgae from Cuatro Cienegas Basin. A microalgae isolated from Cuatro Cienegas, was assessed for cytotoxic and anticancer activities using various in vitro assays and different cell lines of human cancers, including lung, skin melanoma, colorectal, breast, and prostatic cancers, as well as a normal cell line. The cytotoxic effects of microalgal extract tested on the cancer cell lines were close to 20 µg/mL, considered as an active crude extract, according to the criteria of cytotoxicity established by the U.S. National Cancer Institute. In vitro, the microalgal extract exhibits apoptotic activity and marked inhibition of adhesion and cell proliferation.

Pilot study for the evaluation of antimicrobial and antibiofilm activity of two varieties of walnut shell extracts (*Carya illinoinensis*) from Chihuahua State on a clinical *Staphylococcus aureus* strain.

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Background: Nosocomial infections are a common problem present on hospitalized patients that causes a kind of complications in the health that conduce to the dead of many of them, which according to the World Health Organization about 8.7% of hospitalized patients had nosocomial infections. One of the common species of bacteria isolated that generate a hospital acquired infections are *Staphylococcus aureus*, a gram positive bacteria that have the particularity to build biofilms. Plants extracts, like walnut shells, could be a resource to control the replication and construction of the biofilms due to they have bioactive molecules like polyphenols that can act against planktonic cells or interfering on the biofilms formation. In this study we determined the antimicrobial capacity through the cytotoxicity and the antibiofilm activity of two varieties of walnut shell extracts (*Carya illinoinensis*) from Chihuahua State on a clinical *Staphylococcus aureus* strain.
Methods: The antimicrobial and antibiofilm activity was evaluated to prove six crude extracts of two walnut shell varieties (Western [We] and Criolla [C]) by soxhlet extraction (solvents: water [W], methanol [M] and water-methanol 50:50 [WM]). Using a two-fold serial microdilution assay was preparing concentrations of each extract in the range 78-2,500 µg/mL to expose the *Staphylococcus aureus* strain. After 24 hrs of incubation, through the Miles and Misra method, was determined the dose lethal concentration (DL_{50}) for each treatment. Finally, the antibiofilm activity was determined through the crystal violet assay evaluating three extracts concentrations (1000, 500 and 250 µg/mL).
Results: Antimicrobial activity. All the extracts had antimicrobial activity against the *S. aureus* strain, oscillating in the range of 122-1350 µg/mL for the DL_{50} and of 17-189 µg/mL for the MIC (Minimal Inhibitory Concentration), being the best treatment (major cytotoxicity) the extract WeW, which exhibited a DL_{50} and MIC of 122 and 17 µg/mL respectively. Many walnut extracts reported by other authors presents levels of MIC less effective, like 15-46 (shell variety wangenh); 31-125 (root) and 125 µg/mL (leaf) for the same kind strain. Antibiofilm activity. The assays showed two kind of action on the biofilm building: an agonist effect (with the extracts WeW and WeWM) and an antagonist (WeM), being the best treatment the WeM extract (0.25 – 1mg/mL).
Conclusion: The best treatments who exhibited the most antimicrobial and antibiofilm activity was the extracts WeW (DL50=125µg/mL; MIC=17µg/mL) and WeM respectively.

Isolation of Steffimycin from an endophytic *Streptomyces scabrisporus* strain NF3 and screening for its antimicrobial activities

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Endophytic microorganisms reside in the internal tissues and subsist in a symbiotic or mutualistic association with their host plant without causing apparent symptoms of infection. *Streptomyces scabrisporus* strain NF3 is an aerobic, gram-positive with more than 55% GC content bacterium belongs to *Streptomycetaceae* family that was isolated from *Amphipterygium adstringens*, endemic of Mexico. In this study, the mixture of methanol and dichloromethane solvent was used to prepare the extract from strain *Streptomyces scabrisporus* and to separate the compound using column chromatography. The active compound was detected on the TLC plate by visualizing under UV spectra at between 200 to 295 nm. Further, the active compound was determined using ^{1}H NMR spectroscopic data and identified as Steffimycin with their molecular formula derived as $C_{29}H_{32}O_{13}$ (MW 588.6 g/mol). Additionally, the purified steffimycin compound showed significant antimicrobial activity against pathogens. In conclusion, the purified steffimycin compound from *Streptomyces scabrisporus* could be useful in the development of new therapeutic substances for pharmaceutical purposes.

Keywords: Endophytes, *Streptomyces scabrisporus*, TLC plate, NMR, Steffimycin

NP-Hoc protein fusion design and expression in *E. coli* BL21 for a potential influenza A vaccine

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Influenza A virus is the agent responsible of flu disease. Influenza Virus caused the deadly Spanish Flu in 1918 and other pandemics less dangerous. The last register influenza pandemic was in Mexico in 2009 and we don't have the certainty of when is the next one. The current production of the influenza vaccine is based in the 2 glycoproteins present in Influenza virus's capsid: hemagglutinin (HA) and neuraminidase (NA). These two proteins cover almost all virus capsid and when the antibody recognize them, the virus is neutralized and is not able to enter to the cell, so avoiding infection. Nevertheless, the mutation in HA y NA genes is high, producing new variants every 6 - 12 months. The vaccine produced must be updated every time new strain appears. Also, the vaccine is produced in chicken embryonated eggs and it takes 2 days to complete de process. In case of pandemics, would be impossible to cover the demand so it's important to find a new class of universal vaccine against influenza A. All influenza A types has the nucleoprotein (NP) in common which is highly conserved (95%), thus is considered a good candidate for universal vaccine. Hoc protein is present in T4 phage capsid and awards stability in hostile environment. Hoc could be deleted and its viability won't be affected. A fusion protein made of Hoc and another maintains its ability to assemble in all hoc sites available in phage capsid. In this case the fusion of Hoc and NP and generate an immune response. In this work we present the design of the synthetic sequence that encodes NP-Hoc fusion protein through bioinformatics. Three DNA fragments were amplified by high-fidelity PCR: linearized pET30a(+) vector, NP-Hoc synthetic fragment and the hoc gene. To clone the NP-hoc and hoc fragments into pET30a(+) vector, Gap-repair methodology was used, and *E. coli* DH5α was transformed with the new vector. Later, the new vector was isolated and *E. coli* BL21 (DE3) was transformed. IPTG inductor was used to produce NP-Hoc protein, purified by metal affinity chromatography (iMAC) and visualized on a polyacrylamide gel using the SDS-PAGE technique.

Engineering Escherichia coli membrane lipid composition: towards a robust chassis strain

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Ornithine lipids are phosphorus-free membrane lipids relatively common in eubacteria, but apparently absent from archaea and eukaryotes. It has been predicted that about 50% of the sequenced bacterial species have the capacity to synthesize OLs at least under certain growth conditions. *Escherichia coli* has been widely used as a host organism for biotechnology and synthetic biology applications. Previous studies have shown that ornitine lipids and hydroxylated ornithine lipids synthesized by OlsF and OlsC, respectively, may provide *E. coli* membrane stress tolerance, however it has not been tested in a systematic and biotechnology approach. We have previously generated a proteome reduced *E. coli* strain (PFC) that show an increased allocation of cellular resources for biotechnological applications. This strain has a deletion in the *phoB* gene, which is responsible for the response to phosphate limitation. We generated *OlsF* (from *Serratia proteamaculans*) and syntetic operon *OlsF:C* expression plasmids (OlsC from Rhizobium tropici), inducible by AHL and also use a native promoter with box PhoB induced by phosphate limitation. We aim to show if OLs provide interesting properties such as resistance to acid, high temperatures and solvents. Furthermore, OLs replace phospholipids, therefore its use will reduce phosphate requirement of *E. coli* in industrial applications. In this work we will show our advances in engineering the membrane lipid composition of *E coli* and its phenotypic results, in phosphate limitation and in stressful conditions.

Spray drying and antibacterial activity of the aqueous extract of *Agave cupreata*

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Bacterial infections are serious public health problems worldwide, due to this there is a constant demand for new drugs with safer and more stable ingredients, these can be obtained by the spray drying technique that allows obtaining powder products from liquid material. *Agave cupreata*, an endemic plant on the state of Guerrero, has anti-inflammatory and antibacterial activity against Gram-positive and Gram-negative bacteria, these activities are attributed to its secondary metabolites. The aim of this work was to dry the aqueous extract of *Agave cupreta* leaves by spray drying, and to evaluate the antibacterial activity of the microparticles obtained. The aqueous extract was obtained using the infusion technique and was dried by the spray drying technique (inlet air temperature of 180 ± 2 °C and an outlet temperature of 80 ± 2 °C). For evaluating the characteristics of the microparticles, a physicochemical analysis was carried out (humidity, water activity, pH, solubility and scanning electron microscopy). The antibacterial activity was determined by the broth microdilution method against the bacteria ATCC *Escherichia coli* 25923, *Escherichia coli* 35218 and *Salmonella Dublin* 9676, as well as a multiresistant clinical isolate of *Escherichia coli*. The physicochemical characteristics showed that the microparticles presented an irregular spherical shape with a size of 5.93 µm, a pH of 6, the humidity of 4.7%, the water activity of 0.2, solubility of 17.50%. The antibacterial activity, showed that the microparticles has an MIC of 16 mg/mL against all strains evaluated. The results obtained in this study suggest that the microparticles of the aqueous extract of *Agave cupreta* present a good antibacterial activity. In addition, the physicochemical characteristics indicated that they could be suitable for the preparation of phytodrugs.

Isolation and characterization of the antagonistic strain *Alcaligenes faecalis* MNCu3

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Multidrug resistance is one of the main global health problems. Several strains capable of resist common-use antibiotics have been widely disseminated. In 2017, the WHO also published a list of bacteria for which new antibiotics are urgently needed, including *Salmonella* and *Acinetobacter* pathogens. In this study, a strain isolated from nixtamalized mass was characterized as an antagonistic bacteria. Antagonistic assays were performed by the double-layer agar technique. The isolate MNCu3 was identified by 16S rRNA sequencing. Genomic DNA extraction was performed and the *rrs* fragment of the coding gene for 16S rRNA region was amplified by the PCR method. Analysis of the 16S rRNA sequence was carried out by Sanger sequencing, the BLAST tool and the EZBiocloud database. Partial purification of antagonistic metabolites was obtained by sequential solvent fractionation using hexane, ethyl acetate, dichloromethane, methanol and water. Antagonism by the isolate MNCu3 was observed against multidrug resistance strains of *Salmonella* Typhi, *Salmonella* Typhimutium and *Acinetobacter baumannii*. To identify the isolate MNCu3, the *rrs* fragment of the coding gene for 16S rRNA region was amplified by the PCR. After this, an agarose gel electrophoresis was carried out, obtaining a 1500 bp product. The isolate MNCu3 was identified as *Alcaligenes faecalis.* Subsequently, a phylogenetic tree was generated using different 16S sequences from strains of the same species and the strain of interest, defining its place in the species *A. faecalis*. In order to purify antagonistic compounds, the supernatant of batch cultures on potato dextrose broth was separate by sequential solvent fractionation. The extracts were concentrated and their antagonistic activity was tested using the diffusion disc technique. Inhibition of *A. baumannii* ATCC-BAA-747 and ATCC-BAA-007 in the methanol extract.

Finally, the role of siderophores on the antagonistic activity was identified mixing a CAS solution and the bioreactor supernatant and organic extracts. A change in CAS color from blue to red was observed in methanol extract suggesting the presence of siderophores in this extract with antagonistic activity.

Due to the antagonistic activity of *A. faecalis* MNCu3 against strains of *A. baumannii* and *Salmonella*, this strains could be proposed as a new source of antibiotics for treatment of multidrug resistant bacteria.

Production and Partial Characterization of a Cellulase Raw Extract from a Mexican *Streptomyces* Strain

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The energetic demand is in constant increase, this phenomenon is related with the human population rise. This situation has caused the generation of new approaches about the use of energetic sources. Agricultural wastes are viable substrates in the production of biofuels like methane or ethanol. The potential of biomass is due mainly to the chemical energy present in the bonds that bind the glucose molecules in the cellulose polymer. Cellulose needs to be hydrolyzed in order to be used as carbon source in the biofuel production process. This action is performed by especial enzymes named cellulases, which break down the β (1-4) bonds between glucose molecules. The aim of this work was to produce and carried out preliminary characterization of different *Streptomyces* strains and select one with the highest cellulolytic activity. To perform it, different Streptomyces strains, such as *S. griseus, S. hygroscopicus, S. bottropensis, S. nigrescens, S. violaceoruber* and *S. rochei* CC48 were growth in a minimum solid medium supplemented with carboxymethylcellulose (CMC) as carbon source, during 96 h, and immediately Congo red was added. Four strains show clear zones around the colony growth, these strains were classified as positive to cellulolytic activity. The strain that showed the highest cellulolytic activity, in a minimum liquid medium supplemented with CMC was *S. rochei* CC48. Then, the bacterium was subjected to different growth conditions to increase the cellulase production. Three variables with two levels were valued; CMC content (1 and 2% w/v), incubation temperature (28 and 37 °C) and culture media (mineral media and complex media). The optimum pH and temperature activity from raw extract was determined through 2^3 factorial design. CMC was diluted in different buffers in a pH range from 3 to 11, and 30 to 70 °C. The residual activity was determined at 60 °C for 48 h. The cellulases presents in the raw extract were identified through SDS-PAGE and activity gels. The experiments show that *S. rochei* CC48 strain was 95% most active to the other strains assesses. The highest cellulase production was reached in a 2% CMC content, with a complex culture medium at 37 °C. The optimal raw extract cellulolytic activity was at pH 7.0 and 60 °C, however the cellulolytic activity significantly decreases after 4 h to 60 °C. The *S. rochei* CC48 strain expressed three different cellulases, which have molecular weights close to 45, 40 and 30 kDa.

Antifungal activity of *Paenibacillus polymyxa* NMA1017 extracellular metabolites in biological control

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Antagonistic bacteria are considered ideal biological control agents because of rapid growth, easy handling and aggressive colonization of the rizhosphere. Within the biocontrol mechanisms, there is the production of secondary metabolites, these are not essential for the growth of the microorganism, and usually occur in the stationary phase, they play an important role in inhibiting pathogens.Several species of *Paenibacillus* are known to produce metabolites that inhibit growth and/or fungal activity, and these may be influenced by the addition or depletion of carbon, nitrogen or inductor sources. The strain NMA1017 was isolated from the nopal (*Opuntia ficus-indica)* rhizosphere and identified as *Paenibacillus polymyxa* NMA1017. In previous work it was detected that strain NMA1017 exhibited a wide spectrum of action. Therefore, the objective of this study, was to detect the production of extracellular compounds in media with different carbon and nitrogen sources. The results showed that the best carbon sources were glucose and sucrose, and the nitrogen sources were potassium nitrate, yeast extract and soybean paste. The effect of the supernatant observed by microscopy, revealed poor development of the phytopathogenic fungi and damage at the cellular level. The potential antagonistic metabolites were purified from a potato-based medium with sequential extraction using hexane, ethyl acetate, dichloromethane and methanol. The fractions were tested for antifungal activity and on TLC. Four fractions obtained with methanol were detected with antifungal activity. More work, including HPLC, NMR and mass spectrometry, are carried out to determine whether the antifungal metabolites have already been described or they are new molecules.

In conclusion, the use of rhizospheric bacteria can help to detect new antimicrobials for biotechnological use and may offer products that displace or accompany agrochemicals, without modifying or altering environmental conditions

Potential of biocontrol and molecular characterization of a Bacterial Agent of the *Pseudomonas* genus

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The increasing demand on agricultural products on the world implicates a greater exchange of plant material, and also implicates the possibility of introduction of new diseases for the importing country. The development of alternatives to control plant pathogens safer for the environment is a priority in the current production systems. The native microbiota of Mexican soils has shown to be a reservoir of microbiological resources with potential to face this type of phytosanitary risks. The *in vitro* antagonistic potential of the *Pseudomonas* S40 strain isolated form Mexican soils were determined against 13 plant pathogenic bacteria with quarantine status in México. Dual culture bioassays were established to assess the effect of the volatile compounds and the diffusible metabolites in solid medium. The inhibitory index against the 13 bacteria were determined using Image J software, as well as the index of bactericide capacity, and in relation to this the effect on the colonies growth. For the molecular characterization the genes 16S, gyrB, rpoB and rpoD were amplified; sequences information of the same genes was obtained from data bases as NCBI and the Pseudomonas Genome Data Base gathering a total of 221 type strains. The four genes data bases were concatenated and a Multilocus Sequence Analysis (MLSA) were conducted. Volatile compounds produced by strain S40 has bactericidal effect on *Clavibacter* and *Curtobacterium* genus bacteria with inhibitory indexes over 97% (Tukey P≤0.05). Diffusible compounds have significant bactericidal effect only on *Clavibacter nebraskensis* (Tukey P≤0.05). The MLSA showed that strain S40 belongs to *Pseudomonas chlororaphis* group with high phylogenetic similarity to *Pseudomonas protegens.* The results clearly show that the microbial genetic resources contained in Mexican soils have the potential to be used as a solid front for the prevention of introduction of foreign plant diseases to our country.

Effect of fumarate in microbial communities in sediment microbial fuel cells with sediments from Coatzacoalcos River

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The constant activity of the petrochemical industry in the lower basin of the Coatzacoalcos River has contaminated sediments with hydrocarbons (HC). A promising way to remedy polluted sediment is using sediment microbial fuel cells (SMFC). These systems carry out redox reactions, simultaneous pollutant removal and energy production which are catalyzed by microorganisms. However, the knowledge about microbial communities in SMFCs is scarce. Two SMFCs were assembled, using sediment contaminated with HC from the Coatzacoalcos River. The SMFCs obtained the maximum power density of 5.41 W/m^3 and 4.67 W/m^3. The conditions of one SMFC was modified, adding fumarate, where the power density increased from 2 W/m^3 to 74.4 W/m^3. Both SMFCs were disassembled for analyzed the microbial communities by Illumina Miseq sequencing of the V3 and V4 regions16S rRNA gene. The comparative analyses indicated that the microbial community structure of established anode biofilms was clearly differentiated from that in the initial inoculum. Bacterial groups identified were related to those found in contaminated areas with HC or associated with anaerobic metabolisms. One of the main differences between the bacterial community from in startup and end time was the enrichment of some bacterial groups as Desulfomonadales and Desulfobacterales, which are known to degrade HC and transfer electrons to the anode. Between the SMFC without fumarate vs with fumarate; there was a decrease in the Syntrophobacterales order, which perform syntrophy with organisms that use H_2 as an electron donor, possibly due to the change of anode potential. Regarding to the differences between the bacterial communities of anodic sediment vs anode biofilm; the latter was enriched with the Coriobacteriia class a non-cultivable bacterial group, present in anoxic environments contaminated with HC. We conclude that fumarate stimulated the enrichment of microbial communities with the capability to degrade hydrocarbons and transfer the electrons to anodes in SMFC.

PROMOTION OF THE *ZEA MAYS* GROWTH BY MIXED BACTERIAL INOCULANTS ISOLATED FROM JALA MAIZE ENVIRONMENT.

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Maize occupies the first place in consumption, sowing area and grain production in Mexico and worldwide. The microbial populations associated to plants have been widely studied in crops of economic interest, generating knowledge about the role of microorganisms for the plant development. The Promotion Growth Plant Rhizobacterias (PGPR) are a source of nutrient supply, stimulation of plant growth and even biocontrol of phytopathogenic organisms. In this study, nine endophytic bacteria isolated from the tissues of the Jala breed maize presented phenotypic traits commonly associated to promotion of plant growth and fungal antagonism. After antagonism tests among strains, three mixed inoculants were designed and their PGPR capacities were evaluated. The mixture containing *Burkholderia* sp. Z1AL11, *Achromobacter* sp. Z2K8, *Klebsiella variicola* R3J3HD7, *Phytobacter diazotrophicus* Z2WL1, *Pseudomonas protegens* E1BL2 and *Pantoea* sp. E2HD8 presented the best activities such as nitrogen fixation, phosphate solubilization, indolacetic acid production, siderophores production, ACC deaminase production, as well as proteases, cellulases and chitinases *in vitro*. The biocontrol capacities of the mixtures were demonstrated by plaque inhibition assays against *Fusarium culmorum*, *Fusarium oxysporum*, *Fusarium equisetti* and *Fusarium fujikuroi*, which are fungal phytopathogens commonly affecting maize. The growth promotion effect was determined on seedlings of the Conejo landrace in gnotobiotic and greenhouse systems, obtaining a significantly different increase in length and dry weight of stem and root compared to the uninoculated control and individual strains. Also, the increase of the activity of the enzyme Phenylalanine Ammonia Lyase (PAL), an enzyme associated to induced systemic resistance of plants, was detected in root and stem tissues.

Improving functional properties of germinated soybean flour by non-lactic acid fermentative bacteria.

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Fermentation is one of the oldest methods of food preservation. It has been used in legumes such as soybeans to eliminate non-nutritional factors and improve its bioactive properties. Previously, it was determined that germinated soybeans could potentially be a functional food source (González-Montoya et al., 2016; 2017) and even prevent gastrointestinal diseases due to the presence of bioactive peptides released during germination (González-Montoya et al., 2017). In this study, the nutritional properties of germinated soybeans by a non-lactic-acid fermentation process was improved using two microorganisms isolated from Sauerkraut.

Germinated soybean flour fermentation was performed for 96 h at 30ºC. Legumes bioactive molecules are mainly polar, so that supernatant was obtained in order to analyze the nutritional properties. For antioxidant capacity determination of copper chelation percentage and hydroxyl radical scavening were performed. Protein concentration was determined by Bradford method and changes in protein profile after fermentation were analyzed by SDS-PAGE. The isolates were characterized by biochemical and molecular techniques. Biochemical tests such as Phenol Red Broth, MR-VP, MIO, TSI, KIA and Simmon's Citrate Agar were performed. For molecular identification, bacterial DNA was extracted by standard phenol-chloroform protocol and the 16S rRNA gene was amplified by PCR and sequenced by Sanger method.

Biochemical test showed metabolic differences between both strains. This result was taken as a possible primary classification, which in further molecular analysis was confirmed. By their 16S rRNA sequence the isolates were identified as *Enterobacter bugandensis* AC1a (99.97% identity) and *Pseudomonas* sp. AC3a (99.9% identity). The antioxidant activity of fermented flour by AC1a and AC3a showed a lower percentage of copper chelating activity (37.68%, 40.28%) than the non-fermented flour (57.60%). However, they presented a higher percentage of ·OH radical scavening (95.38%, 77.10%, 57.95%). Finally, changes in protein profile between the control flour and the fermented flours were observed using SDS-PAGE analysis. The strains isolated in the present study are capable to improve nutritional and functional properties of fermented soybean flour. The strain *Enterobacter bugandensis* AC1a is related to human pathogens restricting its usage in food processes. However, *Pseudomonas* sp. AC3a is related to some strains used in biotechnological processes. This strain could be proposed as a biological agent to improve nutritional and functional properties of soybean by fermentation.

LIGNOCELLULOLYTIC ENZYMES BY ACTINOMYCETES ISOLATED IN THE EXTREMELY OLIGOTROPHIC DESERT OASIS CUATRO CIENÉGAS BASIN, MÉXICO

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Cellulose bioconversion into second-generation biofuels or other high-added-value products is emerging as the most promising alternative technology to overcome the issues associated with utilization of fossil carbon sources. This alternative is a multi-step process that involves lignin removal, cellulose hydrolysis, and fermentation of released sugars. Among saccharification methods, the use of microbial hydrolytic enzymes is the most eco-friendly system however it is a challenge of high costs since it requires a variety of hydrolytic enzymes working in synergy. Cellulases are a complex mixture of proteins with different specificities to hydrolyze the glycoside bonds, and are globally classified into Endo-β-1, 4-glucanases, Exo-β-1, 4-glucanases and β-glucosidases, being the endoglucanase activity the first stage in the enzymatic process of cellulose breakdown. Actinomycetes diversity in various environments due to its unique biotechnological potential and represent an economic and sustainable biological resource with the potential to obtain this type of enzymes has been widely researched, due to its metabolic diversity, reflected in an overabundance of enzymes available in its genomes. In the present study, biotechnological potential of cellulolytic enzymes of Actinomycetes from Cuatro Ciénegas basin (CCB) was evaluated. A well characterized actinomycete collection from CCB, was reactivated in an ISP culture medium Next, the strains were evaluated by their ability to grow on solid minimal medium with cellulose as sole carbon source. Then, they passed qualitative test to determine their hydrolysis halo in Petri dishes and were analyzed using the *Fiji* image analysis program. Results were analyzed by analysis of variance and comparison of means by *Tukey* test ($\alpha = 0.05$). Isolates with the highest hydrolysis halo were selected for their capacity to produce cellulases in vitro.170 isolates from the collection, had been subjected to the cellulose hydrolysis test; nearly 80% of the isolates tested had shown cellulolytic activity detectable by the Congo red test. The screening allowed the selection of sixteen strains with the greatest activity potential, and they are being evaluated in liquid minimal medium to determine complex cellulolytic enzyme activity.

Molecular detection of *Pseudomonas aeruginosa* by test strips coupled to the LAMP technique

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Pseudomonas aeruginosa is an opportunistic pathogen, of importance at the level of public health, because it is the causative agent of nosocomial infections, burned patients and immunocompromised patients. Traditional methods for identification are microbiological analysis, which includes its isolation, growth and detection by biochemical tests, a procedure that lasts approximately 72 hours. Molecular techniques have been developed in recent years, such as polymerase chain reaction (PCR), although at a high cost. The loop-mediated isothermal amplification method (LAMP) is a technique with high specificity and sensitivity.

In the present work, a diagnostic method for *Pseudomonas aeruginosa* was developed, using the LAMP technique, conjugated to test strips and gold nanoparticles. The samples were obtained from the Mexican Social Security Institute (IMSS) to standardize the PCR and LAMP techniques. Sensitivity was assessed using 30 ng., Obtained from the clinical reference sample. The concentration of the clinical sample was determinate by spectrophotometry with an absorbance 260/280. From the known concentration of 30 ng., serial dilutions 10^2-10^9 were made to evaluate the detection limit and reproducibility of the method. To determine the analytical specificity of the oligonucleotides, LAMP reactions were performed with genomic DNA from six different species of intrahospital bacteria of clinical importance. Additionally, the absence of hybridization with human DNA was evaluated by incubating the LAMP reactions at 0, 15, 30, 45 and 60 min. Subsequently, hybridization of an oligonucleotide probe complementary to the gene of interest was performed, with tests strips and gold nanoparticles.

The detection of *P. aeruginosa*, by hybridization of the oligonucleotide probe, with tests strips and gold nanoparticles, was a faster, less expensive technique with a capacity as efficient as PCR, and biochemical tests for the detection of *P. aeruginosa.* The sensitivity of the LAMP method was 0.03 ng. while in the PCR method it was 0.3 ng. It provides a technique that can be carried out in a simple way, by inexperience personal, achieving a viable alternative to traditional diagnostic methods.

Thematic area: Biotechnology

Physicochemical and Electrophoretical Characterization of Endoglucanases Obtained from a Novel Consortia PM-06 for Lignocelullose Saccharification.

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INTRODUCTION.

Mexico annually produces millions of tons of by-products derived from corn processing, such as the nixtamalized maize pericarp (NMP), which is rich in hemicellulose. The PM-06 consortia have presented a great capacity to degrade this by-product thanks to the enzymatic cocktail that is produced in situ. One of the most important enzymes are endoglucanases, due cellulose is the second most abundant polysaccharide in NMP. The objective of this work was the physicochemical and electrophoretic characterization of the endoglucanases produced by the PM-06 consortia.

METODOLOGY.

Saccharification kinetics was performed using the PM-06 consortia, samples were taking at 8, 24, 96 and 198 hours. Subsequently, endoglucanase activity was determined using DNS assay, using carboxymethylcellulose (CMC) as substrate. Soluble proteins, pH and percent degradation were also measured. The supernatants from each sample were run in acrylamide gels (6% w/v) with 0.1% CMC (w/v) for the zymographic study. The gels were incubated at different pH (4, 6 and 8) and temperatures (40, 60 and 80 °C) for 24 and 48 h, depending on the condition. At the same time, the effect of pH and temperature on the endoglucanases of each sample was evaluated quantitatively.

RESULTS

During saccharification of NMP it presents two pH phases, acid phase (4 and 24 h) and another alkaline phase (96 and 168 h). The endoglucanases secreted by the PM-06 consortium at 8 h of saccharification showed a high activity at 40 °C (0.323 U/mL) in acid pH. Endoglucanases of 24 h presented the highest activity in all process, having a good activity in 6-8 pH at up to 60 °C. At 96 h endoglucanases showed good activity at 4 and 8 pH values at 60 °C. At the end of saccharification (168 h), endoglucanases showed a good enzymatic performance at 6 pH value up to 60 °C. Electrophoresis gel shows two groups of bands, proteins between 200-40 kDa. Zymograms show these bands of high molecular weight with endoglucanase activity, all samples had similar pattern but with different intensities depending pH and temperature values.

CONCLUSIONS

The endoglucanases found during NMP saccharification respond to different medium conditions which are secreted, demonstrating the versatility of PM-06 consortium. Also, the different isoenzymes indicate the diversity in enzymatic crude extracts, and these can act under extreme pH and temperature conditions, proving the potential of these enzymes for their possible application in industrial processes.

Phytochemical profile and antibacterial activity of the acetonic extract of *Phoradendron* sp.

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The genus *Phoradendron* is characterized by having hemiparasite plants used in traditional Mexican medicine, it has been reported that the leaves are a natural source of antihypertensive, hypoglycemic, anticancer and antimicrobial compounds. The objective of this study was to determine the phytochemical profile and antibacterial activity of the acetonic leaf extract of *Phoradendron* sp. The leaves (100 g) dry ground from *Phoradendron* sp were extracted by maceration using solvent acetone (1L, nanograde). The chemical profile was performed by Thin Layer Chromatography (TLC) and Gases Chromatography coupled to Mass Spectrometry (GC / MS). The antibacterial activity was tested using the microtiter broth dilution method against ATCC bacteria and Clinical isolate. The results obtained from the TLC analysis detected the presence of flavonoids, *triterpenes*, saponins, terpenoids, digests, curcubitacins, and pungent compounds. Analysis by CG / MS of the extract detected 5 compounds: 1,6-anhydro-β-D-glucopyranose (1.71%); methoxyacetic acid, tridecyl ester (1.59%); octadecane-1-sulphonyl chloride (9.02%); 4,5-dihydroxy-7-methoxyflavanone (76.29%) and oxalic acid, cyclobutyl pentadecyl ester (4.25%). In the antimicrobial evaluation it was observed that the extract inhibited the growth of the clinical isolates *Staphylococcus hominis* (0433) and *Klebsiella pneumonie* (189) at a minimum inhibitory concentration of 250 and 500 µg / mL respectively. In this study the phytochemical profile and antibacterial activity of the leaves of *Phoradendron* sp. The results obtained show that this species represents an alternative for the search for new compounds that could be used to obtain phytopharmaceuticals.

Characterization of endolysin genes and design of an heterologous expression vector for its use against *Staphylococcus aureus*

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Due to the increase in drug resistance of bacterial pathogens such as *Staphylococcus aureus*, there is an urgent need to develop alternatives to the use of antibiotics for the treatment and control of bacterial infections caused. The endolysins, lytic enzymes from bacteriophages, are able to lyse the bacteria when added exogenously, being an alternative for the fight against pathogenic bacteria. The use of probiotic bacteria, as is the case of lactic acid bacteria, for the expression of these endolysins gives an added value for its use as a biotechnological alternative to antibiotics. The objectives of this work were to analyze the genetic diversity of endolysin genes from bacteriophages against *Staphylococcus aurues*, and generate an inducible expression system in probiotic lactic acid bacteria. The endolisinas encoding genes from bacteriophages isolated against *Staphylococcus aureus*, were amplified and sequenced. Deduced protein sequences were aligned and compared with the UPGMA routine of MEGA software (MEGA-X). Analysis of LysK-type endolysin protein sequences showed low diversity along structural domains associated with peptidoglycan lytic activity. Higher diversity was observed at the C-terminal domain. A plasmid for the expression of a LysK endolysin, was designed with the Smartgene (GenScript) and SnapViewer (GSL Biotech) programs. The plasmid has been synthesized at GenScript. The sequence of an expression vector for a LysK endolysin, plasmid pCipSpHLysKNaeI, which is inducible by NaCl was designed. This vector consists of all the necessary elements for the expression and secretion of the endolysin. It includes origins of replication for both lactic acid and Gram-negative bacteria, making it a shuttle-type vector. It also contains a NaCl-inducible promoter, a signal peptide for the secretion of endolysin, a peptide-tag (Halo-Tag) for its identification and purification, the coding region of the LysK endolysin, and a terminator of the *tpi* gene. The plasmid will be tested for expression in *Lactobacillus plantarum*, *Lactobacillus paraplantarum* and/or *Lactobacillus acidophilus*. This plasmid will allow us to express the endolysin LysK, against *Staphylococcus aureus*, in the probiotic lactic acid bacteria for its use as an antimicrobial agent.

Evaluation of Plant Growth Promoting Bacteria in saline conditions.

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Plant Growth Promoting Bacteria or PGPB's can be defined as bacteria that under some condition generate some benefit to the plants, forming part of the rhizosphere, rhizoplane or as endophytes (Bashan & De-Bashan, 2005). The positive effect that PGPB's provide to plants includes nitrogen fixation mechanisms, phosphorus solubilization, reduced effects caused by stress and the production of plant growth hormones, to name but a few (Bashan & De-Bashan, 2005) . According to Reis et al., (2011) the application of bacterial inoculations with plant growth potential has been widely used, however given the constant adverse conditions for plant growth, PGPBs are also required to be able to withstand the same conditions, being one of the most important salinity. Based on the above, the growth of Glutamicibacter arilaitensis, Pseudomonas chlororaphis and Ewingella americana, isolated compost bacteria and agricultural soil (CIBA), and also described as potential PGPBs by the working group were evaluated. The bacteria were inoculated in semi-solid MS medium (pH 5.8), using concentrations of 50, 100 and 200 mM NaCl and CaCl2, a control was used with no addition of salts and a positive control, that is, growing in nutritive agar. The bacteria were growing for 30 days at 30 ° C. An inoculum of the plaques was taken at days 10, 20 and 30 of bacterial growth to check their viability. A better growth of Pseudomonas chlororaphis was observed in the face of stressful conditions compared to the other bacteria, however it was perceived that the growth of the three bacteria is diminished in salt-enriched media with respect to the positive control, in addition to stressful conditions Bacteria fail to exhibit their characteristic coloration. According to the above, it can be concluded that the three bacteria have the ability to withstand conditions of up to 200 mM of NaCl and CaCl2, however it is important to consider adverse effects caused by salinity such as decreased growth and loss of typical characteristics that The bacteria possess in non-stressful growth conditions.

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In vitro analysis of antagonism of a *Trichoderma atroviride* wild strain towards phytopathogen microorganisms.

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The isolation and characterization of wild *Trichoderma* spp. allows the selection of suitable strains for its use in the biocontrol of specific species/variants of phytopathogenic fungi and oomycetes of local and regional relevance. This work evaluates the *in vitro* antagonistic capacity of a strain of *Trichoderma atroviride* (CMU-08) against different phytopathogens by dual confrontational tests, inhibition by volatiles and antibiosis. All assays were conducted using malt extract agar (MEA), papa-dextrose agar (PDA) and Vogel's minimal medium (VMM). The antagonism of CMU-08 strain towards *Fusarium* sp. in the confrontational trials was of 100% in the three media, with a weaker inhibition by volatiles (30.5%) and antibiosis (51.1%) in VMM. For *Colletotrichum gloespioroides* the highest inhibition (92.8%) was through the production of volatile metabolites in VMM. *Phytophthora cinnamomi* was antagonized efficiently by confrontation and antibiosis, with 70.2% inhibition in VMM; in these trials, CMU-08 strain was less efficient towards *Botrytis cinerea*, with a maximum inhibition value of 38.4% in MEA. The inhibition by volatiles for these pathogens was 67.0% (*B. cinerea*) and 70.2% (*P. cinnamomi*), both in MEA. The CMU-08 strain antagonizes phytopathogenic microorganisms by different mechanisms, and antagonism efficiency is significantly influenced by the culture medium used. The results indicate that the CMU-08 strain produces more metabolites (volatile/antibiotics) in minimal medium than in the complete media and that the availability of nutrients in the latter allows it to efficiently antagonize the phytopathogens tested. Despite being common tool for the characterization of wild *Trichoderma* isolates, antagonism assays are frequently conducted only in one culture medium. Results here obtained highlight the relevance to use different media in order to better knows biocontrol potential of new *Trichoderma* isolates.

Bioassay to evaluate the production of the C4-HSL autoinductor in strains of *Pseudomonas aeruginosa*.

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Many bacteria use acyl homoserin lactone (AHL) type molecules to communicate cell-to-cell and thus regulate the expression of numerous genes. These molecules are also known as autoinducers and are distributed in different groups of bacteria. An example of this is the γ-proteobacteria *Pseudomonas aeruginosa*, an opportunistic pathogen of immune-compromised individuals and it is the main cause of morbility and mortality in cystic fibrosis patients. The expression of different virulence factors such as elastase, pyocyanin, rhamnolipids among others, depend on AHL and the transcriptional activators of the LuxR-type family. *Pseudomonas aeruginosa* produces two types of AHL autoinducers: C12-HSL and *C4-HSL and two lasR and rhlR* regulators, the activity of these transcriptional activators is dependent on their cognate AHL signal, both the LasR-C12-HSL and RHLR-C4-HSL complex bind to their target sequence and activate transcription. To be able to detect autoinducers with simple tests can provide tentative information on the chemical identity, number and concentrations of signal molecules present in bacterial. The objective of this work was to create a simple bioassay for the detection of C4-HSL by *Pseudomonas aeruginosa* PAO1 reference strain. This bioassay is based on measuring the expression of a *rhlA´lux* transcriptional fusión in *Pseudomonas aeruginosa* PAO1. We observed that the bioreporter can detect C4-HSL at nanomolar concentrations (100 nM), and that it responds in a dose-dependent manner; showing that it is a tool to this AHL autoinducer.

Search of extremozymes from halophilic and halotolerant microorganisms for their biotechnological application

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Halophiles are a major class of extremophiles, which inhabit hypersaline environments. These microorganisms have the potential to produce halophilic enzymes ("haloenzymes") with unique structural features and catalytic power to sustain the metabolic and physiological processes under high salt conditions. The aim of this study was to isolate halophilic or halotolerant microorganisms for the production of enzymes with biotechnological or industrial interest.
Methods: The selection of halophilic or halotolerant microorganisms was made through enrichment technique inoculating 1 g of soil in liquid SP or halophilic medium (HM) supplemented with 10% NaCl with shaking at 150 rpm for three weeks. The cultures were inoculated in solid media until pure cultures were obtained. The obtained isolates were inoculated in DNAse medium or SP, HM or mineral medium supplemented with starch, skin milk, Tween 20, 80 or butyric acid and 15% NaCl. The hydrolytic activity was recorded when clear zones appeared around colonies and the results were expressed qualitatively as levels of enzymatic activities (LEA). **Results:** A total of 62 isolates were obtained in enrichment method and only four isolates were able to produce two or more of the enzymatic activities tasted in this study. The LEA showed by these isolates were medium (values between >2.0-5.0) with tolerance and activity until 20% NaCl. **Conclusion:** These data suggest that the microorganisms and their enzymes could be potential candidates for its use in biotechnological or industrial application but more studies are necessary to evaluate its physicochemical and kinetics proprieties.

Metagenomic screening and characterization of bacterial lipases from sediments of the Gulf of Mexico

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Lipases are triacylglycerol hydrolases (E.C. 3.1.1.3) that catalyze hydrolysis and synthesis reactions of lipids. Because their low substrate specificity, high stability and regio- and enantio-selectivity these enzymes are versatile catalysts in biotechnology. The search of lipases by metagenomics in marine environments offers new biocatalysts from uncultured bacteria with high salt tolerance, low temperatures activity and barofilicity, desirable properties in industries proceses. In addition, hydrocarbon polluted environments are known to have high lipolytic activity. The aim of this work is to explore sea-floor sediments through metagenomic approach in order to isolate and characterize new lipases with improved catalytic activities. For this purpose, we elaborated an environmental DNA metagenomic library from the Gulf of Mexico sediments polluted with hydrocarbons. The screening for lipase activity will be performed on plates with triolein substrate. Positive clones will be detected by fluorescence in rodamine test. Further identification of genes coding for lipases will be made building a DNA sub-library of lipolytic clones. Afterwards the genes will be cloned and over expressed and recombinant lipases will be purified in Ni-NTA chromatography. Pure protein catalytic activity will be assessed with *p*-nitrophenyl esters of different sizes as substrates at different temperatures. Analyzing genes coding for lipases by construction of DNA sub-library will allow found lipases with possibly improved functions. It is expected to isolate a lipase capable of catalyst large size *p*-nitrophenyl esters at low temperatures.

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Enzymatic and Metasecretomic Model of Lignocellulose Degradation from a Novel Microbial Consortium PM-06.

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INTRODUCTION.

Saccharification processes using agroindustrial wastes are an ecological and practical alternative that presents two main benefits: first, a reduction of environmental impact resulting from its release to the medium; second, a high value products obtained after its bioconversion. Biological systems like microbial consortia, can be more efficient on saccharification of recalcitrant plant material due their enzimatic diversity and complexity. The aim of this work is understanding the process of enzymatic saccharification carried out by main microorganisms from PM-06 consortium on NMP.

METODOLOGY

A 168 hours degradation kinetic was carried out with PM-06 consortium, under agitation (125 rpm) at 37 ° C. Samples were taken every 4 until 12 h and every 24 h until 168 hours to evaluate the main lignocellulolytic activities and biochemical and physicochemical properties of NMP residues and supernatans. In addition, an electrophoretic and zymographic study of supernatants of each time was conducted.

RESULTS

The enzymatic saccharification by PM-06 consortium allows 88 % of NMP degradation in 168 hours. Hemicellulose and cellulose are the main components consumed by consortium. A sequence was observed between the differents activities secreted by consortium; LPMO-endoglucanases and endoxylanasa-arabinofuranosidase couples were secreted at the beginning, whose generated products that stimulated enzymes such as cellobiohydrolases, glucosidases and xylosidases at 12 h, obtaining at the end fermentable sugar monomers. This sequence showed fluctuations throughout the saccharification, probably attributed to arrangement of lignocellulosic components in the NMP. At 144 hours, peroxidases showed a high activity, removing up to 50% of lignin in the last 24 hours, releasing the most recalcitrant material, which triggers an upturn in all enzymatic activities. Zymograms showed a great diversity of endoxylanases and endoglucanases as the degradation time elapsed.

CONCLUSIONS

The interactions between hemicellulases, cellulases and ligninases during saccharification suggest a possible enzimatic sinergy during all process. Besides, enzymatic systems from PM-06 consortium present a enzymatic diversity due microbial community successions during NMP saccharification.

Endemic igneous rock as a proposal of bracket material media for the growth of a microbial oxidizing sulphate consortium

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Biogas production contains hydrogen sulfide (H_2S) (0.1–3% v / v)[1]. According to the World Health Organization (WHO), the exposure to H_2S above 500 ppm can lead to death[2]. In addition, H_2S corrodes steel, including stainless steel. The biofiltration process is an effective and environmentally friendly technology. It is based on the use of sulphate oxidizing bacteria (SOB), with the ability to transform H_2S in less toxic compounds[3]. The biofilters, can be packed with a variety of adsorbent materials, previously inoculated with SOB. Nevertheless, the oxidation products of the H_2S and the biomass accumulation into the biofilters limit their use[4]. The objective of this research was to evaluate the removal of H_2S of a stream of biogas produced in a biodigestor. It was used a fluidized bed biofilter prototype packed with igneous rock. For this purpose, igneous rock extracted from a mining vein in the state of Chihuahua, Mexico, was used as the support material for a microbial consortium adapted to sulphate oxidation conditions. It was determined that the endemic igneous rock consists mainly of clinoptilolite, quartz and calcite; the material has a surface area of 28.98 $m^2\ g^{-1}$, pore size of 148.7 Å, and a pore volume of 0.1077 cc g^{-1}. The bracket material (sieve on average 3/4 ") was previously inoculated and adapted for a month with the SOB consortium. The fluidized bed biofilter was constructed from a cylinder of PVC of 10.16 cm with a height of 75 cm, and a volume of 5675.13 cm^3. The system was maintained with the following operating conditions: 30% humidity by recirculation (20 mL every 5 minutes) of a reducing sulphate culture media, maintaining a pH between 5.5-6.6, and a temperature of 25°C. Concentration of H_2S input to the system was on average 2881.3 mg dm^{-3}. Under these conditions, the biofilter removed H_2S in a range of 75-100% during 87 days of operation. During this process, the fluctuations of H_2S removal were observed as well as changes in the color of the circulating culture medium every 18 hours. It was concluded that the proposed biosystem is an efficient alternative technique for the removal of H_2S under the operating conditions mentioned.

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Biodirected phytochemical fractionation and antibacterial activity of fraction from *Agave cupreata*

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The indiscriminate use of antibiotics has led to the emergence of multidrug-resistant bacteria. According to WHO "Antibiotic resistance is growing, and we are fast running out of treatment options". For this reason, it is important to searching compounds with antibacterial activity that its can used as antibiotics to helping to fight this world problem. Plants have been a source of traditional drugs during millennia, and their pharmacological benefit is because they have a big diversity of secondary metabolite. The discovering the chemical structures in these plants is representing great potential for new drugs. These are highly complex and difficult to synthesize in an industrial scale. *Agave cupreata* is an endemic plant of the state of Guerrero, with medicinal properties. However, few studies determine its phytochemical profile and biological activity. The aim of present work was to determine the phytochemical profile and the antibacterial activity of the fractions obtained from the dichloromethane extract of *A. cupreata*. The phytochemical profile was determined by thin-layer chromatography using specific reactive for the family of compounds, while the antibacterial activity was evaluated with the techniques: bioautography (*Staphylococcus aureus* ATCC 25923) and microdilution in liquid Mueller Hinton medium using sensitive bacterial and drug-resistant clinical isolates. The purification of the fractions was performed through open glass column chromatography. The results of the phytochemical profile showed the presence of phenolic compounds, terpenoids, pungent compounds, and alkaloids. The bioautography indicated that fractions 40 to 92 avoided the growth of *S. aureus*. Fraction 92 inhibited sensitive strains and clinical isolates with a MIC of 8 mg / mL. The 69G fraction showed a mayor inhibitory activity against Gram-positive strains: *Staphylococcus aureus* and *S. haemolitycus* with a MIC of 10 mg / mL. While fraction 41 only showed activity against the susceptible strains *Staphylococcus aureus* and *Escherichia coli* with a MIC of 32 and 16 mg / mL respectively. From the purification of fraction 41, two pure compounds **A5** and **A6** were obtained. Compound **A5** exhibited antibacterial activity against drug-resistant and sensitive *Staphylococcus aureus* strain with MIC of 300 and 6.25 µg / mL respectively. The data obtained in this study suggest that the dichloromethane fractions obtained from the leaves of *A. cupreata* possess chemical compounds with antibacterial activity and potential pharmacological use.

Search of genes encoding for bioactive components by transposon mutagenesis in *Burkholderia cenocepacia* TAtl-371.

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Currently, the search for new antimicrobial compounds has increased due to the emerging of multidrug resistant microorganisms. On the other hand, it is known that the species of the *Burkholderia cepacia* complex are producers of compounds that inhibit the growth of various pathogens of agricultural and medical interest. In our laboratories we have studied *Burkholderia cenocepacia* TAtl-371, a strain producing compounds that inhibit the growth of *Tatumella terrea* SHS 2008^T, *Acinetobacter baumanii* BAA 007, and *Salmonella* Typhimurium, to mention a few. When analyzing the genome, genes related to the synthesis of some antimicrobials were found. However, due to the wide antagonistic spectrum and the production of a metabolite of unknown nature by this strain, it is suggested that there are genes unidentified involved in this activity. Thus, in this project a bank of transposition mutants was generated by using a Tn5 derived transposon that confers resistance to chloramphenicol (Cm). The insertional mutants were isolated by their resistance to Cm and tested for antagonism towards *T. terrea* SHS 2008^T. So far, 3 insertional mutants with a reduced inhibitory activity have been obtained. The location of these insertions is being conducted to define which genes are involved in the antagonism activity for Tatl-371. Moreover, in order to determine the feasibility of directly using strain *B. cenocepacia* TAtl-371 for biocontrol and to determine its potential toxicity it was tested in a *Galleria mellonella* animal model. Results showed that this strain had a pathogenic behavior similar to the type strain of *B. cenocepacia* J2315, indicating that the TAtl-371 strain is potentially virulent and cannot be considered for field experiments.

Analysis of the enzyme Lytic Polysaccharide Monooxygenase during the enzymatic degradation of the maize pericarp

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INTRODUCTION.

Lytic polysaccharide monooxygenases (LPMO) are key enzymes in the enzymatic conversion of lignocellulosic biomass, due to its oxidant activity in glycosidic bonds allowing the activity of other enzymes in the substrate. They have gained interest since their discovery because of their ability to improve the enzymatic cocktail activity. However, there are still many unanswered variants of the LPMO. The present work is considered a contribution to the research on the degradation of biomass by the LPMO in a microbial consortium denominated PM-06 with a potential for biotechnological applications.

METHODOLOGY.

PM-06 consortium was cultivated in a medium containing maize pericarp and yeast extract. Samples were taken at intervals pertinent, to measure the weight of the residual, enzymatic activities and pH. mgDNA sequencing was done Sequencing data were assembled and aligned with against the NCBI nr database for taxonomic classification. The activity of the LPMO enzyme was determined in each time interval and its temperature (40°,60° and 80°C) and optimum pH (4, 6 and 8) were characterized, an enzyme purification was performed and an analysis of the sequence alignment of the bacteria expressing for the LPMO enzyme activity.

RESULT

Potential for enzymatic degradation was 86% of the biomass in a time of 168 hours, the highest activity by the LPMO was at 8 hours with 0.001 U / mL, by measuring the response of the LPMO enzyme in various pH and the temperature was obtained at an optimum temperature of 40 ° C with a pH of 8. The purification of the LPMO was carried out where the enzymatic activity was better appreciated comparing the data obtained with the supernatant without purifying. With the analysis of the mgDNA, the *Bacillus Cereus*, *Bacillus paranthracis* and *Paenibacillus macerans* strains were identified as responsible for the expression of LPMO belonging to the AA10 family with conserved regions between them.

CONCLUSION

Significant progress has been made towards the degradation of biomass through the investigation of microbial consortium, in this study the percentage of degradation of a native microbial consortium with LPMO activity is demonstrated, providing a better understanding of the functional activity of the recently discovered enzyme, as well as allowing the analysis of the role played by the LPMO during the degradation of lignocellulosic biomass.

Generation of an immune library fragments of single-chain antibodies of mouse against prostate specific antigen

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The prostate is an important gland in the male reproductive system. The uncontrolled growth of these glandular cells can become a cancer. In 2018, the World Health Organization reported 1,276,106 cases of prostate cancer worldwide. Thus, becoming the fourth more common type of cancer and the second more among men, affecting 1 in 7 men above the age of 65 years old. Currently, the most used diagnostic method for this type of cancer is the detection of Prostate Specific Antigen (PSA) in blood, however it lacks a necessary high level of reliability specificity. The generation of fragments of single chain antibodies of mouse origin against human PSA with higher specificity is proposed as a solution to improve the precision of this diagnostic method; furthermore, it could be used in immunotherapy against prostate cancer. Three mice were immunized with PSA in different concentrations in order to get immune response. The affinity measured by an immunoenzymatic assay (ELISA) from serum of an immunized mouse showed the highest antibody titles. Afterwards, the extraction of total RNA of the removed spleen demonstrated good integrity of nucleic acid, and it was used for synthesis of cDNA. Subsequently, the sequence that encode the heavy variables regions (VH's) and the light variables regions (VL's) of the immunoglobin were amplified by the Polymerase Chain Reaction (PCR), resulting in DNA bands of 400 bp and 350bp lengths, respectively. These two amplicons were cloned in frame together with an intermediate sequence that encodes a flexible peptide linker; the resultant constructs (800 bp) encodes a single chain fragment variable (scFv) against PSA. Finally, the library was cloned inside a pComb3X vector to select the right constructions. This investigation is in the area of molecular biology & biotechnology, and I want to participate presenting a poster.

Kinetic characterization of the radial growth of *Ganoderma lucidum* on different supplements and media culture

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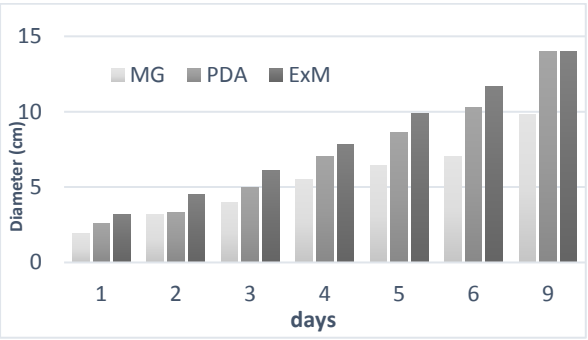
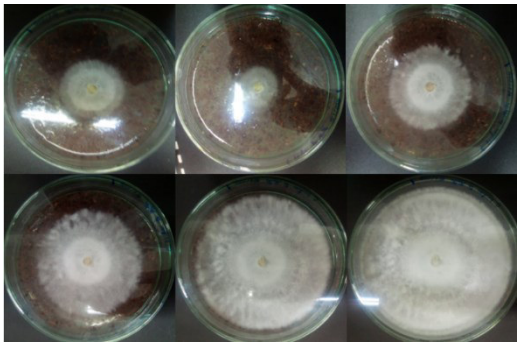
Introduction. White rot fungi are of great interest for their ability to degrade lignocellulosic material (Hernández Mendieta, *et al.*, 2013). Many basidiomycete have a great commercial value for their nutritional content, the production of spawn is an important process in the cultivation of edible fungi, so it is sought to optimize the obtaining methodology (Suárez A. & Holguín H., 2011).

Methodology. Three culture media, malt extract agar (ExM), potato and dextrose agar (PDA) and m-Green agar (MG) were used in the process, and, pine sawdust (*Pinus* sp.) and oak sawdust (*Quercus* sp.) as a supplement. For each of the medium three treatments were performed, in duplicate.

Results. The figure 1b, show a higher radial growth rate in the ExM supplemented with oak sawdust, obtaining a growth rate of 0.648 mm / day (figure 1a), while in the ExM sole, a speed of 0.541 mm / day.



(a)



(b)

Figure 1. Radial growth of mycelium in solid medium (a), mycelium growth in ExM supplemented with oak sawdust (b).

Conclusions. The fastest growth, was obtained in the ExM supplemented with oak sawdust; the characteristics that developed the mycelium, varied according to the culture medium and the supplement used. In the Petri dishes with pine, sawdust, slow growth was observed, while those supplemented with oak sawdust shown a cottony mycelium and speed growth, may be due to the tree lignocellulosic constituents used like supplement.

Morphological and electrogenic analysis of bacteria isolated from the Lagos de Moreno river, Jalisco.

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At present, the use and consumption of fossil fuels has a dramatic increase due to industrialization in developing countries, population increase and modification of lifestyles. This demand for fossil fuels generates an energy crisis marking interest, in the promotion of alternatives to meet the world's energy needs. The systems bioelectrochemical it´s a technology of broad interest because it complies with two functions of environmental significance, the production of renewable energy and the treatment of effluents. The technologies used to generate bioenergy are microbial fuel cells (CCMs), which are a technology capable of producing electricity through the oxidation of organic compounds by microorganisms. This technology offers a promising solution to meet growing energy and wastewater treatment needs. Among the microorganisms used in these technologies is the gender Geobacter, which generates a "excess" of electrons during its metabolism and are driven to the outside of the cell, morphologically are bacteria, Gram-negative. For insulation, it carried out a sampling of water from the river of Lagos de Moreno, Jalisco. This biological sample is inoculated in an environment of selective culture containing as carbon source. It incubated to 35ºC between 18 and 24 hours. Analysis and characterization of the bacteria through, optical density, spectrophotometry, staining Gram and Atomic Force Microscopy (AFM), were done. The results presented are similar in morphology and staining Gram with bacteria of the genus *Geobacter* of Interest. The isolated crop was used to generate electricity in microbial full cells constructed with glass container. The isolated crop electrogenic potential were observed.

Metagenomic and chemical analyses of microbial communities from coastal karstic swamp sediments to assess their biosynthetic potencial for PKS and NRPS production

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In the last decades the use of metagenomics to study microbial communities has revealed their crucial role in sustaining ecological balances. Moreover, this technique can also be used as a strategy to identify genes involved in the biosynthesis of compounds of special interest, for example, those with biological activities. Mangrove sediments are, in general, environments with high diversity and richness of microorganisms which are adapted to particular environmental conditions involving changes in salinity and fresh water inputs. When analyzing three metagenomes from microniches in these sediments (canal, roots, middle) the three were basically replicates of each other with Proteobacteria, Cyanobacteria, Firmicutes, Bacteroidetes and Actinomycetes as the dominant Phyla in the three sites and them sharing the same main functions. A deeper analysis of genes involved in secondary metabolism suggested that sediments from the canal had the best biotechnological potential since they showed the highest number of genes for PKS (106) and NRPS (86) biosynthesis. From these sediments, 114 strains with actinomycete morphological characteristics were isolated and 90% showed presence of PKS genes by PCR amplification. Further targeted massive sequencing of 20 representative strains showed they possess genes involved with PKS and NRPS biosynthesis similar to those involved in the production of scabichelin, epothilone, actinomycin and oxazolomycin, amongst others. Taxonomic identification shows these strains belong to the genera *Streptomyces*, *Nocardiopsis* and *Pseudonocardia*. Further work on the chemistry in extracts from these strains will be presented. This work highlights the importance of using an interdisciplinary approach for natural product discovery.

Proyecto Semilla, Facultad de Química, UNAM

Isolation of wild bacteria from nodules of *Phaseolus vulgaris* and analysis of their potential use as biofertilizers

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Common bean (*Phaseolus vulgaris*) represents an important proteins source in the Mexican population diet. The production of common bean is severely affected by drought and other abiotic stresses, however, more than 60% of world production comes from arid areas with little water availability. As an example, it has been calculated that only during 2012, the drought was the cause of a drop of around 60% in the production of common bean in México, which represents around 800 million dollars in losses. To increase the production of this legume, synthetic fertilizers and pesticides have been used. Nevertheless, such practices increase production costs, in addition to the ecological impact and damage to soils and public health. The application of plant growth promoting rhizobacteria (PGPRs) as biofertilizers can improve the yield of *P. vulgaris* with respect to grain production, biomass, N content and a better moderate response to abiotic (including drought and salinity) and biotic factors. The objective of the present project is the identification of new wild bacterial strains that could be applied in the near future as biofertilizers designed to be used in arid and saline soils. Here, we show the isolation of 76 wild-type rhizobacteria from arid and saline areas of central Mexico. The whole collection resulted to be constituted by Gram- bacteria, and based on cell appearance the collection contains cocci and bacilli. Our bacterial collection was analyzed on their ability to grow at different concentrations of NaCl as a saline agent, and this tolerance test showed that 14.47% of the isolates tolerated until 0.2 M, 17.10% tolerated up to 0.4 M, while 26.31% tolerated 0.6 M and interestingly 11.84% tolerated up to 0.8 M. We are currently analyzing the ability of the full collection to grow under osmotic stress mediated by PEG. Additionally, our isolates were tested to carry out phosphate solubilization showing that 23.6 % of bacterial isolates solubilize phosphate with different solubilization rates ranging from 2.12 to 2.99. As a first strategy to know the type of bacteria that constitute our collection, we are performing a metagenomic analysis of the mixture of the entire collection. The obtained data will be showed.

MAVL wish to thanks to SIP-IPN 20181190, SIP-IPN 20196334, SIP-Innovación 20164857 and SIP-Innovación 20172308 grants for the financial support to develop the present work. CMR thanks the CONACYT fellowship to study his master degree.

BACTERICIDAL EFFECT OF PURIFIED PRODUCTS OF SOIL BACTERIAS AGAINST GRAM-POSITIVE BACTERIAS

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One of the biggest problems of the plant *in vitro* cultures that compromises the growth of explants is the several contaminations with bacteria and fungus. Hence the application of antibiotics in the media, however this has been contextualized in groups as dangerous or overexploited, so that the searching for new alternatives is currently carried out, which have led to the exploration of microbial diversity in each ecosystem . Therefore, the task of finding a natural antibiotic for the eradication of phytopathogens was given. For this, five phases were carried out, where the first phase consisted of taking soil samples, obtaining the inoculum, dissolving it in water, centrifuging and inoculating the supernatant in nutrient agar, incubating at 32 ° C for 72 hours. For phase two, three bacterial strains were selected that prevented bacterial growth around them. During phase three, the bacterium that presented the greatest inhibition was grown in Muller-Hinton broth for 7 days at 32 ° C, until reaching the stationary phase, it was subsequently filtered on a 0.2 micron membrane filter to separate excreted metabolites from biomass (Fraction F2). Phase four consisted of a Kirby Bauer antibiogram of disk diffusion inhibition test against a wild strain of *Staphilococcus* spp. contaminant of an MS medium from an in vitro culture of sorghum. The results were analyzed by a completely randomized design, taking the average of the inhibition halo, having as a positive inhibition control the commercial antibiotic oxytetracycline, the broth and the centrifuged biomass as well as sterile water as a negative control. Additionally, an E. coli strain sensitive to oxytetracycline was used as growth control.The results were analyzed using a MANOVA analysis with the PSPP software (GNU / GPL license). The analysis of the data motivated that according to the average that the greatest inhibition was observed with the F2 treatment, followed by the treatment with oxytetracycline, in the same way this was supported by the multiple comparisons test, which shows that the F2 treatment shows a significant difference of 0.048. In this way and confirming with the test between subjects, it was determined that there were differences between the treatments tested. For all the above, it can be said that it was possible to observe inhibition with a Staphilococcus spp. with F2, which indicates that the bactericidal substance is excreted, according to the statistical analysis of the Kirby Bauer antibiogram and the guidelines of the Institute of clinical and laboratory standards, the substance produced by the bacteria has a high degree of inhibition of bacterial development Other studies could demonstrate the potential use of the antibacterial product of Bacillus spp. isolated in field and industries.

Isolation and molecular characterization of a strain with 2,3-extradiol dioxygenase activity and heterologous expression of the enzyme responsible of the activity

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The degradation mechanisms of aromatic compounds generally converge to central intermediaries such as catechol -highly persistent in the environment and toxic-., in which it is possible to observe the incorporation of oxygen atoms by specific enzymes (dioxygenases), causing the ring to be open into what is known as the lower degradation pathway of aromatic compounds. The 2,3 extradiol dioxygenases catalyze the incorporation of two oxygen atoms to the catechol ring outside the hydroxyl groups of the molecule, leading to the formation of semialdehyde 2- hydroxymuconate. It has been widely reported that aromatic hydrocarbons can be degraded by marine bacteria. In this work we analyzed the 2,3 extradiol dioxygenase activity screnning in 32 bacterial isolates, 67 bacterial consortiums and two metagenotecas obtained from water and sediments of the Gulf of Mexico.
The activity was detected in 11 bacterial consortiums and the consortium with the highest activity was selected, dispersed using rich medium supplemented with phenol and corroborated by microscopy.
To identify a strain with the activity, catechol was added, and the change of coloration was monitored spectrophotometrically by reading the absorbance at 375 nm using crude protein extract. The 16S rRNA gene was amplified to generate a phylogenetic tree using MEGA 7, which allowed us to identify Pseudomonas genus. The complete genome strain was sequenced with technology Illumina, and its assembly was carried out finding a homology of 98.08% with Pseudomonas stutzeri DSM 50227. The presence of the 2,3 extradiol dioxygenase gene was identified, which was cloned and expressed in *E. coli*

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SEARCH FOR LACTIC ACID BACTERIA CAPABLE OF DEGRADING GLUCOSINOLATES

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Keywords: lactic acid bacteria, glucosinolates, myrosinase

Summary

Introduction: Crucifers contain unique compounds called glucosinolates (GSL) which promote health. Their enzymatic hydrolysis by myrosinase releases several beneficial products, so this enzyme is the most important factor in the transformation of GSLs. However, the vegetable enzyme has the disadvantage of inactivation by heat, pH and high pressures, so an alternative source such as bacterial myrosinase should be taken into account. It has been shown that lactic acid bacteria (BAL) have the ability to hydrolyze GSL through fermentation processes obtaining greater bioavailability of the benefits offered by secondary metabolites. [1].

Methodology: Microorganisms were isolated from four commercial fermented products in LB medium enriched with 10 mM sinigrin (SN), which were cultured every 3 days in Petri plates with M9 medium and SN until day 15 at 37 °C. From the plates where growth was observed, the colonies in LB broth with SN were isolated and kept in incubation for a period of 72 h at 37 °C. Afterwards, the medium was removed and the degradation of this compound was observed by means of an HPLC analysis. From the first isolations a second selection was carried out, leaving under analysis only those microorganisms that were able to survive the study conditions. These isolates were subjected to microscopic identification by means of a Gram stain and the catalase test was performed, followed by DNA extraction and PCR analysis of the rRNA 16S gene.

Results and discussion: A total of 40 isolations of microorganisms capable of growing in a medium enriched with 10 mM SN were obtained from two of the four products evaluated. The HPLC analysis allowed us to observe the partial or total degradation of the SN carried out by the isolated microorganisms, as well as the production of secondary metabolites among which we find indol -3-carbinol. The second selection was carried out by the capacity to survive in the studied conditions, from which 22 microorganisms were obtained, which were tested for identification. It was found that the bacteria were gram positive with the presence of bacilli and cocci, all catalase negative. The amplification of the 16S ribosomal gene allowed the identification of *Lactobacillus* spp and *Bacillus* spp by sequencing.

Conclusions: It was possible to isolate microorganisms capable of degrading SN glucosinolate from commercial fermented products. The tests to which the microorganisms were subjected indicated that they belong to lactic acid bacteria.

Reference: [1] V. Luang-In, S. Deeseenthum, P. Udomwong, W. Saengha, and M. Gregori (2018) Formation of sulforaphane and iberin products from Thai cabbage fermented by myrosinase-positive bacteria. Molecules, 23, 1–1

Bacterial hydrocarbon-responsive transcriptional factors and their application for the design of biosensors

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Hydrocarbonoclastic bacteria contain transcriptional factors (TFs) able to sense hydrocarbons and regulate the gene expression necessary for their degradation. Some of these TFs have been used for the design of whole cell biosensors (WCBs), which are devices that sense chemical compounds producing a measurable signal with applications for environmental monitoring. The WCBs are composed of a TF and a reporter gene (e.g. a fluorescent protein) whose expression is driven by the TF-recognized promoter. Thus, in the presence of a chemical analyte (hydrocarbon), the TF can activate/repress the expression of the reporter gene. We are interested in the structural and functional study of TFs able to recognize alkanes and aromatic compounds.

AlkS from *Pseudomonas putida* is a TF which has been used for the construction of WCBs to detect short-chain alkanes. Although the AlkS's activity has been widely studied, there is no information about how the protein recognizes the hydrocarbons. Therefore, we overexpressed AlkS in the *E. coli* BL21(DE3) strain for its purification and subsequent *in vitro* analysis. The heterologous protein was insoluble, to overcome that, we tested different conditions including host strain, temperature and time of induction. We obtained a soluble fraction of AlkS inducing its expression in the *E. coli* Rosetta-gami strain, previously incubated at 42°C in the presence of 3% NaCl (heat and osmotic stress). Additional experiments are being carried out to purify AlkS.

For detection of aromatic compounds, we constructed a WCB based on the benzoate-sensing BenR TF from a marine *Pseudomonas stutzeri* and the mNeonGreen fluorescent protein. Early experiments in *E. coli* demonstrated that the expression of the fluorescent protein only occurred in the presence of benzoic acid. We will evaluate the activity of the biosensor in different host strains including environmental bacteria.

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Characterization of cultivable yeasts in the production of a traditional mezcal from Oaxaca

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Mezcal is a traditional Mexican alcoholic beverage, obtained through an artisanal process that has 7 stages of production: selection and raw material cutting, agave cooking, must grinding, fermentation of fructose juice, distillation, rectification and maturation of distillate. Any alteration during any of these stages could affect the final organoleptic properties, as well as alcohol yield. Identifying yeast and bacterial strains involved in the fermentative process is crucial in order to determine population dynamics (biotic factor) as well as the abiotic factors such as temperature, pH, content of sugars and mineral salts that affect the aforementioned process. Mexico has the PDO (protected designation of origin) of Mezcal, which is hand made in the states of Oaxaca, San Luis Potosí, Durango, Zacatecas and Guerrero. Depending on the production location, different *Agave* species are used as raw material and the process may vary, causing a wide range of fermentational profiles caused by differential volatile compounds production that affect the characteristical taste and aroma of the final distillate from one region to another. Nevertheless, the quality is regulated by the Standard Mexican Officer: NOM-070-SCFI-94.

Currently, there are few studies on the production of Mezcal, therefore we seek to sample the fermenter tank to determine the composition of cultivable yeasts (*Saccharomyces* and no-*Saccharomyces*) populations present from the start of the fermentative process until the end and, identify how these changes reflect on the organoleptic and physicochemical properties of the drink. In this study we will assess the population composition, purify and, identify through MALDI-TOF mass spectrometry and sequencing of the 18s gene the different strains obtained and characterize the biochemical properties.

Analyzing the composition of the microbial community found in these kind of products paves the road to understanding the interactions between the biotics and abiotics factors, like the relationship in the fermentations temperatures and the production of specific compounds by isolated yeast, related to the aromatic properties of this distilled beverage (Martell Nevárez et al., 2011).

Martell Nevárez, M. A., Córdova Gurrola, E. E., López Miranda, J., Soto-Cruz, N. O., López Pérez, M. G., & Rutiaga-Quiñones, O. M. (2011). Effect of fermentation temperature on chemical composition of mescals made from Agave duranguensis juice with different native yeast genera. *African Journal of Microbiology Research*, *4*(22), 3669–3676. https://doi.org/10.5897/ajmr11.467

Anticancer Potential of Two Sediment Actinobacteria Extract from Puerto Vallarta and Veracruz, México

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Cancer is the second cause of death worldwide, is a term that involve a group of more than hundred diseases that arise when defects in physiological regulation cause unrestrained proliferation of abnormal cells. Conventional cancer therapies such as chemotherapy cause deadly secondaries effects. Therefore, the research for novel anticancer compounds is one of the major emerging areas for research in the past decades. Marine actinobacteria have been looked upon as potential sources of bioactive compounds. They hold a prominent position as targets in screening research due to their ability to produce metabolites and other molecules of pharmaceutical importance. Have been reported that actinobacteria produce antibiotics, antitumoral agents, immunosuppresses and enzymatic compounds. In this work, extraction of two actinobacteria CNY-484 and CNY-528 (previously isolated from marine sediment in Isla Verde, Veracruz and Los Arcos, Puerto Vallarta, México) was carried out with ethyl acetate. Two extract were concentrated by rotoevaporation to evaluate the cytotoxicity in five human cancer cell lines. Antitumoral activity in five human cancer cell lines; HeLa, H1299, H1487, Caco-2, HepG2 and MCF7, was proved using MTS colorimetric reactive. 2.5x105 cells were cultured in 96 well plates and incubated at 37ºC with 5% CO_2. After 24 hours 50 μg of each extract was added, etoposide was used as positive control. Results were expressed as mean±SD compared to negative control (untreated cells). The results shown that CNY-484 and CNY-528 extracts decrease the cell viability of all five cell lines up to 93% (**p<0.01) in all cancer cell lines. Both extracts present the major

Screening of cellulose-degrading wild molds for cellulase production from lignocellulosic agroindustrial residues.

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Cellulases are important enzymes that hydrolyze cellulose for industrial purposes, specifically for biofuel. They are produced by many organisms, mainly bacteria and fungi, and are obtained through fermentative processes. Therefore, cellulase contribute significantly to the world enzyme market. Growing concerns about crude oil depletion and greenhouse gasses emissions have promoted the production of bioethanol from hydrolysates of lignocellulosic materials, making the screening for high cellulase-producing microorganisms a high-interest research topic. The aim of this work was to select cellulase producing molds and evaluate their ability to grow in cellulose-rich agroindustrial residues. Nine fungal strains were obtained from the Instituto Tecnológico de Colima's collection, and grown on Carboxymethyl Cellulose Agar (CMC) for 72 h at 28°C. Afterwards, the Potency Index (PI) of each strain was determined based on the radial enzymatic halo using the Congo Red Test, the best performing isolates were selected to evaluate their radial growth rate on mineral-supplemented, cellulose-rich, low cost and accessible materials in the state of Colima, México: green sugarcane bagasse, burnt sugarcane bagasse, wheat straw and coconut coir.

The four isolates with highest Potency Index belong to the *Aspergillus* and *Penicillium* genera, and the top two were identified as *Aspergillus niger* (PI= 0.35) and *Aspergillus sydowii* (PI= 0.75) based on morphological traits. When these two *Aspergillus* strains were grown on the cellulose-rich materials, *Aspergillus niger* showed the best performance, especially on coconut coir (growth rate= 0.84 mm/h) and green sugarcane bagasse (Growth rate= 0.29 mm/h).

Bacillus subtilis as promoter of volatile organic compounds (VOCs) applied in blackberry crop (Rubus fruticosus).

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Aroma and flavor of blackberry (*Rubus fruticosus*) are characteristics that are used by consumers when selecting high quality fruits, these attributes are given by the concentration and variety of volatile organic compounds (VOCs), VOCs are metabolites secondary causes of the different aromas and flavors given off by the fruits. The concentration of VOCs depends on the availability of nutrients in the soil and the management of the crop, various techniques are used to increase the availability of these nutrients and thus improve the quality of the fruit, such is the case of the use of Plant-growth-promoting bacteria (PGPB), such as *Bacillus subtilis* (BS), BS is a microorganism that has a positive effect on crops, is attributed to a better production yield and improves nutrient availability. Although the effect of PGPB on crop yield and disease prevention has been evaluated, their effect on the concentration of blackberry VOCs is unknown. The objective was to determine the effect of the application of BS on the concentration of VOCs related to aroma and flavor in blackberry fruits (*Rubus fruticosus*) through gas chromatography coupled to mass spectrometry (GC-MS) using phase microextraction solid (SPME). 20 g of blackberry samples were collected without application of BS and with application of BS, stored in refrigeration at 4 ± 1 ° C until analysis. The VOCs analyzed were: 2-Heptanol (7.33-8.61 µL / 20 g of blackberry, equivalent to 1-nonanol), camphene (3.45-4.00 µL / 20 g of blackberry, equivalent to 1-nonanol), myrcene (0.81 µL / 20 g of blackberry, equivalent to 1-nonanol) cimeno (1.52-2.17 µL / 20 g of blackberry, equivalent to 1-nonanol) and terpinen (1.51-1.72 µL / 20 g of blackberry, equivalent to 1-nonanol). It is concluded that the application of BS is capable of promoting VOCs in blackberry cultivation.

Keywords: Volatile compounds, *Bacillus subtilis*, GC-MS, SPME, blackberry

Whole-genome sequence of strain *Streptomyces thermocarboxydus* and their heavy metal resistance biosorption property

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Streptomyces thermocarboxydus is a Gram-positive, rod-shaped with high GC content bacterium belonging to the *Streptomycetaceae* family that was isolated from the arid soil sample in Mexico. In this study, we report the genome sequence of *Streptomyces thermocarboxydus* that consists of 7.39 MB with a G+C content of 72.22% containing 6929 protein-coding genes. Putative heavy metal resistant genes are found in the genome. Interestingly, totally 13 biosynthetic gene clusters (BGCs) for putative secondary metabolites were found based on the antiSMASH 3.0 program. In addition, isolate *S. thermocarboxydus* was screened for their heavy metal resistance potential against copper, zinc, cadmium, and cobalt. Moreover, heavy metal biosorption was determined by analytical techniques such as atomic absorption spectrophotometry and scanning electron microscopy (SEM). Hence, isolate *S. thermocarboxydus* which could be used to develop a biosorbent for absorbing the heavy metal ions as well as a great potential to bioremediate heavy metal contamination and biotechnical application.

Keywords: *Streptomyces thermocarboxydus*, antiSMASH 3.0, Biosynthetic gene clusters, Heavy metal, Biosorption.

Molecular identification of yeasts in cider from Zacatlán

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Introduction. Yeasts represented the most important group of microorganisms to winemakers, because without Saccharomyces, producing quality wine would be Impossible (Fugelsang & Edwards, 2007). They are ubiquitous unicellular organisms present in all fermented beverages and among them the Zacatlán cider recognized by its apples. **Materials and methods.** The yeasts were quantified under NOM-111-SSA1-1994. 17 strains of apple juice were isolated at five days of age. Through the technique of the striatum in PDA medium (Bixon, Mexico), they were subsequently reproduced in YPD liquid medium (yeast extract 1 %, peptone 2 % and glucose 2 %). DNA was extracted with Pérez's protocol (2017). The ITS (Internal Transcribed Spacer) region of the rRNA of the 17 yeast strains was amplified through the PCR technique using the ITS primers 5HP (forward) 5'-GGA AGG AGA AGT CGT AAC AAG G-3'and NL-4 (reverse): 3'-GAC TCC TTT TTC CCG AGG TAG GTC AGA T-5'. The thermocycling program was as follows: for the initial denaturation, 4 minutes at 95 ° C, followed by 35 cycles [94 ° C, 1 min; 57 ° C, 1 min; 72 ° C, 2 minutes], the amplification concluded with a final extension of 72 ° C, 10 min. The PCR products were visualized on a 1.4 % agarose gel, in TAE 1x buffer (0.04 M Tris-acetate, 0.00 l M EDTA-pH 8.0), were revealed in ethidium bromide (7×10^{-5} µg · μL^{-1}) and photodocuments. **Results and Discussion.** Of the 17 strains isolated only 7 had similarity with Saccharomyces cerevisiae with 98% identity according to GenBank, the other 10 were not possible due to the fact that the extracted DNA presented contamination. The yeasts isolated in apple juice have an economic potential for the Zacatlán cider industry, because they can be used as initiators to speed up the fermentation phase and improve the sensory qualities of the final product. **Bibliography**. Fugelsang C. K. & Edwards G. C. (2007). Wine Microbiology Practical applications and procedures. Ed (2nd). Springer. NY.

Influence of heavy metals on biofilm production in bacteria isolated from contaminated soils

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Heavy metal contamination is a problem that has been increasing due to anthropogenic activities, therefore new bioremediation techniques have been searching. Recent studies suggest that some bacteria resistant to metals can reduce the concentration of these, through several mechanisms, such as biofilm formation. The aim of this work is to evaluate the biofilm production as a mechanism of resistance to metals. From 116 metal-resistant strains, a primary selection was made considering only the exopolysaccharide producing strains, subsequently a secondary selection was carried out from the selected strains by minimum inhibitory concentration (MIC), biofilm formation. The exopolysaccharide-producing strains were 24 of genera *Bacillus*, *Cellulomonas*, *Citrobacter, Delftia* sp., *Enterobacter*, *Flavobacterium*, *Klebsiella*, *Kluyvera*, *Microbacterium, Pantea*, *Pseudomonas*, *Staphylococcus*. The 24 strains showed the following resistance to metals: As^{5+}>As^{3+}>Ni^{2+}>Zn^{2+}>Co^{2+}>Cu^{2+}> Cr^{6+}. *Delftia sp*. was selected for its capacity for biofilm formation with Cr^{6+} 10 mM in 48h.

Effect of *Pseudomonas aeruginosa* rhamnolipids over mature biofilms of multirresistant clinical isolates

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Rhamnolipids are a group of molecules classified as glycolipid biosurfactants; due to their surfactant properties, they have different industrial and biological applications. Rhamnolipids are produced by different bacteria, mainly by strains of the genus *Pseudomonas*, with different physiological functions. The aim of this work was the production and extraction of rhamnolipids of *Pseudomonas aeruginosa* RN19a from liquid cultures, and the evaluation of the effect of this biosurfactant on mature bacterial biofilms of multiresistant strains isolated from patients with urinary tract infections. The identification of the rhamnolipids was done by thin-layer chromatography and the determination of the Critical Micelle Concentration by surface tension measured using the pendant drop methodology. Biological activity was tested against mature biofilms developed in polystyrene microplate wells of three strains of *Escherichia coli*, two strains of *Pseudomonas aeruginosa* and one strain of *Enterococcus faecalis*; all strains were multi-resistant and identified by phylogenetic analysis from 16Sr DNA gene nucleotide sequences. The treatments with rhamnolipids were done with three different concentrations (27.5, 55, and 110 mg / L) for 1.5, 3.0, and 4.5 hours. The quantification of biofilm formation was measured by spectrophotometry. Results demonstrated that rhamnolipids at low concentrations and in short incubation times, decrease absorbances up to 87.3% in *Pseudomonas aeruginosa* strains, 90.1% in *Escherichia coli* strains and 93.6% in *Enterococcus faecalis* strain, compared to the control biofilm without rhamnolipids. In another experiment, the bacterial sensitivity on biofilm was determined by the exposure of a disinfectant for medical use (activated glutaraldehyde), applied for 15 minutes after the treatment with rhamnolipids. Vitality was determined by the ability of live bacteria to metabolize the resazurin dye to resofurin, with a change in color quantified by spectrophotometry. Results indicated a significant higher antimicrobial effect of the disinfectant on the strains that were pretreated with rhamnolipids. Even though bacteria in biofilms have a higher resistance to disinfectants than planktonic cells, the treatment with rhamnolipids expose the cells to the disinfectant. The use of rhamnolipids can have applications in hospitals for the control of biofilm-forming multiresistant bacteria.

A Case Study: Obtainment of Minimally Functional Microbial Consortia from CR1 Consortium Using Dilution to Extinction for Lignocelullose Degradation.

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INTRODUCTION

The dilution to extinction method has the potential to be applied in the development of minimally functional microbial consortiums (MFMC) from a native mixed culture to preserve its degradative capacity with a smaller number of microorganisms. The CR1 consortium, derived from PM-06 consortium, native to the nixtamalized maize pericarp (NMP), which degrades up to 88.4% of the NMP in 168 hours. The objective of this work was obtaining a MFMC from the consortium CR1 and conserves the high capacity of degradation on the NMP.

METHODOLOGY

CR1 consortium was diluted in sterile saline serially from 10^{-1} to 10^{-11} and inoculated in NMP medium. The degradation process was carried out under aerobic conditions and under agitation (125 rpm) at 37 ° C for one week (168 h). At the end of the experiment, pH and degradation percentage were measured as efficiency parameters. Soluble protein, peroxidase, xylanase and cellulase activity assays of each consortium obtained were evaluated. All the experiments were performed in triplicate and subjected to a one-way analysis and analysis of variance (ANOVA).

RESULTS

The CR1 consortium diluted 10^{-9} showed a greater degradation capacity (60 %) than the undiluted consortium (53 %), reflected in the cellulase (0.560 U/mL) and peroxidase (13.500 U/mL) activities, while the undiluted consortium showed the highest xylanase activity (9.285 U/mL). The other diluted consortiums (10^{-7} and 10^{-11}) showed very low enzymatic activities and poor degradation percent, probably mains bacteria involved on NMP degradation weren't inoculate due high dilution values. The dilution factor may affect strongly the bacterial populations and therefore decreasing enzymatic saccharification process over NMP.

CONCLUSIONS

The obtaining of a new diluted CR1 consortium (10^{-9}) was relatively less diverse in microorganisms but does not lose its enzymatic capacities and even increasing in comparison with the original consortium. The application of dilution to extinction method in a microbial consortium allowed the development of novel consortium candidate to be a MFMC, which retains the ability to produce enzymes involved in the degradation process of NMP, with a lower microbial diversity.

Identification, expression and characterization of a biosynthetic cluster involved in the production of a lantipeptide in *Micromonospora purpurea*

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The abuse in the use of antibiotics causes selection of resistant bacteria, which limits the effectiveness of the necessary drugs for the treatment of infections. Thereby, discovery of new antimicrobials is necessary. Genomic mining of biosynthetic clusters has become a method that accelerates the identification and characterization of bioactive compounds such as bacteriocins. These natural products are a diverse class of ribosomally synthesized antimicrobial peptides lacking of side effects in the human body. Members of this group are the lantipeptides, which own their antimicrobial activity disrupting the integrity of the bacterial cell wall. The proteins involved in the biosynthesis of these peptides are the precursor peptide *lanA*, the dehydratase enzyme *lanB* and the cyclase *lanC.* In order to identify and express a cluster putatively involved in the production of a lantipeptide in *Micromonospora purpurea*, we used the bioinformatic programs AntiSMASH and Bagel. *In silico* analysis of the *M. purpurea* genome, suggested this microbe has the putative capacity to synthesize 29 secondary metabolites such as polyketides, non-ribosomal peptides, terpenes, aminoglycosides and bacteriocins, among others. In this study, a cluster encoding for a putative type I lantipeptide was selected. Bioinformatic tools allowed us to determine the lantipeptide putative sequence, as well as predict the region that would correspond to the leader peptide. The fully processed peptide would have an expected molecular weight of 4.2 kDa and the immature peptide 7 kDa. The genes of interest were amplified by PCR from the *M. purpurea* genome and *lanA* and *lanB* subcloned into the vector pCR™2.1-TOPO™, while *lanC* in pJET1.2. In order to express the lantipeptide and characterize its putative biological activity, these biosynthetic genes will subsequently be cloned into the expression vectors pRSFDuet-1 and pET22b.

A tRNA-utilizing enzyme is involved in the synthesis of the protease inhibitor thiolstatin (livipeptin) in *Streptomyces lividans* 66

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Abstract:

Small peptides aldehydes (SPA) are natural products (NPs) typically synthesized by Nonribosomal Peptide Synthetases (NRPS) with potent protease inhibition activity. SPAs are widely used during recombinant protein production and have large potential as therapeutic molecules. They also play important biological roles in the producing organisms, such as in *Streptomyces*, where they are believed to protect the substrate mycelium from proteolysis by competitors. By means of comparative analysis of the genomes of closely related *Streptomyces* strains, we identified an unusually small biosynthetic gene cluster (BGC) in *Streptomyces lividans* 66, which is absent from the derived industrial strain TK24. Through a series of 'synthetic biology' experimental approaches we show that this unusual BGC directs the synthesis of the previously isolated SPA thiolstatin (livipeptin) via a tRNA-utilizing enzyme (tRUE). This key tRUE belongs to the L/F transferase family and it is responsible for transfer a phenylalanine from a Phe-tRNA into an arginine, leading a dipeptide that is then reduced to produce the functional aldehyde. Phylogenomics analysis of L/F transferases in Actinobacteria confirms the presence of divergent enzymes under diverse genetic contexts, suggesting the occurrence of BGCs with high potential to drive the synthesis of similar compounds. This molecule is the first example of a tRUE enzyme involved in the biosynthetic assembly line of a SPA, opening the door for the exploration of tRUE enzymes following genome mining. Our results are also relevant to understand *S. lividans* physiology, and its role as a host for production of recombinant proteins in industrial settings.

Evaluation of astaxanthin production by *Xanthophyllomyces dendrorhous* XR4 in saccharified lignocellulosic biomass.

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ABSTRACT

Astaxanthin is a carotenoid that has gained importance over time, due to its high antioxidant capacity. It is implemented in the pharmaceutical, food and cosmetics industry, however, most of the commercial astaxanthin is obtained by chemical synthesis, which can represent a risk of toxicity. Due to the increase in demand for products of natural origin, biotechnology has made it possible to obtain astaxanthin from microorganisms. The yeast *Xanthophyllomyces dendrorhous* has been noted for its ability to produce astaxanthin in commercial substrates as glucose, however, this increase the cost of production. In order to reduce costs, the growth and carotenoids production by the hyperproducer astaxanthin strain XR4 were evaluated in pure sugars that are part of the lignocellulosic biomass. Xylose, cellobiose, and glucose were used as substrates. For the culture, a medium composed of yeast extract (10 g/L), bactopeptone (20 g/L), and pure sugar as a carbon source (10 g/L) was implemented. The culture conditions were 20 °C, 600 rpm, and 0.2 vvm. The highest growth and production of total carotenoids was observed in cellobiose, followed by xylose and glucose. This behavior suggests that *X. dendrorhous* XR4 can be cultivated in saccharified lignocellulosic biomass with a high content of cellobiose and xylose. While, when the substrate is glucose, the carbon flux is being directed towards the production of astaxanthin and other metabolites.

Keywords: *Xanthophyllomyces dendrorhous,* saccharified, cellobiose, xylose.

Effect of the growth temperature on proteomic and structural response of the rHuGM-CSF inclusion bodies of *E. coli* under thermoinduction

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The λpL/pR-cI857 expression system induced by temperature (>37°C) is one of the most studied strategies to produce recombinant proteins (RP) in *E. coli*, since do not require the addition of chemical inducers like IPTG and contamination risks are minimized. However, an increase above 34°C cause activation of the Heat Shock Response (HSR), triggering the expression of molecular chaperones involved in the correct folding of the RP. It has been reported that according to the heat induction strategy, the mRNA levels of heat shock and RP genes may change, but few studies refer to variations at proteomic level. The objective of this study is to evaluate the effect of the growth temperature (30°C *vs.* 34°C) on the HSR, determining changes -after thermoinduction at 42°C- in the protein composition and structure of the aggregates known as inclusion bodies (IBs) when producing the human recombinant Granulocyte and Macrophage Colony Stimulating Factor (rHuGM-CSF). To achieve this objective, were carried out submerged cultures of the recombinant strain *E. coli* W3110 in bioreactor with mineral medium at two growth temperatures (30°C and 34°C) with induction at 42°C in pre-stationary phase of growth. Biomass growth was measured by optical density ($OD_{600\ nm}$), glucose consumption was quantified in the biochemical analyzer YSI 2900 and acetate production by HPLC. The IBs were isolated and purified to determine changes in the expression of rHuGM-CSF and in the molecular chaperones DnaK/J, GroEL/ES, IbpA/B by SDS-PAGE, Western blot and Mass spectrometry. The structural characterization of IBs was performed by FTIR-ATR, transmission electron microscopy, denaturation with chaotropic agents and solubilization with proteinase K. Similar maximum biomass was reached under both thermoinduced cultures, but at 34°C there was an increase of ~40% in the specific growth rate (0.90±0.07 h^{-1}) compared to 30°C (0.53±0.01 h^{-1}). In both cases, glucose was completely consumed and ~6.0 g/L acetate was accumulated. The rHuGM-CSF (~14 kDa) was aggregated in IBs, observing more endogenous proteins at 34°C than 30°C. There was no heterologous expression in the wild-type strain and non-induced cultures, neither in the soluble fraction. In the Western blot, DnaK increased its concentration in the IBs as the cultivation time increased, while GroEL presented a constant expression during the cultures. GroES was not observed in IBs. Mass spectrometry analyzes reveal the presence of IbpA in the IBs.

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Growth evaluation of lentinan producer mycelium of *Lentinula edodes* (Berk.) Pegler, in solid and submerged fermentation medium

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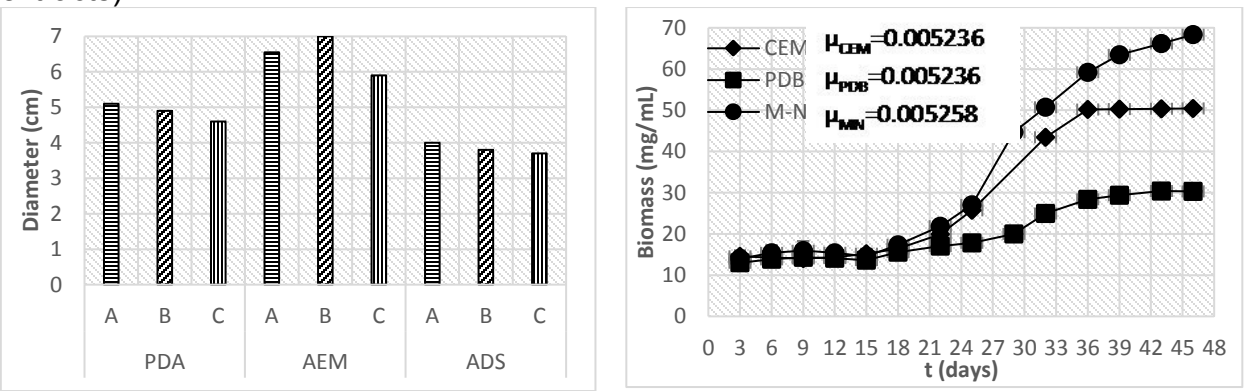
Introduction. Mycelial growth in solid medium involves low activity of water (Aw), but the substrate must have enough moisture to support the process and the metabolism of the organism. In cultivation by submerged fermentation in the case of fungi, these can form small spheres of mycelium called "pellets" when there is agitation, otherwise, they grow on the Surface (Suárez and Holguín, 2011, López-Peña *et al.*, 2013).
Methodology. For the growth of the mycelia of *L. edodes* in Petri dishes, sowed on malt extract agar (AEM), potato and dextrose agar (PDA) and agar Sabouraud (ADS). The strain passed from the solid medium in AEM to a flask with broth Malt extract (CEM) in destructive type kinetics coding each flask with the time of sampling shows. The μ_{max} was determined based on the logistic model.
Results. The best solid culture medium for *L. edodes* inoculum was the AEM with an average diameter of 6.48 cm (Figure 1a), as reported by Veleska et al., 2007 the best growth was presented in agar yeast malt extract (MYA) and malt and oat extract agar (OMYA). In submerged fermentation, the higher biomass production of 68.33 mg/mL (Figure 1b) higher than the reported value by López-Peña et al., 2013 with 16.57 mg/mL in the T2 treatment (extract from vine wood supplemented with aqueous and ethanolic extracts).



(a) (b)

Figure 1. Radial growth of mycelium in solid medium (216 h) (a) obtaining biomass by submerged fermentation (46 days) (b).

Conclusions Although the best growth diameter of *L. edodes* was obtained in CEM the mycelium with the best characteristics was observed in the ADS with mycelium air, white and cottony. However, the fastest growth rate was in the CEM, said medium was used in the kinetics in liquid medium where the phase of adaptation up to 18 days, an exponential phase until 33 days and a phase stationary after 36 days.

Characterization of biological control agents for diseases of importance in cacao crops in Chiapas.

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The cacao constitutes one of the plants with the greatest cultivation and commercial value in the tropical regions of the world. Cacao is the base for industrial processing to obtain various products. Different factors affect the cacao production around the world. Within the biotic factors are those phytosanitary problems. The diseases caused by fungi and oomycetes are the most important in the state of Chiapas, within these moniliasis caused by *Moniliophthora roreri*, anthracnose by *Colletotrichum gloesporoides* and black pod rot by *Phytophthora* spp. are very important phytopathogen microorganisms. An eco-friendly approach to tackle the previous pathogens are biocontrol agents. In this study, samples were taken from the surface of apparently healthy pods, leaves and flowers from cacao plants, in a crop where there were diseased plants. The aim of selecting these samples was that potential biocontrol agents could be exerting protection in the cacao. We performed antagonist qualitative and quantitative tests on potato based medium to select bacteria with antagonistic activity. As results 40 bacteria were obtained to inhibit *M. roreri*, *C. gloeosporioides* and *Phytophthora* spp. One out of 40 antagonistic isolates, was selected due to an inhibition percentage greater than 70%, for the three phytopathogenic fungi. This value could suggest a potential use as biological control agent. The antagonistic microorganism was identified by 16S rRNA as *Fructobacillus,* a member of the *Leuconostocaceae* family, Gram positive with few species in the genus. Moreover, the pathogenicity of the fungi was corroborated on pear fruit with the objective of establishing a study model to analyze the biocontrol agent *in vivo*. Currently, the in vivo experiment is carried out, using a tripartite assay with pear-fungi-bacterium.

Isolation and characterization of the lipolytic activity of a marine *Pseudomonas alcaligenes* from Gulf of Mexico.

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Marine microorganisms have adapted to extreme conditions, such as salinity, temperature and pressure, their enzymes have characteristics of thermostability, halotolerance, barophilia and scalability at the level of production and industrial application. Marine biotechnology continues to advance mainly in the search for microorganisms and enzymes for its application, a large percentage of the latter have been characterized from isolated organisms applying marine microbiology strategies. Although there are technologies that allow us to dispense with cultivation, there is still an undefined number of cultivable organisms that can be potential carriers of new lipolytic activities. In 2015, the Gulf of Mexico Research Consortium (CIGoM, acronym in Spanish) was consolidated through its research lines, a reservoir of information was obtained that represents an important source of potential marine biotechnological tools. Derived from the first oceanographic campaign, a *Pseudomonas alcaligenes* was isolated, which presented an interesting lipolytic activity on tributirin, and also positive on tricapriline, which shows the presence of lipases and esterases. A library was constructed to obtain the genes involved in lipolytic activity, with a title of 5346 CFUs, only three were positive for screening on tributirin, and only one positive for tricapriline. Each clone was named as Lip1, Lip2 and Lip3, with an insert size: 3 Kb, 5Kb and 4.5 Kb, respectively. Once sequenced each clone contains a particular arrangement. Lip1 is a triacylglycerol lipase, Lip2 is a triacylglycerol lipase of the EstA family and forms a small operon with its own chaperone, while Lip3 is an esterase fused to its autotransporter. Lip1 and Lip2 are labile in the presence of detergent, while Lip3 has been robust and can be purified in the presence of high detergent values. Each enzyme has particular qualities and potentially each, an application.

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Marine Dioxygenases: Who thinks crude oil is delicious?

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Catechols are abundant environmental pollutants because of their long-term persistence and toxicity. Catechol dioxygenases play a central role in degradation of aromatic compounds because they catalyse the critical and chemically difficult aromatic ring-cleavage reaction. Catechol oxidation can occur through the two ring-cleavage pathways: ortho and meta cleavage, catalysed by C12DO (catechol 1,2-dioxygenase) and C23DO (catechol 2,3-dioxygenase), respectively. During the last step, the ring cleavage products are metabolized to carbon dioxide during tricarboxylic acid cycle. Microorganisms that contain catechol dioxygenases are able to utilize aromatic molecules as their sole source of carbon and energy not only to survive in polluted areas but in doing so, to decontaminate the soils. Subsequently, catechol dioxygenases have a high biotechnological potential in treatment of wastes contaminated with aromatics compounds.

The aim of our study is the cloning, expression and characterization of two catechol dioxygenases from *Pseudomonas* strains, obtained from sediment and water samples of the Gulf of Mexico (GoM) for its use in bioremediation.

We analyzed the activities of C12DO and C23DO in 40 bacterial isolates and 160 bacterial consortia that were able of utilize crude oil and kerosene as the only carbon source. Two *Pseudomonas sp*. were isolated, their genomes were sequenced and the bioinformatic analysis showed the presence of an operon for benzoate and phenol catabolism. The activity of both catechol dioxygenases was assayed spectrophotometrically. We cloned, expressed purified and tested C12DO enzymatic activity to determine the optimal ranges from this marine dioxygenase. This C12DO isolated from a *P. stutzeri* from GoM has important features that distinguish it from all previously reported dioxygenases: The purified enzyme is still active after 4 months storage at 4 C without an apparent loss of its activity, active at high saline concentration due to the origin of the sample and also showed high tolerance to certain inhibitors that severely affect the activity of other reported enzymes.

We are performing X-ray crystallography studies to determine the protein structure and carrying out enzyme immobilization tests on calcium alginate beads for its use as biosensors in the detection of aromatic compounds from pesticides and industrial wastewater, as well as biodegradation assays in contaminated soil samples.

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Growth promotion in wheat (*Triticum turgidum* L. *subsp. durum*) by co-inoculation of native *Bacillus* strains isolated from the Yaqui Valley, Mexico

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Plant growth-promoting rhizobacteria (PGPR) are a group of rhizospheric bacteria with the ability to promote plant growth and health, as well as to restore soil fertility. The objective of this study was to evaluate wheat (*Triticum turgidum* L. *subsp. Durum*) growth promotion by co-inoculation of native *Bacillus* strains isolated from the Yaqui Valley, Mexico, for the potential use in a microbial inoculant. Three bacterial strains obtained from the Collection of Edaphic Microorganisms and Native Endophytes (COLMENA), isolated from wheat cultivation in the Yaqui Valley, were studied. First, the molecular identification of the strains was performed by sequencing the 16S rRNA gene, using the Sanger platform. In addition, bacterial strains were metabolically characterized by functional activities associated with the promotion of plant growth (production of indoles, solubilization of insoluble phosphorus, and production of siderophores). Finally, the impact of the inoculation of these individual strains, and in consortia was determined in wheat (*Triticum turgidum* L *ssp. Durum*), simulating the edaphoclimatic conditions of the Yaqui Valley. The morphometric variables measured were aerial and root length, aerial and root dry weight, and biovolume index. The studied strains (TRQ8, TRQ65, and TE3) were taxonomically affiliated with *Bacillus megaterium*, *Bacillus licheniformis*, and *Bacillus subtilis*, respectively. Those *Bacillus* strains showed the ability to produce indoles, strain *B. licheniformis* TRQ65 showed the highest production of this metabolite, 39.29 µg/mL. For the insoluble phosphorus solubilization test, the 3 strains showed this ability ranged from 1.37 to 1.43, and only strain *B. megaterium* TRQ8 showed the siderophores production, index 8.17. Inoculation of the *B. megaterium* TRQ8 + *B. licheniformis* TRQ65 consortium to wheat plants showed the greatest increases in the 5 measured variables, significant difference ($p < 0.05$) *vs.* the non-inoculated treatment, aerial and radical length showed an increase of 6 and 10%, respectively, while aerial dry weight increased 60%, and radical dry weight had an 82% increase. In the biovolume index, this consortium showed an 18% higher than the un-inoculated treatment. The strains TRQ8, TRQ65, and TE3 showed growth promotion traits, *in vitro* and *in vivo*. In conclusion, co-inoculation of these strains increased their ability to promote growth in wheat. Therefore, the mechanisms associated with this effect, as well as their ecological functions and interaction with the biotic and abiotic factors of agro-systems must be studied for extensive use as a microbial inoculant.

Effect of volatile organic compounds synthesized by *Pseudomonas rhodesiae* GRC140 on the root architecture of *Arabidopsis thaliana*

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Plant-associated bacteria contribute to plant growth through different microbial activities such as phosphate solubilization, nitrogen fixation, siderophores production and emission of volatile organic compounds (VOCs), which play a very important role in the interaction with the plant. In this study, the effect of VOCs emitted by *Pseudomonas rhodesiae* GRC140 on the growth and development of *Arabidopsis thaliana* grown *in vitro* was investigated. For this, Petri dish (I-plates) containing 0.2x MS medium supplemented with sucrose was used. Five seedlings of *A. thaliana* Col-0 previously germinated were transferred on one half plate and *P. rhodesiae* GRC140 was inoculated on the opposite half of plate. After 10 days of incubation, primary root length, lateral roots number and total dry weight were measured.

After 10 days of co-cultivation, it was found that *P. rhodesiae* GRC140 increasing the plant biomass of seedlings of *A. thaliana* compared with control seedlings. In addition, it was observed that VOCs emitted by GRC140 has a marked effect on the root system architecture of *A. thaliana.* It was observed that the primary root length and the lateral roots number of the seedlings exposed to GRC140 were increased significantly ($P < 0.05$) compared to the seedlings non-exposed. These results suggest that *P. rhodesiae* GRC140 synthesizes volatile organic compounds that are involved in the modification of root architecture.

Synthetic bacterial consortium to degrade monocyclic aromatic compounds

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Aromatic compounds can be defined as organic molecules conformed by one or more aromatic rings. These compounds are present naturally as lignin, amino acids and tannins. Other sources include those derived from anthropogenic activities as agriculture, domestic sources and industry. Many of these aromatic compounds are catalogued as environmental priority pollutants. Has been found that several microorganisms have the capacity of degrade these compounds, being aerobic bacteria the most active degraders. The transformation of these compounds structurally diverse, through peripheric metabolic pathways results in the formation of key intermediates as catechol, protocatechuate and gentisate that in some cases cannot be degraded completely. Our study area is the Southwest of the Gulf of México due the oil extraction processes that are carried out here. We have obtained three bacterial in the highly contaminated coastal region capable of growth in presence of phenanthrene belong to the genders *Alcanivorax* sp., *Arthrobacter* sp. and *Halomonas* sp. Their genomes were assembly to identify the possible metabolic pathways used into the aromatic compounds degradation. To corroborated if metabolic pathways enzymes related in the benzoate, protocatechuate, gentisate degradation were active, we grow the bacterial in compounds mentioned, our results showed that *Alcanivorax* sp. does not have the capacity of growth in any of these compounds while *Halomonas* sp. and *Arthrobacter* sp. are able of grow in two or three compounds respectively (benzoate, protocatechuate, gentisate). Finally, in the mixture of the three aromatic compounds we observed a biggest growth in *Arthrobacter* sp. than *Halomonas* sp. and the consortium. We do not observe a mayor grow or degradation in our consortium as we expected, it could be explained due both isolates counts with all enzymes necessaries to degrade completely each compound, being Arthrobacter sp. the isolate where we obtained the best results.

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Polyhydroxybutyrate production by heavy metals-resistant bacteria strains

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Keywords: polyhydroxybutyrate, heavy metals- resistant bacteria

Introduction. Polyhydroxybutyrate (PHB) is produced in several microorganisms in response to environmental conditions of physiological stress such as nitrogen limitation or presence of heavy metals [1]. For the detection of PHB granules, it uses staining with lipophilic dyes such as Black Sudan B [2]. In this work, heavy metal resistant bacteria (Mn, Pb, Zn, Cd) were analyzed, to verify if heavy metal stress increases its ability to produce PHB. ***Methodology.*** The bacterial strains from mining wastes of Guanajuato used in this study were: *Microvirga subterranea, Bacillus thuringensis, Brevibacterium frigoritolerans, Streptomyces durmitorensis, Streptomyces sp, Rhodococcus sp., Pseudomonas sp., Exiguobacterium sp*. and *Microbacterium sp*. The strains were grown in nutritious broth with and without heavy metals. The cultures were incubated at 28 ° C and 130 rpm, taking an aliquot at different growth times for the detection of PHB granules by staining with Black Sudan B. ***Results.*** Intracellular PHB granules were detected in the strains analyzed. Likewise, growth curves with and without metal were determined. ***Conclusions.*** So far, the conditions for the production of PHB granules by means of bacteria resistant to heavy metals were established for the following strains: *Bacillus thuringensis, Brevibacterium frigoritolerans,* and *Microvirga subterranea.*

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Selection of Operating Conditions for Palm Oil Mill Effluent Treatment in Microbial Fuel Cells

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Palm Oil Mill Effluent (POME) is the wastewater produced in palm oil agroindustry which has a large amount of organic matter (COD> 55 000 mg-O_2/L); consequently, it is required an effective treatment before discharge. Traditional POME treatment is based on methanogenic and facultative series of ponds which require large areas and long retention times. Microbial Fuel Cell (MFC) is a suitable alternative to treat POME since it is a clean technology that turns organic matter into electric energy due to bacterial digestion. In addition, MFC conditions need to be enhanced to treat this kind of wastewater. Therefore, this work evaluated three different operational factors over electric energy generation while reducing the organic content of POME. Diluted POME (COD= 1000 mg-O_2/L) was treated in the anodic chamber of dual chamber MFCs inoculated with an anaerobic sludge from a POME treatment plant. The cathodic chamber was filled with phosphate buffer (PBS) 0.1 M containing $K_3Fe(CN_6)$ 0.05 M as the final electron acceptor. Carbon felt were used as electrodes, wired by titanium and a 1 kΩ external resistor. It was compared a heat treatment over electrode material, membrane pretreatment (NaCl or H_2SO_4) and the addition of PBS into the anodic chamber. Cell potential was continuously monitored (10 min intervals) until voltage decreased below 70 mV. COD removal and coulombic efficiency (CE) was calculated to determine the best configuration. Subsequently, best condition was compared to controls (diluted POME and sterilized) through power curves and wastewater parameters were obtained.

In MFCs which electrodes were heated, shows a higher voltage, what suggest that carbon felt presents a better behavior when it is heated in comparison to electrodes without treatment. Voltage generation in not treated electrodes achieve similar values just after third fed batch cycle, which indicates possibly biofilm formation is slower than in heated carbon felt, but finally it could be the same generation from then on. Membrane treatment does not show a substantial difference in voltage generation of MFC. The evaluation of PBS in the anolyte is evident that enhance the voltage generation of MFCs. In all cases, a comparison between the same MFC using PBS 0.1 M to these in which does not in the anodic chamber, shows better performance. This is attributed to pH amortization and electric conductivity. Heat treatment of carbon felt electrodes, addition of PBS to anolyte, and membrane immersed in H_2SO_4 were selected as best configuration to treat POME in MFCs. This configuration reached values of COD removal and coulombic efficiency of 76 % and 20 % respectively. Afterward, inoculum effect was tested resulting in a maximum power density of 3.69 W/m^3 (1.20 W/m^2) with an organic matter removal represented by COD, TSS, and VSS (1.1, 4.4, and 3.5 times respectively) greater than control without inoculation.

Operational conditions in MFCs such as material treatment, and electrolyte composition affected performance in the voltage response. Enhances in factors evaluated reveals a rapidly biofilm formation allowing to reach stable maximum voltage in less time. The utilization of anaerobic sludge indicates a better electrochemical, and wastewater treatment performance for POME in dual chamber MFCs.

Expression of a 3-hydroxyacyl-ACP thioesterase and a mcl-CoA ligase in *Azotobacter vinelandii* for the production of Medium Chain Length polyhydroxyalkanoates*:* new degradable bioplastics

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Polyhydroxyalkanoates (PHAs) are natural polyesters synthesized by many bacteria, as a carbon and energy reserve. These polymers accumulate intracellularly as insoluble granules. PHAs can be extracted, moulded and used as a biodegradable plastics. These materials are formed by polymerization of hydroxyalkanoates of diverse number of carbon atoms, and the physical and thermal properties of these materials as bioplastics depend on the type of hydroxyalkanoate monomers incorporated; for instance, there are short-chain-length PHAs (scl-PHA), formed by monomers of 4-5 carbon atoms, and medium-chain-length PHA (mcl-PHA), with monomers having more than 6 carbon atoms. mcl-PHA are more flexible, elastic materials and have a higher elongation at break than scl-PHA. The composition of the PHA produced is affected by the metabolic routes present in the microorganism.

The bacterium *A. vinelandii* accumulates high amounts of PHAs (up tu 90% of its dry weight) but produces only short chain length PHAs. On carbohydrates it synthesizes the polyhydroxybutyrate homopolymer (4C monomers), whereas on mixtures of carbohydrates with valerate, heptanoate or nonanoate it accumulates the copolymer polyhydorxybutyrate-co-hydroxyvalerate (4 and 5C monomers). In comparison, some *Pseudomonas spp.*, like *P. putida*, are able to synthesize medium chain length PHAs from carbohydrates only, obtaining the precursors for the synthesis of PHAs from the fatty acids synthesis pathway (FAS). The link between between these two pathways in this bacterium is provided by a β-hydroxyacyl ACP:tioesterase and a mcl-CoA ligase, that convert intermediates of the FAS pathway to the hyroxyacyl-CoA substrate for the polymerization reaction.

To allow the synthesis of mcl-PHA from carbohydrates in *A. vinelandii*, a recombinant strain was constructed that bears *phaG* and *PA3924* genes from two *Pseudomonas* species under a sucrose inducible promoter. When growing on carbohydrates and under non-inducing conditions (glucose as carbon source), this strain produced a polymer composed only of hydroxybutyrate units. When the *phaG* and *PA3924* genes were induced, the monomer composition changed, obtaining a copolymer composed of hydroxybutyrate and hydroxyhexanoate (~94 and 6% respectively). This result shows that the two genes introduced confered *A. vinelandii* the ability to synthesize a bioplastic not previously produced by this bacterium that has better mechanical and thermal properties. Although the enzymes introduced have been shown to be able to generate precursors of longer chain length in other bacteria, no other monomers were detected in the polymer of the recombinant *A. vinelandii*. These results suggest that the PHA synthase of *A. vinelandii* has no capacity to polymerize hydroxyalkanoates containing more than 6 carbons.

Pseudomonas stutzeri MLA9, a marine bacterium with high potential to degrade pyrene

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are one of the most toxic components present in crude oil and they show low bioavailability and high persistence in the environment. Furthermore, PAH can associate with other pollutants as heavy metals.

Coastal areas are prone to contain elevated PAHs concentration as a result of several anthropogenic activities. One way to remove PAHs from the marine environment is to design efficient, feasible and "green" strategies. The use of hydrocarbonoclastic marine bacteria is a hopeful alternative. In this sense, the aim of this work was characterize the marine bacterial strain MLA9 with potential to degrade pyrene.

The isolate was previously identified using MALDI-Biotyper and, in this study, its identity was confirmed by 16S ribosomal gene sequencing as *Pseudomonas stutzeri*. The bacterial strain can grow in minimal medium supplemented with pyrene, phenanthrene or naphthalene as sole carbon and energy source, being pyrene the best substrate. *P. stutzeri* MLA9 possesses the gene that encodes for the enzyme that acts in the first step of the PAH degradation pathway. Moreover, MLA9 seems to be a microorganism with a very versatile catabolism, since the genes encoding for catechol 1,2- and catechol 2, 3-dioxygenases are present in this strain, suggesting that it can cleave the ring in *ortho* and/or *meta*, respectively.

Qualitative assays indicate that MLA9 is a rhamnolipid biosurfactant producer strain. The above was supported by the amplification of the *rhlab* operon fragment to encode for rhamnosyl transferase I. In addition, *P. stutzeri* MLA9 forms biofilms and harboring, at least, four plasmids. Therefore, *P. stutzeri* MLA9 appears to be an excellent candidate to continue PAH degradation studies and its potential application on bioremediation processes.

Keywords: *Pseudomonas stutzeri,* Hydrocarbonoclastic bacteria, PAH, pyrene.

Phenotypic analysis of the *fur*$_{1398}$ mutant in *Gluconacetobacter diazotrophicus* Pal5 strain

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Introduction: Iron in high intracellular concentration causes oxidative stress, since it reacts with ROS produced normally in the aerobic metabolism of bacteria, producing highly reactive hydroxyl (HO•) radicals. Due to this, the bacteria have developed iron capture, storage and regulation mechanisms, which are carried out by the *fur* transcriptional regulator (*f*erric *u*ptake *r*egulator). The Fur protein was the first member described of the Fur superfamily of metalloregulators, first described by Ernst *et al*. (1978) in a *fur* mutant strain of *Salmonella typhimurium*, it has also been described in Gram positive bacteria; the Fur protein is considered as a global regulator. *G. diazotrophicus* Pal5, a Gram-negative and endophytic bacterium and member of the family *Acetobacteraceae,* included within the PGPB group of agronomical interest. Bertalan *et al*. (2009) completely genome sequence of this bacterium and find two *fur*-like genes, GDI_1248 and GDI_1398. The results found by Soares *et al.,* 2012 and Pérez Rodríguez in 2015 suggested that the *fur*$_{1398}$ gene is functional. In this work we provide results obtained from the generation of an isogenic *fur*$_{1398}$ mutant of *G. diazotrophicus* Pal5 strain. **Methods**: it was constructed by deletion of the *fur*$_{1398}$ gene by inverse PCR of pTOPO:*fur*$_{1398}$, generated pTOPOΔ*fur*$_{1398}$; this was subcloned in pSUP202 and inserted a Kanamycin resistance cassette and then was obtained the recombinant plasmid pSUP:*Gdi*Δ*fur*::Km^R, this was used for allelic replacement in strain *G. diazotrophicus* Pal5. The mutant *Gdi*Δ*fur*$_{1398}$::Km^R obtained and the wild type *G. diazotrophicus* Pal5 strains were used for the analysis of phenotypic growth curves in the presence and absence of iron, phosphate solubilization assays $Ca_3(PO_4)_2$ and qualitative production of acids in LGIE culture medium (with the blue bromothymol). **Results and discussion**: The isogenic mutant *fur*$_{1398}$ compared with wild type strain in the presence and absence of iron showed a dramatic decrease in growth, phosphate solubilization and qualitative production of organic acids. **Conclusion**: data obtained suggest that the effect of the deletion of the *fur*$_{1398}$ gene in *G. diazotrophicus* Pal5 exert an important role in some survival mechanisms.

Characterization of *Cupriavidus* strains isolated from nodules with biotechnological potential

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Cupriavidus is a genera knowed by your biotechnological potencial and your simbiosis with leguminoses (*Mimosa* genera). The nodulating species are *C. necator, C. taiwanensis, C. numazuensis* and *C. pinatubonensis*. The mexican Mimosa are preferentially nodulated by alphaproteobacteria. In Mexico were isolated five distincts isolates from nodules of plants of Leucaena and Acacia genera that were identify as Cupriavidus by sequencing of 16s rRNA. Objectives: Caracterization as PGPR and analized the tolerance to grow in diferents metals, test the ability of nodulate y analyse phylogenetically the siolates. To the PGPR activity was evaluated by the production of syderophores, indol acetic acid , phosphate solubilization and nitrogen fixing. The metal tolerance was evaluated growth the isolates in plates with diferents metal concentration. To the nodulated experimentes the seeds were scarified and desinfected. The seeds were growth in pots with bermiculte and icoculated with 1mL of culture adjusted to 1 D.O. The phylogeny of the isolates were analysed with the sequense of 16s rRNA using the software PhyML. Four isolates nodulated *Phaseolus vulgaris* and *Leucaena* plants. The isolate LEh25b produced siderophores and 56 ug/mL de AIA but thise isolated can´t nodulating. All isolates growth in Cu 2.5 mM, Pb 1mM, As3+ 1mM and Cr 5mM. The isolalte Leh25b was identify as *C. metallidurnas*. The isolates LEh25a and LTz26 are close to *C. oxalaticus* and the isolates 19.1a and 19.6 are close to *C. alkaliphylus*. The isolated 19.1a, 19.6, LEh25a and LTz26 can represents new species to genera *Cupriavidus* with a ecological and biotecnological potencial.

The pH shift affects the production and architecture of inclusion bodies of recombinant-phospholipase A2 expressed in *Escherichia coli*

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The aggregation of recombinant proteins (RP) in inclusion bodies (IB) is the main drawback of heterologous expression in *Escherichia coli*. This due to overexpression that could cause inefficient folding and aggregation. These aggregates are important in bioprocessing as a raw material, because contain a large amount of RP. Since RP are desirable in their monomeric and functional form, they are extracted from IBs in many bioprocesses using chaotropic agents and refolding strategies. However, the recovery of proteins in a native and soluble state of IBs depends on their grouping characteristics and solubilization conditions. Therefore, the structural analysis of IBs has become an important field of study. The objective of our project contemplated the study of the effect of the variation in pH on the architecture of IB formed by a phospholipase A2 (PLA2) of the snake venom *Micrurus laticollaris*, expressed in *E. coli* Origami (DE3). The cultures were carried out in 1.2 L bioreactors, using minimum medium, 37 ° C and 30% dissolved oxygen. The production of PLA2 was evaluated under 4 pH strategies: pH change from 7.5 to 6.5 or 8.5, without pH control (NC) and constant. The glucose and lactate concentration were quantified in a biochemical analyzer. Organic acids were quantified by HPLC. The protein concentration was determined by the Bradford method. IB were obtained and visualized by electron transmission microscopy and we define the differential amyloid structures, resistance to degradation by proteinase K and solubilization with chaotropic agents and the secondary structure was analyzed by FTIR. The proteins in IBs were separated by 2D SDS-PAGE and the differential spots were identified by MS/MS. The results showed that the cultures at pH without control and 7.5 achieved a maximum biomass (Xmax) of ~ 3 g / L. While at pH 6.5, they only reached 66% of Xmax. In cultures at pH 8.5, the basic pH favored the exit of acetate from the cells. At pH 8.5, the total protein yield and productivity of rPLA2 in insoluble form were twice as much compared to the other conditions. Those IB formed when pH was shifted to 8.5 revealed greater amounts of proteins extractable by proteinase K compared with others IB. In general, IB did not have an enzymatic activity. The Th-T binding assay indicated amyloidogenic characteristics or β-strand content in the rPLA2-containing IBs produced at acidic pH. By contrast, ∼50% less Th-T bound to IBs that formed when pH was shifted to 8.5. The comparison of IBs proteomic analysis between the pH shift of 6.5 and 8.5, showed at least 107 differential expressed proteins, being important the differential accumulation of two structural chaperones (DnaK and GroEL), Omp and CsgE proteins in the IB formed at pH 8.5. This finding suggests that pH modifies the stress response and their participation in the regulation of RP aggregation IB.
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Resonant acoustic mixing improves oxygen transfer in shake flasks and production of a recombinant phospholipase A2 in *Escherichia coli*

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Shaken flasks are widely applied in bioprocesses due to their flexibility and ease of operation. Resonant Acoustic Mixing (RAM) enables non-contact mixing by the application of low frequency acoustic energy, and is proposed as an alternative to solve oxygen limitations in orbital mixing (OM). In RAM system, flow patterns oscillates in one dimension through low frequency acoustic resonance. Aeration is accomplished by diffusion, the entrapment of droplets that separate from the liquid bulk, and the formation of small bubbles. Oxygen participates as a nutrient during aerobic growth, with the main function to act as the final electron acceptor of the respiratory chain, then mass transfer variations have impact on the growth of *Escherichia coli.* For example, cultures with a glucose excess and low oxygen transfer show incomplete glucose oxidation, resulting in the accumulation of metabolic acids like acetate, whose accumulation leads to a reduction in culture growth and lowered recombinant protein accumulation. Hence, novel culture strategies using shake flasks are needed.

With the aim to analyze the effect of the aeration improvement in shake flask cultures, we analyzed cultures of *E. coli* BL21-Gold (DE3) producer of recombinant phospholipase A2 (rPLA2) from *Micrurus laticollaris* snake venom, under OM and RAM. We observed ~69% less biomass under OM compared with RAM (200 rpm vs. 7.5*g*) at the same initial volumetric oxygen transfer coefficient. We analyzed two more agitation conditions at 12.5*g* and 20*g*, and ~1.6- and ~1.4-fold greater biomass was obtained as compared with cultures at 7.5*g.* In all cultures carried out in RAM, the specific growth rate was statistically similar, but ~1.5-fold higher than that in cultures carried out under OM. Furthermore, glucose was consumed in RAM cultures, doubling biomass per glucose yields, while only half of the glucose was consumed in OM. Interestingly, acetate accumulation was prevented at the maximal RAM (20*g*). The amount of rPLA2 in both, OM and RAM cultures, represented 38 ± 5% of the insoluble protein. The rPLA2 was found in inclusion bodies (IBs). At maximal agitation by RAM, internal *E. coli* localization patterns of protein aggregation changed, as well as, IBs proteolytic degradation, in conjunction with the formation of small external vesicles, obtaining structures of an approximate size of 105-147 nm.

In conclusion, the agitation in RAM was not enough to avoid the classical oxygen limitation that happens in OM shake flasks, resulting in a favorable effect on biomass growth and volumetric rPLA2 production.

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Emergent properties of a synthetic community of *Bacillus* or how the context changes the dynamics in the study of interactions

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Historically, the interactions between bacteria in a community has been studied in pairs in artificial conditions. This approach is highly limited, but setting up a natural experiment, considering all the variables and the numerous and diverse bacteria in a community represents a great difficulty. We designed a synthetic community of three bacteria to study the interactions between them. Although three bacteria may not seem to be a big difference, we have observed emergent properties not observed in a paired interaction. Three different species of Bacillus spp. were chosen to set up this community. Each has a different role in an interaction network: non-antagonist and resistant (R); antagonist and resistant (A); non-antagonist and non-resistant (S). The experiments were performed in a liquid medium for the interactions to occur in a well-mixed interplay (non-structured environment) and then plated on a semisolid medium to quantify the Colony Forming Units (CFU). Our results showed that the interaction between A and S strains caused a decline of the colony forming units of the S strain in the first 5 minutes of the interaction, but that its viability did not decrease further within the next 30 min. Unexpectedly, when the R strain was included (interaction among the three strains, R, A, and S), the antagonism against the S strain was not observed. These results suggested that the presence of strain R interfered with the antagonism of A against S, and this constitutes an emergent property. Experiments were performed using membranes to separate the bacteria from each other but allow metabolite exchange. A decline of the CFU of strain S, even when strain A was out of the membrane. When the S and A strain were in the membrane and R out of the membrane, the CFU of the S strain did not decrease. In the present work, it is proposed that the social context of the interaction is important to determine the dynamics of a community. We also explored why the antagonistic paired interactions were stabilized with time. With the data obtained, we made a model for the interactions observed in this synthetic community, which we will discuss.

Etiological agent prevalence for ecosystem health monitoring in mangroves of the Yucatán peninsula, México

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Mangroves are interface ecosystems between terrestrial and aquatic environments of fresh and saltwater with multiple functions including habitat of a great diversity of organisms, soil stabilizer, storage and carbon transformer. Mangrove's degradation by deforestation and changes in land use could promote the prevalence of infectious diseases' etiological agents. This work was aimed at the study of the presence of etiological agents in mangroves with different degrees of affectation in the Yucatán Peninsula of Mexico using a DNA microarray as a tool for ecosystem health monitoring. Water and sediment samples were collected at 11 localities in the States of Yucatán and Quintana Roo between February and September 2018. Physicochemical parameters in water such as temperature, pH, salinity, conductivity, dissolved oxygen, total dissolved solids and redox potential were measured *in situ.* Metagenomic DNA was extracted, fragmented, labeled and hybridized in a DNA microarray developed by our team that contains 38,000 probes to detect 270 etiological agents. Total coliforms, fecal coliforms, *E. coli* and enterococci were detected with the most probable number method. A total of 215 etiological agents were identified in water and sediment samples, including bacteria, microalgae, fungi, yeasts, viruses, protozoa, arthropods, flatworms, and nematodes of which bacteria were the most abundant, followed by microalgae. These microorganisms could cause infectious diseases involving several systems in humans such as digestive, respiratory, urinary, nervous, osteoarticular, cardiovascular, immunological, otic, ophthalmological, skin and soft tissues. Some etiological agents identified could affect marine organisms as well. The detection of etiological agents in mangroves could be a useful tool for monitoring ecosystem health and promoting the prevention of infectious diseases in the surrounding localities in the Yucatán Peninsula.

Key words: Etiological agents, DNA Microarrays, Mangroves, Yucatan Peninsula.

Isolation and pre-characterization of microorganisms from "Cerro El Toscano" soil, Michoacán

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This work is part of a prospective study focused on sustainable exploitation and conservation of biodiversity in a natural enviroment known for its cultural and historical heritage for two different counties in the developing northwestern area of Michoacán; so that, soil samples from three different locations with a variable degree of enviromental perturbation in "Cerro El Toscano" (Latitude:20.013915/Longitude:-102.74764), were studied in order to measure and characterize the portion of the microbial communities existent. Then, several strains of microorganisms were found, described and cuantified, acording to basic microbiological methods of analysis, wich included: direct culturing bacteria in nutrient media, microbial quantification curves following a one week kinetics, the pre identification screaning of microorganisms through colonial morphology and microscopic observing of cultured media in addition to Gram stains. The results show that each of the three zones studied (highly perturbated, mid perturbated and barely perturbated), have a particular microbiome and the quantity and variability of each group of microorganisms is strongly related with its place of primary isolation, wich is characterized for a diversity in vegetation, soil physico-chemical properties and geographical ubication. This mainly states the complex interrelation between microbiomes ant its enviroment; although some of the strains, such as *Bacillus* and *Brochothrix* species, have been described in other places in the country, there are native species wich yet remain crypted. Regarding the counting of microbial cells, it can be mencionated that the higher counts come from the highly perturbated area in opposition to the barely perturbated soil; however this late shows a higher diversity in colony formation. Meanwhile, the mid perturbated zone, seems to be a kind of transition area between the former two spaces. Furthermore, as one of the most diverse habitats on the planet, soil microbiomes provide rich opportunities to develop novel applications for its study and preservation, since most ecological habitats are still to be characterized.

A new biosynthesis pathway for the sulfolipid sulfoquinovosyl diacylglycerol in *Sinorhizobium meliloti*

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Sulfoquinovosyl diacylglycerols (SQDG) are polar membrane lipids containing sulfur and lacking phosphorus. Membranes of *Sinorhizobium meliloti* grown in media with sufficient phosphate are mainly formed by glycerophospholipids. However, under phosphate limitation, as occurs in other soil bacteria, glycerophospholipids are partially replaced by membrane lipids that do not contain phosphorus. In *S. meliloti* these membrane lipids are SQDG, ornithine-containing lipids, and diacylglyceryl-*N*, *N*, *N*-trimetylhomoserine. Four structural genes for SQDG biosynthesis, *sqdA* and the operon *sqdBDC*, were identified in *Rhodobacter sphaeroides*. The *sqdB* gene product generates UDP-sulfoquinovose, from UDP-glucose and sulfite, while the function of *sqdA*, *sqdC* and *sqdD* genes remains poorly understood. We have identified SMc02490 of *S. meliloti* as the orthologous ORF to SqdA of *R. sphaeroides* and mutants in *smc02490* are unable to form SQDG. Expression of the *S. meliloti sqdBDC* operon in *Escherichia coli* results in formation of sulfoquinovosyl glycerol (SQGro), while co-expression of this operon together with *smc02490* results in formation of significant amounts of the sulfolipid SQDG. SQGro was obtained by deacylation with mild alkali treatment of the purified sulfolipid. Addition of SQGro to an *E. coli* culture expressing *smc02490* led to SQDG formation. Furthermore, *in vitro* enzymatic assays using the substrates [^{35}S]-SQGro and palmitoyl-ACP with crude extracts of *E. coli* expressing *smc02490* result in SQDG formation. In summary, our results show that SqdA is an acyltransferase involved in acylating SQGro. Presence of SqdA in bacteria allows them to use environmental SQGro for the biosynthesis of the membrane lipid SQDG.

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Geographic distribution and diversity of *Fusarium* species in the state of Colima, México.

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Fusarium spp. are important phytopathogenic molds that decimate agricultural production in temperate and tropical regions of the world, where their growth is particularly favored by high humidity and warm temperatures. Some strains also possess the ability to produce toxic metabolites, termed “mycotoxins”, that impact human and animal health. The aim of this work was to characterize both morphologically and through molecular biology the occurrence and diversity of *Fusarium* species in the state of Colima, México. Air, soil and tissue samples from plants showing symptoms of *Fusarium* disease (mainly root, stalk and/or fruit rot) were taken from the three most important agricultural regions of the state: Tecomán Valley, Colima Valley and the northern municipalities of Cuauhtémoc, Comala and Villa de Álvarez. Fungal isolates were obtained through serial dilutions and growth on Dichloran Rose Bengal Chloramphenicol agar (DRBC) for 72 h at 28 °C. Monosporic cultures from colonies with macroscopic morphology (texture, pigmentation and colony diameter) typical of *Fusarium* spp. were transferred to Dextrose Sabouraud Agar (DSA) and incubated for seven days at 28 °C for further analysis. So far more than 400 isolates have been obtained, with 65 of them showing microscopic structures typical of *Fusarium* spp., such as fusiform to sickle-shaped multicellular macroconidia, one or two-celled microconidia and chlamydospore production. The region with the highest incidence of these molds was the Tecomán Valley, especially in banana plantations. The molecular identification with the ITS1/ITS4 and EF1/EF2 primers is currently underway, and mycotoxigenic capability of the isolated fusaria will be evaluated by HPLC further on.

Bacterial diversity in karst sinkholes (cenotes) from the Puerto Morelos and Tulum touristic zones

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The Yucatan Peninsula is an extensive karst system in Mexico that encompasses a unique topography characterized by the absence of surface water bodies and the presence of karst sinkholes, locally called cenotes. These sinkholes are not only for touristic activities such as swimming and diving, but they also represent a source of drinking water, especially in rural areas. Microbiological surveys in these aquatic systems have been mainly focused in quantifying pathogenic bacteria. Yet, there is a lack of knowledge about the groundwater bacterial diversity. In this study, we surveyed the groundwater bacterial diversity in water samples from five cenotes from the Puerto Morelos and Tulum touristic zone by 16S rRNA sequencing (Quintana Roo, Mexico). Water samples were taken for hydrochemical and bacterial characterization by massive amplicon sequencing. We found that the principal classes were Acidomicrobiia, Actinobacteria, Alphaproteobacteria, Betaproteobacteria, Cytophagya, Epsilonproteobacteria, Flavobacteriia, Gammaproteobacteria and Thaumarchaeota. The 16S rRNA diversity varied according to the electrical conductivity, yet the samples showed wide number of amplicon sequence variants (ASVs) from 10 to >3000 and Shannon values (*H'*) from 1 to 7.5. Also, our results showed that the intrusion of marine water affects the bacterial diversity in these anchialine systems. Yet, further studies must be done to understand the role these groundwater microbial communities in biogeochemical processes and pollutant degradation.

Keywords: bacteria, aquifer, karst
Área Temática: Otras
Indicar la preferencia: Cartel (X)

Experimental evolution to explore phenotypic plasticity to temperature in wild type strains from a natural environment

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Phenotypic plasticity allows organisms to face environmental stress through temporary regulatory changes that can eventually lead to evolutionary adaptation. We wanted to know if different lineages exhibited the same capacity to face a stress, such as temperature. We also explored if the strains of this lineages exhibited parallelism in the strategies evolved to withstand higher temperatures. Bacteria are excelent models to study of evolution, due to their rapid regulatory response and rapid mutation fixation. We studied isolates from sediment in the Churince lagoon in the desert of Cuatro Ciénegas Coahuila, Mexico. The selected strains belonged to two *Bacillus* lineages, three strains to *Bacillus cereus sensu lato* and three more to *Bacillus subtilis sensu lato*. A lab strain was included, so seven bacterial isolates were studied. Six populations of each were used for experimental evolution, 26 populations were evolved at constant mesophilic temperature (37oC) and 26 populations with incremental temperature (up to 46 oC) for 1000 generations. The populations established in the invariable temperature of 3 oC is a control for changes in populations due to a novel environment, the culture medium.Although all strains evaluated bacteria have presumably evolved in the same environment, the two lineages exhibited differences in plasticity. Colonies of the evolved strain exhibited differences in pigmentation, and many had lost the ability to sporulate. The *B. subtilis* lineage was more tolerant than that of *B. cereus* to increases in temperature. The maximum temperature that the strains could reach was 47 ^{o}C. The phenotypic plasticity to temperature of ancestral and evolved lines was analyzed by reaction norms. We also evaluated whether co-variation in their responses to NaCl concentration, and pH could be explained as tradeoffs to adaptation to growth at high temperature. The genomes of all the evolutionary lines were sequenced, to determine whether the different populations for each strain and the two lineages exhibited parallelisms in their adaptation strategies. We will discuss the results obtained in this work.

Identification and biochemical characterization of the putative XPG/Rad2 nuclease of *Giardia duodenalis*

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Giardia duodenalis is a flagellated protozoan parasite that causes giardiasis. During its life cycle, several rounds of DNA replication, cytokinesis and karyokinesis occur associated with DNA damage inherent to cell metabolism. In these processes DNA repair mechanisms must be functional to maintain genomic stability. In the vast majority of organisms, nucleotide excision repair (NER) is the main mechanism to remove adducts and bulky lesions all over the genome and also during transcription, such as the ones that block the RNA polymerases progression. During this repair mechanism XPG/Rad2 is the principal nuclease involved. Thus, the aim of this work was to identify and perform the biochemical characterization of the putative XPG/Rad2 nuclease of *Giardia duodenalis.*

By *in silico* analysis, the putative *Gd*XPG/Rad2 nuclease sequence was identified as GL50803_16953 in GiardiaDB using human and yeast XPG and Rad2 sequences as query. The putative gene sequence was amplified from *G. duodenalis* genomic DNA, cloned in the expression vector pET100 and transformed in *E. coli* Solu-BL21. The expression of the putative *Gd*XPG/Rad2 nuclease was induced during 6 hours using IPTG, verified by western blot and then purified by affinity chromatography. Next, the ability of the putative protein to bind to DNA simulating a bulky lesion or to normal double stranded DNA (no damage, dsDNA) using a fluorescent labeled bulky or normal probe (bDNA, nDNA) was evaluated and visualized through electrophoretic mobility shift assay (EMSA), then the *Gd*XPG/Rad2 nuclease activity on bDNA and nDNA probes was also studied and compared.

Results showed that the putative *Gd*XPG/Rad2 nuclease at concentrations of 0.5 µM to 8 µM bind to 500 nM of bDNA probe in the presence of 1 mM ATP when incubated for 30 minutes. For the nuclease activity on probes bDNA or nDNA, 100 mM $MgCl_2$ and 100 mM $MnCl_2$ were used as cofactors and 100 mM $CaCl_2$ negative control.

These results indicate that the putative protein *Gd*XPG/Rad2 binds with more affinity to DNA containing a bulky lesion when compared to a nDNA (no damage). Further, GdXPG cleaves the bulky probe preferably with respect to nDNA probe, this strongly suggests that the putative protein *Gd*XPG/Rad2 has the biochemical characteristics predicted and could act as a nuclease in *Giardia duodenalis* having a role in the NER pathway.

Study of RNA Degradosome *in vivo* dynamics using FRET.

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Introduction. The RNA Degradosome is a protein complex discovered in *E. coli* and with analogs in gram-negative bacteria. The canonic complex, discovered in *E. coli*, is formed by RNase E, PNPase, RhlB and enolase and it effectuates post-transcriptional regulation by mRNA decay. RNA Degradosome works by detection of complexes formed by small RNAs protected by Hfq and bound to target mRNAs. Hfq helps sRNA-mRNA hybrid to interact with the protein complex and to be degraded by RNase E and PNPase with help of helicase activity of RhlB, enolase function in the complex has not been well described. Recently, it has been shown that RNA Degradosome is located at cell membrane and in cell cytoskeleton by a mix of *in vitro* and microscopy assays. Despite the knowledge we have of RNA Degradosome and its function, there are few works about the complex *in vivo* dynamics and protein's interactions of its members and how this data is related with RNA decay. In this work, we show how RNA Degradosome assembles *in vivo* for RNA decay after specifics stimuli using Fröster Resonance Energy Transfer (FRET) technology. **Methods.** Strains of *E. coli* were genetically modified using lambda phage technology to fuse fluorescent proteins to canonic RNA Degradosome proteins and regulators and these strains were used for studying RNA Degradosome assembly during different growth phases. Also, modified bacteria were exposed to specific stress conditions and RNA Degradosome assembly and RNA decay was studied using FRET and qPCR. Same experiments are planning to be done using Total Internal Reflection Fluorescence (TIRF) technology to study RNA Degradosome-membrane dynamics. Some of the stress conditions studied were glucose stimuli, iron starvation and oxidative stress. **Results.** FRET strains were characterized at phenotype and genotype level, looking for fluorescent protein expression and its gene insertion fused to RNA Degradosome proteins. After this, FRET experiments were done under different stress conditions, observing different RNA Degradosome assembly after stimulation. Also, a growth curve was done, observing the RNAD behavior under the different growing phases. All this while we were observing target mRNA degradation. **Conclusions.** RNA Degradosome assembles *in vivo* under different conditions and this assembly changes from stimuli to stimuli, allowing post-transcriptional adaptation of bacteria to environmental changes by RNA decay. **Acknowledgments.** This work was financed by Cinvestav, and CONACyT-255374 CB-2015-01. CONACYT doctorate scholarship 556942 to MEJV.

A highly conserved *16S rRNA* region reveled a new strain of *Acidithiobacillus ferrooxidans* from a biohydrometallurgy residual solution

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Abstract

Metal recovery from minerals has been practiced since prehistoric times, it has developed into new and more efficient ways of searching for less negative environmental impact. An example of this is the use of microorganisms like bacteria, to biooxidize minerals and bioleach metals, known as biohydrometallurgy. During pyrite (FeS_2) biooxidation in aqueous medium, Fe^{2+} and S^{2-} bonds break because of ferric oxidation; then, Fe^{2+} is reoxidized by iron-oxidizing prokaryotes, catalyzing the process and acidifying the medium, making it an advantageous environment for acidophiles. The 16S ribosomal RNA gene is commonly used for identification of prokaryotes. While sequencing of *16S rRNA* hypervariable regions permits to explore microbial community dynamics over temporal and spatial scales (Huber *et al*. 2007), the use of a highly conserved region of *16S rRNA* reveals subpopulations. In mining environments, bacteria from the genres *Acidithiobacillus, Acidobacterium* and *Leptospirillum* have been identified (Casas-Flores *et al*. 2015; Sánchez-Andrea *et al*. 2011). In this study, we analyzed the bacterial community present in two samples. The first bacterial sample was obtained from a remnant solution of biohydrometallurgical process at a mining company in northem Mexico, this solution was used to isolate and cultivate a second sample. This second and cultured bacterial sample was adapted to pyrite (8% w/v) per 13 days, when a concentration of $1.2x10^8$ cells/mL was reached. A set of primers from a highly conserved region of the *16S rRNA* gene was designed to amplify a 404-516 bp PCR product. Total RNA was extracted from both samples and the 16S rRNA PCR product obtained. Amplicons were then cloned, sequenced and results subjected to a BLAST search to identify the species present in each culture. Remarkably, *Acidithiobacillus ferrooxidans* was the only specie we found in both industrial and adapted to pyrite samples. However, analysis of the sequence for the conserved region of the *16S rRNA* allowed us to identify two different unreported strains. The importance of our results lies in the fact that, “a noticeable genetic diversity exists within *Acidithiobacillus* genus, especially within *A. ferrooxidans* (Pristas *et al*. 2017).

Horizontal transference of virulence genes in *E. coli* strains isolated from a same geographical area.

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Introduction. *Escherichia coli* is a microorganism with a wide genetic variability, which is part of the normal microbiota, however the acquisition of genetic material produced pathogenic variants of this bacteria with the ability to infect humans and animals. The onset of pathogenic variants is a decisive factor for the ability to integrate new genetic material into genome (genetic plasticity). These evolutionary processes have allowed *E. coli* infect a wide range of hosts, including ruminants and wildlife animals, which are reservoirs of *E. coli* pathogenic strains such as STEC and non-STEC. **Material and methods.** In this study, 13284 strains of *E. coli* isolated from wildlife carnivores and bisons of the Biosphere Reserve of Janos Chihuahua Mexico (RBJ) were analyzed through PCR, in order to detect 10 virulence genes. All isolates with one or more virulence genes were serotyped and their phylogroups were stablished using standard procedures. **Results.** The results showed 62 isolates with at least one virulence gene and 68 with two or more virulence genes (v.g.). Carnivorous strains showed a lower diversity in v.g. ($<p = 0.05$), in comparison with bison strains. Meta-community analysis showed that the strains isolated form bisons and carnivores had a Clementsian structure ($p<0.05$), suggesting that both Taxa belong to the same group, considering only the virulence genes. Also, these genes were relocated between different strains of both taxa, proposing the existence of horizontal gene transfer processes. The meta-community analysis also showed that these events were independent of the phylogroup and serogroup. Strains isolated from carnivores and bisons correspond to different serogroups in all animals, but showed a same distribution in virulence genes, confirming the results obtained in the meta-community analysis. Many of the strains isolated in this study were not associated with a particular phylogroup, suggesting the existence of more genetic groups. **Conclusions.** In this geographical area there is a continuous process of gene transfer of *E. coli* between strains of different taxa, this phenomenon was not dependent of phylogroup and serogroup.

Modalidad: cartel

Área: Ecología y genética

Specific genetic background is required for acquisition of virulence genes in *Escherichia coli*

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Introduction. *Escherichia coli* is one of the most important pathogens associated with gastroenteric diseases. *E. coli* enteric infections have a different pathogenesis. This phenomenon is the result of the wide range and combination of virulence factors (VF) in pathogenic *E. coli* strains. Virulence factors patterns allow to classify *E. coli* in different pathotypes (intestinal and extraintestinal). Many of these virulence genes have been acquired through a horizontal gene transfer process (HGT). Acquisition of these genes has been associated with variations in the serogroup, phylogroup and other genetic determinants. However, these associations are not clear whereas the presence of virulence genes in some strains seems to be a random process. The study of pathogenesis evolution in wild type strains recovered from natural microenvironments represents an opportunity to understand the molecular determinants associated with virulence gene transfer. **Material and methods.** In this study, 123 strains of *E. coli* with one or more virulence genes were isolated from bison and carnivores were used. The phylogroups were determined using the methodology carried out by Clertmot. The serogroups of each strain were determined by using standard procedures. The *rpo*D sigma factor was analyzed by High Resolution Melting in order to identify possible genetic variants in order to associate them with a particular virulence genes pattern. **Results.** Correlation analysis showed that the serogroup and the phylogroup could not stablished the presence of virulence genes in all strains analyzed in this study. High resolution melting analysis showed the presence of genetic variants of *rpo*D sigma factor in *E. coli* strains. A principal component analysis showed that virulence genes patterns were associated with these genetic variants of *rpo*D sigma factor. **Conclusions.** In this study, the presence of virulence genes was associated with genetic variants in the *rpo*D sigma factor, suggesting that genetic variations in different transcriptional factors (genetic background) could be necessary for the acquisition of virulence genes.

Modalidad: Cartel

Área: Ecología y genética.

Isolation of halophilic archaeas from Cuatro Ciénegas Basin: the lost world hotspot

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Cuatro Ciénegas Basin (CCB) represents an unexplored niche of wide microbial diversity, making it an attractive site for ecological and bioprospecting studies. The marine origin of these ponds suggests that these microorganisms can be halophilic and extreme, since they had to adapt to thrive in conditions such as phosphorus deprivation and the high incidence of solar radiation. The main goal of this work is to isolate, describe and characterize halophilic-endemic archaea from Cuatro Ciénegas ponds in Chihuhuan desert. The sampling of water and sediment, was carried out on April 2016, October 2016 and February 2017. Enviromental conditions (pH, temperature and salinity) were measured and samples of each sampling points were taken, with their respective coordinates. Halophilic culture mediums (12.5% and 25% NaCl) were designed from these conditions, subsequently these media were inoculated with water and sediment sample and incubated at 28°C and 50°C. Once the colonies were developed after 3-6 months, biochemical tests and the macroscopic and microscopically description were realized. DNA extraction was carried out from the isolates (using a protocol standardized in our laboratory), PCR-amplification of 16S rRNA marker was performed, then cloned-transformed and sequenced. Furthermore, phylogentic analysis with its 16S sequence were done to identify distance from the reported archaea. The isolate strains mostly belong to Archaea order Halobacteriales being classified as uncultured archaeaons; also some of them presented an irregular pleomorphic morphology including rods and cocci, displaying pigmented red and rose colored colonies. The species with these morphological features are known to possess and S-layer (surface layer), which has an important role maintaining their shape. It has been described that this morphology tends to change according to the salt concentration (Javor, 1989). Isolated strains correspond to halophilic prokaryotes from Archaea Domain, and 16S sequence obtained for phylogenetic analysis indicates their long distance and ancestry from what it is been reported. This could prove the CCB has a real ancient past with endemic communities.

Assessing the biotechnological potential of Antarctic and sub-Arctic sediment cores

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Polar and subpolar ecosystems are highly vulnerable to global climate change with consequences for biodiversity and community composition. As bacteria are directly impacted by future environmental change; it is therefore essential to have a better understanding of microbial communities in fluctuating ecosystems. Exploration of these environments, specifically sediments, represent a unique opportunity to discover new species of bacteria with biotechnological applications. In particular, Actinobacteria account for the production of over 10,000 bioactive compounds, accounting for over 45% of all bioactive microbial metabolites. Culture-dependant studies of 12 sediment cores from the Antarctic and sub-Arctic generated a culture collection of 46 strains encompassing the genera *Microbacterium, Rhodococcus* and *Pseudonocardia* from the phylum Actinobacteria. We were able to isolated taxonomically unknown strains that represent an exciting resource for bioactive metabolite discovery. This work exploits the phylogenetic diversity (bacterial taxonomy and evolutionary relatedness) and secondary metabolite variation using cutting-edge comparative omics approaches for accelerated and efficient discovery of bioactive molecules against commercially valuable organisms.

Biofertilization of *Bonellia macrocarpa* with native bacterial strains and its effect on the growth and content of bonediol

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ABSTRACT

In nature the interaction plant, soil, microorganism has been witnessed since remote years, and has been the main object of study in genomic ecology, associating soil microorganisms with the growth of plants. In this work, the diversity of bacteria associated with the Bonellia macrocarpa plant was studied and its potential was determined as plant growth promoting bacteria (PGPB). A total of 95 strains were isolated from the rhizosphere of B. macrocalix. The majority of the isolates were in the form of Bacilli, Gram negative, aerobic, with mobility by flagella, rapid growth. The phylogenetic analysis of the 16S gene grouped the strains within the genres: Rhizobium, Pseudomonas, Sphingomonas, Caulobacter, Microbacterium, Achromobacter and Massilia. All strains showed the ability to fix N2, solubilize phosphate and synthesize auxins. Rhizobium sp. BON-1 stood out for its ability to fix N2 and the strain Pseudomona sp. BON-2 recorded the highest phosphate solubilization. The use of native bacteria is a biotechnological alternative for the generation of biofertilizers with high efficiency, replacing fertilizers or plant growth regulators.

KEYWORDS

Auxins, symbiotic bacteria, biofertilizers.

Design and validation of a multiplex PCR assay for MAT idiomorph determination within *Fusarium fujikuroi* species complex.

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The ascomycete genus *Fusarium* is estimated to comprise over 300 phylogenetically distinct species, representing one of the most important groups of mycotoxigenic plant pathogens. Of these, members of the species-rich *Fusarium fujikuroi* species complex (FFSC) represent one of the greatest threats to global agricultural biosecurity due to the large number of economically important plant diseases that they cause. Notable plant diseases induced by members of the FFSC include corn ear rot and seedling blight caused by *F. verticillioides*, foolish seedling disease or bakanae-byo of rice caused by *F. fujikuroi*, pitch canker of *Pinus* spp. caused by *F. circinatum*, mango malformation caused by *F. mangifera*, *F. tupiense*, *F. sterilihyphosum*, *F. mexicanum* and *F. pseudocircinatum.* Because plant pathogens with the ability to reproduce both sexually and asexually pose the greatest threat to overcoming control method based on fungicides and host resistance, numerous studies have investigated the reproductive mode and genetic architecture of the mating type (*MAT*) locus within the FFSC. It was found that previously published PCR assays for determining *MAT* idiomorph failed to genotype some of the FFSC isolates recovered from malformation disease in mango and neotropical trees in Mexico. Thus, the primary objective of this study was to design and validate a robust multiplex PCR-based diagnostic for typing *MAT* within the FFSC. To accomplish this objective, we mined the *MAT1-1* or *MAT1-2* locus from the genomes of 60 FFSC isolates, representing 56 phylospecies, and from four species in its sister group, the *F. nisikadoi* species complex (FNSC). Bioinformatic searches were facilitated by targeting DNA lyase (SLA2) and apurinic endonuclease (APN1), the genes that flank the *MAT* locus in *Fusarium*. As expected, three genes were identified within *MAT1-1* (*MAT1-1-1, MAT1-1-2* and *MAT1-1-3*) and two in *MAT1-2* (*MAT1-2-1* and *MAT1-2-9*), using the prediction tool AUGUSTUS (http://augustus.gobics.de). Three multiplex PCR assays were designed and tested. The assay targeting *MAT1-1-2* and *MAT1-2-1* successfully genotyped the entire 71-isolate validation panel, which included 56 FFSC and 4 FNSC phylospecies. Sanger sequencing confirmed the identity of the amplicons following published protocols that included BLASTn queries of GenBank. By contrast, the previously published PCR assays produced positive genotypes for 46.5-59% of the 71-isolate validation panel, but only when they were run as a uniplex assay. The *MAT1-1-2* and *MAT1-2-1* primer pairs were tested on a 48-isolate/47 species panel that spanned the phylogenetic breadth of *Fusarium* to assess how broadly they worked within this genus. Positive genotypes were only obtained for three near relatives of the FFSC/FNSC: *F. oxysporum, F. foetens* and *F. hostae*. Although only one-fifth of the FFSC/FNSC are known to reproduce sexually, our results suggest that if they possess a sexual cycle, it is heterothallic (self-sterile).

Environmental conditions and organisms associated to the coloration change in the "La Salina" lagoon of Bajos de Coyula, Oaxaca.

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Extreme environments have been considered to be populated almost exclusively by prokaryotic organisms. Some coastal lagoons at Oaxaca are natural hypersaline environments. The present work studied the environmental conditions and the microorganism that flourished in the "La Salina" Lagoon of Bajos de Coyula, Oaxaca, and that turned on pink for a short time the watercolor. The physicochemical parameters and water nutrients were evaluated and the microorganisms were isolated, identified and cultivated in Pfennig´s and marine medium at salinities of 0.5 to 4.2 M, in aerobic and anaerobic conditions at 35 ºC in darkness. The results showed high salinity levels (51-53), with low levels of oxygen (0.2 to 0.62 mg L^{-1}), temperatures from 28.5 to 31.3 ºC and pH values of 7.65 to 7.89. The nutrients showed values of NO_2^-+NO_3^- from 13.64 µM to 23.84 µM, NH_4^+ from 0.73 µM to 1.25 µM, PO_4^{3-} from 10.41 µM to 32.08 µM and SiO_2 from 70.96 µM to 88.97 µM. The microorganisms isolated correspond to different phylum considered extreme halophiles, the red haloarchaea *Haloterrigena hispanica*, and two bacterias, *Halobacillus halophillus* and *Halomonas salina*. The presence of these halophiles organisms is evidence of the sedimentation process and closure of a coastal lagoon of ancient usage as artisanal saline, with conditions for massive growth of salt-loving organisms principally.

***Keywords**: halophiles, red archea, bacteria, coastal lagoon, hipersalinity, Oaxaca.*

pH-dependent Predation by *Bdellovibrio bacteriovorus* 109J

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Bdellovibrio bacteriovorus is a gram-negative predator microorganism which is known to exist in various environmental samples such as river, sea, and soil. In recent years, anti-microbial resistance (AMR) became ineffective against antibiotics, and this is now recognized as a serious issue all over the world. As a means to solve the issue, *B. bacteriovorus* is worthy of attention because it has a unique life cycle by which prey cells will be killed; therefore, it may be used as a new-type bactericidal agent. However, the nature of *B. bacteriovorus* is still unknown, and no research has been conducted focusing on changes in predatory effects caused by changes in pH. In this study, we investigated the effect of pH on the predation of *B.bacteriovorus* 109J. Co-cultivation of *B.bacteriovorus* 109J and *E. coli* BW25113 as a prey in HEPES buffer solution from pH 6.6 to pH 7.6 for 10 days showed a decrease in turbidity under the pH conditions higher than pH 6.7; however, no predatory activity was found at lower pH conditions than pH 6.6. Plaque forming unit (pfu) of *B. bacteriovorus* was compared at pH 6.6, pH 6.8, pH 7.0, pH 7.3, and pH 7.6 by using a two-layer plating method. As a result, a low number of *B. bacteriovorus* cells were detected at lower pH conditions. Furthermore, the assay of metabolic activity using WST reagents showed that *B. bacteriovorus* 109J has a low metabolic activity at lower pH conditions. Taken together, we found that the predation by *B. bacteriovorus* is active at an alkaline condition rather than an acidic condition.

Sphingolipids required for survival of *Caulobacter crescentus*

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Sphingolipids are essential and common membrane components in eukaryotic organisms, participating in many important cellular functions, such as the organization of lipid rafts or cell signaling. In contrast, only few bacteria are known to harbour sphingolipids in their membranes. Whereas in eukaryotes the distinct steps of sphingolipid biosynthesis have been resolved, only the first step, catalyzed by serine palmitoyltransferase, has been characterized to some extent in bacteria. Presumptive structural genes for a serine palmitoyltransferase are also found in bacteria in which no sphingolipids have been identified so far. For example, the well-studied α-proteobacterium *Caulobacter crescentus*, which serves as a model for asymmetric cell division and cellular differentiation, has a structural gene for a presumptive serine palmitoyltransferase (*spt*). Here we report that *C. crescentus* wild type produces several molecular species of dihydroceramides, which are not produced in a mutant lacking the presumptive *spt*. Whereas growth of the *spt*-deficient mutant and wild type are indistinguishable during the exponential phase of growth, survival of the *spt*-deficient mutant is much reduced, in comparison with wild type, during stationary phase of growth, especially at elevated temperatures. The structural gene for *spt* is located within a cluster, comprising another 16 genes and which, like *spt*, are important for fitness of *C. crescentus*. Mutants were generated that lack genes for a presumptive 3-oxo-sphinganine reductase, a presumptive dihydroceramide synthase, a presumptive acyl-CoA synthetase, or a presumptive acyl carrier protein. Similarly, as the mutant deficient in *spt*, the other four mutants were unable to produce dihydroceramide and were susceptible to thermal, oxidative and acid stress. These results show that at least five structural genes are required for dihydroceramide biosynthesis in *C. crescentus* and that sphingolipid biosynthesis is needed for survival of this bacterium.

Phenotypic plasticity evaluated in bacteria in a classic G x E study comparing lineages from contrasting natural environments

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One of the significant challenges in evolutionary genetics is to understand how genetic architecture affects the potential of individuals to respond to selective forces. It could be expected that species collected from similar environments may be more similar in their environmental response, their thermal tolerance, for example, as a result of their adaptation to such environment, rather than as a result of their shared genetics. This important question has hardly been addressed in bacteria from natural environments. Here, we analyzed reaction norms to temperature of strains from two lineages of *Bacillus, Bacillus cereus sensu lato,* and *Bacillus subtilis sensu lato*, that evolved in two contrasting environments, a temperate lagoon (T) and a hot spring (H). Through norms of reaction, it was possible to describe the phenotypic response of individual strains across a range of environmental conditions. Our results showed that all strains from the H environment exhibited more tolerance to temperature (a shifted norm of reaction). However, no convergence of norms of reactions was observed among the two lineages co-occurring in the same environment. The two lineages maintained distinct features in both environments, suggesting that genetic architecture imposes a constraint to phenotypic plasticity. The thermotolerant strains from the hot spring cannot withstand an increase of temperature beyond a couple of degrees of those from the temperate lagoon. Maybe this should be a strong warning to the increase in temperature that the planet will experience from climate change.

Molecular identification and characterization of bacteria isolated from biofilms in Atetelco, Teotihuacan.

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The biodeterioration of cultural property is the physical or chemical damage caused mainly by microorganisms in monuments or buildings that belong to the cultural heritage. Frequently they are observed forming communities or biofilms of bacteria, algae, fungi and lichens inside and on the substrate.

The aim of this work was to isolate, molecularly identify and characterize bacteria from samples of biofilms collected in areas with obvious biodeterioration in the Atetelco palace of the archaeological zone of Teotihuacan. Biofilm samples were collected aseptically using a sterile wet swab and placed in a sterile polypropylene container with screw cap, serial dilutions of 10^{-1} to 10^{-3} were taken from each sample, taking 100 µL to inoculate by dispersion in nutrient agar. Isolates were obtained based on their morphological differences and incubated at 30 ° C in a range of 3 to 5 days. DNA extraction was performed using the technique proposed by Hoffman and Winston in 1987. The identification was made by phylogenetic and similarity analysis of the 16S rRNA gene, in order to evaluate them phenotypically tests were performed: mobility in SIM medium, catalase, lactose fermentation in Mac Conkey medium and those included in the criteria of Cowan and Steel, growth in nutritive broth with 3% NaCl, use of citrate and mannitol, DNAse test, liquefaction of gelatin, starch and sculin hydrolysis, nitrate reduction, amino acid decarboxylation and carbohydrate fermentation.

The isolated and molecularly identified strains were: *Sphingomonas aerolata* (2 isolates), *Pseudomonas poae* (2 isolates), *Massilia plicata*, *Sphingomonas aerolata*, *Variovorax paraduxus* (2 isolates), *Methylobacterium marchantiae*, *Pseudomonas poae, Ochrobactrum pseudogrignonense* (2 isolates), *Lelliotia nimipressuralis* (2), *Epilithonimonas tenax*. Microscopic morphology in all bacterial strains corresponds to Gram-negative short bacilli, mostly mobile which gives them a survival strategy in hostile environments; its colonial morphology is similar in size and color, with yellow predominant, which could be of importance due to the physical deterioration that pigmentation can cause on murals and buildings. It was found when studying the metabolism of strains that carbohydrate fermentation is an important phenotypic trait since there is formation of organic acids that are part of the possible causes of wall biodeterioration due to solubilization or chelation of the material. Based on the results, the strains *Epilithonimonas tenax*, *Lellitotia nimipressuralis*, *Ochrobactrum pseudogrignonense* and *Massilia plicata* are considered to have the greatest potential for chemical damage to the murals.

The knowledge of the phenotypic profile of the existing microbial diversity and the application of techniques such as molecular identification, will allow us in the future a better protection of the cultural heritage.

Isolation, and molecular identification of plant growth promoting bacteria in vegetables and *Salix sp*. from Chinampa agricultural system in Mexico City, Mexico

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"Chinampa" is the Aztec name for an ancestral agro-productive system, consistings in plots of land inside the lake. They offer high agricultural productivity, surrounded by canals ditches and rows of "ahuejotes" (*Salix bonplandiana*) native willow species that performs several functions, keep the soil in the plots, whose roots protect the borders chinampa from erosion. Also, in the roots there are plant-microbe interactions in the rhizosphere that are determinants for plant health, productivity and soil fertility. Plant growth-promoting bacteria (PGPB) are bacteria that can enhance plant growth and protect plants from disease and abiotic stresses through a wide variety of mechanisms; those that establish close associations with plants, such as the endophytes, that could be more successful in plant growth promotion. In this work, we isolate a total of 33 strains from vegetables and from trees of *Salix sp*. by serial dilution and inoculating on nutritive agar incubated at 28 ºC, and their characterization by general microbiology indicates that the cells of the isolates were Gram-positive, aerobic and some strains were endospore-forming rods with possible promoting growth.

Phylogenetic analysis of 16S rRNA gene revealed that the organisms belonged to the genus *Bacillus*, sharing from 97% to 99% of sequence similarity with *Bacillus megaterium* and *Bacillus licheniformis, Paenibacillus amylolyticus, Exiguobacterium sibricum* and *Exiguobacterium acetylicum, Acinetobacter lwoffii, Moraxella osloensis* and *Micrococcus sp.* These species, according to the literature, promote the growth of plants. Thus, the isolates obtained could be potential strains. The studies of physiological characteristics of isolates to be identifycated as the bacteria promoting plant growth are in progress.

Therefore, isolates are likely to be potential species to promote plant growth. So to identify this capacity of the isolates, its physiological characterization is being carried out.

Subsequently, once the growth promoting bacteria have been identified, they will be added as biofertilizer, thus helping to make crops more efficient and avoid contamination due to chemical fertilizers.

STRUCTURAL GENOMICS AND MOLECULAR CHARACTERIZATION OF SYMBOL BACTERIA RESISTANT TO HEAVY METALS

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The changes in the disposition of the soil, together with the toxic effects of heavy metals, represent a stressful condition in the establishment of the vegetation cover, since, due to these inappropriate characteristics, a negative effect on physiology has been observed and a obstacle to germination and growth in plants mainly caused by biochemical imbalance and genetic damage. In the last decade, it has been shown that symbiotic microorganisms allow the host plant to improve its growth under conditions of heavy metal stress. This phenomenon occurs by stimulating the growth of plants indirectly through the induction of defense mechanisms against phytopathogens and / or directly through the solubilization of mineral nutrients (nitrogen, phosphate, potassium) or the production of phytohormones that promote growth like auxins, gibberellins and ethylene. It can also change the bioavailability of metal in the soil through various mechanisms such as acidification, precipitation, chelation, complexation and redox reactions that can relieve the phytotoxicity of the metal and stimulate plant growth.

In this study 111 endosymbiotic bacteria were isolated from two *Acacia* species, predominant in two mining jales located in the mining area of the town of Huautla, Morelos. From a qualitative test of resistance to heavy metals by means of the disk inhibition technique, isolates AF16 and AC44 were selected, for their ability to resist: 45 mM Pb, 207 mM Mn, 7200 uM Cd and 84 mM of Zn in addition to being multi-resistant to antibiotics. The two isolates were characterized by phylogenetic methods based on multilocus sequence data revealing that strain AF16 is grouped with the genus *Microvirga sp.* and strain AC44 with the species *Stenotrophomonas maltophilia*. Based on this, the genome of these two isolates was sequenced and assembled to study the evolution and genomic basis of heavy metal resistance, identifying efflux pumps, P-type ATPases and oxidizing-reducing enzymes. Regarding the interaction of these two strains with leguminous plants, they were transformed with the vector pSEVA537R-pBBR1 that carries a fluorescent red protein and nodulation tests were carried out with *P. vulgaris* in a flask, to visualize the location of the bacteria in the plant. Currently, the accumulation and transformation of Pb, Cd, Mm and Zn is being evaluated, as well as the production of plant growth and nitrogen fixation promoters to know the different molecular mechanisms of resistance to heavy metals, in addition to the role that bacteria are symbiotes in the establishment of *P. vulgaris* under stress conditions.

ISSR-based assessment of the genetic diversity of *F. mexicanum,* causal agent of mango and big-leaf mahogany malformation diseases in Mexico.

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Fusarium mexicanum has been reported as the causal agent of mango malformation disease (MMD) and big-leaf mahogany malformation disease (BLMMD) in Mexico. MMD is one of the most important diseases of mango in the country; while BLMMD was recently described and represents a potential threat to the current reforestation projects in Mexico. Due to the importance of this pathogen, our objective was to determine the genetic diversity of *F. mexicanum* isolates obtained from malformed tissue of big-leaf mahogany and mango trees, using an analysis of molecular variance (AMOVA) based on the fingerprints obtained with internal simple sequence repeat (ISSR) primers. We selected five out of 14 ISSR primers that generated the highest number of polymorphic bands to genotype 61 isolates of *F. mexicanum,* 32 from mango and 29 from mahogany. The five selected primers generated 49 polymorphic bands (85.96%) from a total of 57 fragments ranging in size from 250 to 2800 bp, with an average of 11.4 bands per primer. The AMOVA indicated that the variation within populations (isolates grouped by host and geographic origin) was significant (43%), followed by the variation between the big-leaf mahogany versus mango isolates (34%), while among populations the variation was the lowest (22%). The genetic fingerprints suggested that genetic variability of *F. mexicanum* populations are structured by the host of origin rather than the geographic region.

Lambda red system adjustment for *pilA* gene mutation in *Klebsiella pneumoniae*.

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Klebsiella pneumoniae is frequently isolated from a wide variety of environmental sources such as soil, vegetation and water, even as part of the human and animal microbiota. But it is also a severe infectious agent associated with serious hospital acquired infections such as pneumonia, septicemia and urinary tract infections among others. *K. pneumoniae* affects immunocompromised, healthy, neonatal and elderly patients. Furthermore, it is currently one of the most drug-resistant pathogens, limiting the treatment options.

Prior to this study, our group isolated strains of *Klebsiella pneumoniae* from blood samples of patients with neonatal sepsis. Transmission electron microscopy (TEM) analysis of two of these strains (003 and 021) revealed the presence of a filamentous structure that resembles a type IV pili (T4P). The *pilA* gene (putative pilin) was amplified by PCR, and its expression was determined by RT-PCR.

To understand the role of this T4P pilin of in *Klebsiella pneumoniae,* we aim to construct a *pilA* gene mutation in strain 003 using the lambda red system with the plasmid pKD46. This thermosensitive plasmid expresses the operon of the lambda red system that encodes three components under the control of an inducible promoter: Redγ (gam), Redα (exo) and Redβ (bet). These components allow the homologous recombination between the gene of interest (*pilA*) and a PCR product containing the kanamycin resistance cassette and an homologous region of the target gene (*pilA*). The pKD46 has an ampicillin resistance cassette and *Klebsiella pneumoniae* is intrinsically resistant to this antibiotic, which makes it necessary to adequate this mutagenesis tool for *K. pneumoniae*. For that purpose, the plasmid pKD46 was treated with the *Xmn*I restriction enzyme. On the other hand, the tetracycline resistance cassette was released from the pBSL193 plasmid cut with the *Sma*I enzyme. Both products, (pKD46) and insert (ΩTc^R cassette) were purified, and then the ligated and transformed in *E.coli*. Once the cloning of the ΩTc^R cassette in the vector pKD46 was obtained, the construction pKD46ΩTc^R was checked by restriction and PCR.

Plant growth-promoting bacteria associated to pioneer plants from El Chichón volcano, Chiapas (Mexico)

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The search for microbial inoculants may be an effective biotechnological alternative to nourish crops reducing the negative impact of chemical fertilizers to the soil. We studied the diversity of the Plant Growth Promoting Bacteria (PGPB) associated with the pioneer plants *Cheilanthes aemula* (Pteridaceae) and *Andropogon glomeratus* (Poaceae) that grow into the crater-lake of El Chichón volcano. The bacteria were isolated using selective culture media, and then characterized phenotypically. The taxonomic status of the isolates was determined by phylogenetic analysis of the chromosomal 16S rDNA gene. Plant growth promoting characteristics and in vitro plant inoculation assays were evaluated to know the potential of the strains as PGPB. The isolates grew in high concentrations of salinity, at different pH levels and in moderate concentrations of heavy metals. Based on 16S rDNA gene sequences isolates were classified within the genera *Burkholderia*, *Brevibacillus*, *Chryseobacterium*, *Dyella*, *Enterobacter*, *Exiguobacterium*, *Microbacterium*, *Pandoraea*, *Pantoea*, *Ralstonia*, *Serratia*, *Sinomonas*, *Sphingobium*, Methylobacterium, *Kocuria*, *Rhizobium*, *Bacillus* and *Sphingomonas*. The strains were distinguished by their ability to produce extracellular enzymes, indole compounds (IAA), solubilize phosphate, synthesize siderophores, ACC desaminase and nitrogenase activity, also showed a positive effect on the growth of *Phaseolus vulgaris*. The diversity of bacteria associated to the pioneer plants had multifunctional qualities as PGPB. that may contribute to their adaptation and proliferation at this active extreme volcano.

Prevalence Of *Campylobacter jejuni* and *Campylobacter coli* From Environmental Samples in Culiacan, Sinaloa, Mexico

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Area Ecología y genética. Presentación Cartel.

Campylobacter is a leading foodborne pathogen causing gastroenteritis in humans. Most human *Campylobacter* infections are caused by either *Campylobacter jejuni* or *Campylobacter coli*, Complications such as the Guillain-Barré syndrome can be the result of campylobacteriosis. The main reservoirs of *Campylobacter* are birds due to their high body temperature which provides an optimum growth temperature for these thermotolerant species. Consumption of undercooked or raw poultry meat, unpasteurized milk and dairy products are the primary vehicles of human infections. However, exposure to the environment through rivers, domestic animals, contaminated food and water as well as feces, also represent a source of human infections. Thus, the aim of the present study was to evalute the prevalence of *C. jejuni* and *C. coli* from environmental samples. Thirty environmental samples including surface water and domestic animal feces were analyzed. Water samples were collected using the Moore sampler proposed by Cooley et al., 2015. 5mL of surface water sample and 1g of animal feces were enriched in a sterile cell cultrue flask with a 0.2µm vent cap with antibiotics, and incubated at 42°C with gentle shaking under microaerobic conditions. After incubation of the enrichment broth for 24h, 250µl sample was filtered for 30min in a sterile mixed cellulose ester membrane filter (47mm in diameter and a pore size of 0.65µm), which was placed on the surface of ABA plates. After incubation filters were removed, and plates were incubated for 24 to 48h at 42°C under microaerobic conditions. The recovered isolates with a *Campylobacter*-like colony morphology (pale orange colonies) were inspected visually for a very small, curve (S-shaped) single-cell morphlogy and corkscrew motility by light microscopy. A descriptive analysis was perfomed to determine the prevalence of *C. jejuni* and *C. coli*. A total of 80% (24/30) of the environmental samples were *Campylobacter* presumptive species. From the total isolates recovered 21.3% (16/75) were positives for *Campylobacter*. The multiplex PCR results showed 81.2% (13/16) of the isolates were *Campylobacter jejuni* and 18.7% (3/16) were *Campylobacter coli*. 41.1% (7/17) were taken from surface water and 5.8% (1/17) were taken from domestic animal feces. This is the first study carried out in the Northwestern part of Mexico identifying the presence of *C. jejuni and C. coli* in

Development of SCAR markers by Inter-Simple Sequence Repeat (ISSR) analysis for identification of *Fusarium mexicanum* and *F. pseudocircinatum*.

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Mango malformation (MMD) is one of the main diseases of this crop, with at least five species of *Fusarium* as the causal agents worldwide. In Mexico, *F. mexicanum* and *F. pseudocircinatum* has been reported causing MMD. Recently, it was reported that these two *Fusarium* species cause big-leaf mahogany malformation disease (BLMMD) in the central western region of Mexico. The goal of the present study was to develop SCAR markers derived from ISSR analysis for a rapid identification of *F. mexicanum* and *F. pseudocircinatum*. Genomic DNA of *F. mexicanum, F. pseudocircinatum, F. mangiferae, F. sterilihyphosum* and *F. tupiense,* two species of *F. solani* species complex and two of *F. incarnatum-equiseti* species complex, were amplified with fourteen ISSR primers. The exclusive polymorphic bands for *F. mexicanum* and *F. pseudocircinatum* were cut from the agarose gel, purified and the DNA fragments were ligated into the pGEM-T Easy vector and electroporated in *Escherichia coli*. The cloned fragments in *E. coli* were sequenced and nine and seven pairs of SCAR primers were designed and synthesized for *F. mexicanum* and *F. pseudocircinatum*, respectively. Oligonucleotide universality, specificity and sensitivity were analyzed by PCR. Two primer pairs specifically amplified genomic DNA of *F. mexicanum*, but not from other species that cause MMD, regardless of whether they were isolated from MMD or BLMMD, with a sensitivity range of 1 pg to 250 ng of DNA per reaction. An additional pair of primers amplified all isolates of *F. pseudocircinatum* in a specific manner, with a sensitivity range of 10 pg to 100 ng of DNA per reaction. Identity of pathogenic *Fusarium* species is usually confirmed by sequencing of nuclear and ribosomal genes. The use of specific primers will reduce the time and cost involved in the identification of the causal agents of mango and mahogany malformation in Mexico, and foster the study of the biology of these pathogens.

Plasmid construction for CRISPR genetic edition in a no model fungal phytopathogen *Sclerotium cepivorum* Berk

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CRISPR-Cas9 it has become the most powerful editing tool we have today, its effectiveness has been reflected in the successful editing cases in model and no model organism and offer a different tool for editing in different ways.

In our group we are interested in *Sclerotium cepivorum* Berk a particular fungus that only affects *Allium* plants and that is because only can germinate with diallyl disulfide compounds secreted by these plants and causes a disease called white rot, that have an economical importance. This fungus presents highly persistence because the fungus produces resistance structures known as sclerotia, which remain viable for up to 20 years in the soil and the resistance is attributed to a melanin layer.

In the work group the melanin DHN pathway was determinate by spectrophotometrically assays were Scitalone dehidratase (SDH) is a key enzyme in melanin production in this fungus and can be an excellent target for study the process of melanization and melanin importance in the survival process.

In *Sclerotium cepivorum* Berk stable transformant have not been obtained, so it is desirable probe genetic edition techniques like CRISPR/Cas to understand biological processes through generating mutants. In order to apply the CRISPR technique in our fungi model, we directed CRISPR towards SDH from *S. cepivorum* with the porpoise to generate a physical probe of correct CRISPR/Cas mutation.

For CRISPR edition we decided a variant were a plasmid carrying a promotor and terminator functional have an optimized Cas9 fungal protein and the sgRNA will be inserted with this plasmid in a protoplast transformation.

Up to now, we synthesized the gRNA for *Sclerotium cepivorum* Berk by fusion PCR from the plasmid pFC334. Primers with the SP6 RNA polypeptide recognition sequence have been designed to allow *in vitro* synthesis of the gRNA.

Study of the microbial communities of the swamp de Sisal and the El Palmar State Reserve in the Yucatan Peninsula using Next Generation Sequencing tools.

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Microbial communities are of great importance in coastal areas because they carry out vital functions in biogeochemical cycles such as nitrogen and sulfur. Therefore it is important to know the microbial biodiversity due to the role they play in these ecosystems. Next generation sequencing methods have helped improve the study of microbial communities since there is a greater characterization and knowledge of the organisms present in ecosystems.

In this work, metagenomic studies were carried out in two sites of the Yucatan Peninsula, in the State Reserve of El Palmar and in the swamp of the town of Sisal during the years 2017 and 2018. The sites selected for sampling represent two opposite environmental states. The Sisal swamp represents a state of contamination by anthropogenic activities carried out in the town, while El Palmar represents a conserved state due to its status as a State Reserve. The samples were extracted from the sediment of both sites at 20 cm deep, then they were placed in 50 ml tubes with buffer for preservation until they were taken to the laboratory for DNA extraction and subsequent sequencing using Illumina's MiSeq platform. The reads obtained were analyzed for the identification of the taxonomic groups present in the samples, their relative abundance and diversity index estimation. The analyzes were realized with the Qiime 2 program and using the SILVA database. Statistical analyzes were carried out with the STAMP program.

The phyla *Spirochaetes* and *Proteobacteria* presented a significantly greater abundance in Sisal samples during the years 2017 and 2018, respectively. Both taxonomic groups have pathogenic bacteria among their representatives, such as the *Vibrionaceae* family, which had a relative abundance of 40% in the Sisal samples. Both phyla have been associated with environmental states of contamination by human activities. In the case of the El Palmar samples, the phyla that presented a significant abundance were *Gemmatimonadetes, Chloroflexi* and *Actinobacteria,* which have been associated to places whose environmental states are considered conserved.

Through the results obtained in the present work, we have been able to identify in a preliminary way taxonomic groups of bacteria that could be consider associated to environmental states of contamination or conservation, which can be used as biological markers of the health states of ecosystems.

Shift of the species in a microalgae community exposed to Cu and Zn

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Abstract

Biodiversity is an inherent factor to biosphere and refers to the different organisms co-existing in an ecosystem, which, in case of biotic or abiotic stress, would be restored itself because of such biodiversity. Ecotoxicology is focus on the study of biotic and abiotic factors that negatively affect distribution and abundancy of organisms. After exposing microalgae to chronic concentration of Cu and Zn, García-Meza *et al*. (2005) proposed that differential survival among individual species in communities (biofilms) is due to specific metabolic activity. Microalgae (as Cyanobacteria and the Eukarya Chlorophyte, Rhodophyte and Bacillariophyte) are phototrophs that have been widely studied in ecotoxicology due to its role as primary producers in photic zones found in aquatic ecosystems. A biofilm is defined as a complex and highly organized community (prokaryotes and eukaryotes) that coexist space-temporarily, immersed in an extracellular matrix constituted by extracellular polymeric substances (EPS). In this study, we present results of the effect of Cu and Zn biofilm structure, specific composition and photosynthetic efficiency in microalgal biofilms. Firstly, we isolated and cultured microalgae biofilm followed by their identification; after, microalgae were exposed per 8 days to 100 µM Cu, 200 µM Zn or 100 µM Cu and 200 µM Zn. All experiments were performed by triplicates. Analyses were performed after 8 days of culture. Community of microalgae mainly composed by *Scenedesmus* spp., showed and overall response to Cu and Zn: No statistically significant toxicity effect of each metal evaluated was detected in the photosynthetic efficiency after 5-day trials, while the specific composition of the communities changed because the decrease of sensitive ones.

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"Study of the interaction between RNase II and RNase PH with RNase E of *Escherichia coli in vivo*"

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Introduction: RNA degradosome is a multiproteic complex which is responsible for mRNA maturation in bacteria, it is formed by several enzymes some of which are: the helicase RhlB; an enolase; PNPase and RNase E, this last component is divided in two portions, the amino terminal end which is the catalytic domain effecting a 3' endoribonucleolytic activity and the carboxyl terminal end which posses the binding site por the three first mentioned enzymes, among others. On the other hand, there have been reported the existence of different RNases in the same bacteria and also among other bacteria and in some cases RNase E is not present at all, in this case it is replaced by its analogues like RNase Y or J. RNase PH and II are also present in *Escherichia coli* but their function is more related to rRNA and tRNA maturation or degradation depending on the environmental conditions and developmental stage, however, several studies *in vitro* have demonstrated an interaction between both RNases and RNase E, so we asked if this phenomena occurs *in vivo* and under which conditions this happens, to address this we develop the following methodologic process. **Methods:** we created several *E. coli* strains containing a cyan fluorescent protein (CFP) binded to the RNase E and phi-yellow fluorescent protein (YFP) binded to RNase II or RNase PH, this was done applying the lambda phage homologous recombination system. These strains were summited to normal growth conditions and under stress using a hydrogen peroxide survival curve and M9 medium, this interaction under these conditions was determined using Fröster Resonance Energy Transfer (FRET) technique. **Results:** Both RNase II and RNase PH have been demonstrated to realize an interaction with RNase E *in vivo*, this interaction is higher under stress conditions propitiated by hydrogen peroxide and a minimum medium. It is expected that in the future we can determined specific mRNAs affected by these RNases in order to determine a connection with stress related mRNAs and stress conditions. **Conclusion:** RNase E can interact with RNase PH and RNase II under normal conditions, this interaction increase under stress conditions and can possibly affect the mRNA turnover of several transcripts related with these conditions. **Acknowledgements:** This work was financed by CINVESTAV and CONACyT-255374 CB-2015-01. CONACYT master scholarship 919105 to ESR

Characterization of antagonistic bacteria of phytopathogenic fungi obtained from a strawberry orchard with organic management

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Bacteria are part of the large number of microorganisms that exist as control agents for fungal and bacterial diseases. In addition, some of them can favor the growth and development of plants. These bacteria are known as plant growth promoting bacteria (PGPB) and play a major role in crops, reduce the use of chemical fertilizers, increase yields, shorten cycles and, consequently, reduce environmental pollution. The objective of this work was to isolate and characterize bacteria from samples of soil and strawberry plants (*Fragaria* spp.) collected from an orchard with organic management, located in the ejido La Calera, Irapuato Guanajuato. Based on this, it is intended to take advantage of the bacteria characterized as potential biological control agents for various crops of economic importance. To isolate the bacteria serial dilutions of soil and root were performed to obtain axenic cultures resulting in 13 bacteria. Antagonism tests were carried out against six phytopathogenic fungi that attack several economically important crops such as *Alternaria alternata, Botrytis cinerea, Colletrotrichum gleosporoides, Fusarium oxysporum, Rhizoctonia, sola*ni. and *Sclerotium* sp., inhibition of fungal growth was observed, in addition, to delay the formation of sclerotia in the last two fungi mentioned. These bacteria did not inhibit the growth of the beneficial fungus *Trichoderma* sp. The biochemical characterization indicated that some of the bacteria produce secondary metabolites such as organic acids for the solubilization of phosphates and the secretion of siderophores, these promote plant growth because they release iron from organic compounds in forms assimilable for plants, which it improves its growth and productivity, although the indole production test was negative. Their identification was made by analysis of their DNA sequence of the 16S region. Among the identified genera highlight *Variovorax* spp. and *Pseudomonas* spp. which are used as biological control agents and promoters of plant growth. Characterized bacteria have a high potential for the agricultural sector, which can help reduce the application of pesticides and chemical fertilizers.

Bacteriological quality in fresh fish from different aquatic systems from Tabasco State.

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Tabasco is located in the southeast of Mexico, limited to the north with the Gulf of Mexico, to the east with Campeche, to the southeast with Guatemala, to the south with Chiapas and to the west with Veracruz State. The state has a coast of 191 km between the River mouths of Tonalá and San Pedro, besides it has 29,800 hectares of stereos, lake, lagoons and so many rivers and streams, which have great possibilities for the fishing exploitation both in salt water and fresh water. For the determination of bacteriological quality of fish and water, the total coliforms (TC) and fecal coliforms (FC) were used as indicators of contamination. Samples were collected in nine sites during the dry (S) season and rain (LI) 2016 to 2017. The water samples were collected in sterile jars and fish in sterile bags and both were preserved at 4°C. Fish were disinfected with alcohol at 70% in the gills, Intestine and anus areas; analyzing only the dorsal muscle according to NOM-210-SSA1-2014. The TC and FC was conducted by the Most Probable Number method (MPN) according to the NOM-242-SSA1-2009 for organisms and for water the NMX-AA-042-SCFI-2015. In rain 2016 was observed that the fish species with the most concentration of FC were *Oreochromis niloticus* (Rosario y Cangrejera lakes), *Dorosama petenense* (Limones lake), *Eugerres plumieri* (Machona lake); and in dry of the same year *Cichlosoma urophtalmum* (Yucateco lake) and *Cathorops aguadulce* (Tonalá river). In 2017, it was in drought *Theraps persei* (Cangrejera lake). These species exceeded the 400 NMP/g de FC which was established by NOM-242-SSA1, 2009, as maximum limit fresh fish. In the water test, the highest value of FC were found in Limones lake en S 2016 and LI 2016 and 2017, Machona lake in LI 2016 an S 2017, San Felipe y Zanapa rivers S 2016 and LI 2017; Tonalá river drought and rain seasons 2016 and 2017, Carmen lake LI 2016 until LI 2017, Yucateco and Cangrejera in LI 2016 and 2017. All these water bodies exceeded the 200 MPN/100 ml of FC that is established by ecological criteria of water quality, 1989 (CE-CCA-001/89) as allowable limit for the protection of aquatic life. According to these data, we can conclude that in 22 studied fish species, six of them obtained superior values to the Mexican normativity of Coliform bacteria. So, it is concluded that the bacteriological quality of fish studied is influenced by the environmental quality of the water bodies where these organisms live, constituting a risk for human consumption.

Pathogenic bacteria in tilapia (*Oreochromis niloticus*) cultivation

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Aquaculture is a multidisciplinary activity and with great importance as productive systems which uses the knowledge of biology, engineering and ecology to help to solve health problems according to the type of organism which is cultivated, such as the tilapia (*Oreochromis niloticus*), commercial species with great demands worldwide. The abusive health practices in installations for the cultivation of this type of organisms, affect the dissemination of bacterial diseases in the organisms cultivated in farms, causing important economic loss. The search of pathogenic bacteria en *O. niloticus* was made in 15 young individuals, besides the water of the cultivation systems where the number of bacteria was quantified Total Coliforms (TC) and Fecal Coliforms (FC). Fish skin and organs were analyzed such as spleen, kidney and liver. The taking sample in the skin was done by swept with a sterile hyssop in all the fish body, inoculating in Tripticasein Soy Broth (TSB). The organs were extracted and put separately. In 2 ml of salt water at 0.85% to its maceration. From this suspension 0.3 ml were transferred to TSB. All the test tubes were incubated a 35°C± 2°C for 24 h. This time passed, they were inoculated in the media of Mac Conkey (MC), eosin methylene blue (EMB), cetrimide (C), and Tripticasein Soy (TS). The former units of colonies got characterized morphologically and were identified through the system of identification of *Enterobactereaceae* and other Gram- negative rods, api 20E y 20NE, completed with conventional biochemical (Rodríguez *et al.*, 2001, Morales *et al*., 2004, Soriano *et al*., 2010 y Rivera A. H, 2014). The determination de TC and FC was made through the most probable number method (MPN), NMX-AA-042-SCFI-2015. The bacteria identified in skin and organs were *Aeromonas spp* (26%); *Pseudomonas flourescens* (13%); *Pseudomonas aeruginosa* (9%)*; Klebsiella pneumoniae ssp. Pneumoniae*, (9%); *Enterobacter cloacae* (9%); *Pseudomonas putida* (7%)*; Bacillus spp* (7%)*; Citrobacter spp* (7%)*; Enterobacter spp* (7%); *Aeromonas sobria* (4%); *Pseudomonas stutzeri* (2%) y *Morganella spp* (2%). The number of TC in test of water varied between 90 to 2,400 MPN/100 ml of TC and FC from 40 to 430 NMP/100 ml. In fish, 12 bacterial species were isolated; being *Aeromonas spp* and *Pseudomonas spp* the most abundant and its presence is related with a bad handle of organisms and its consequent stress in the ponds.

Ribosome profiling analysis of a Pth (Ts) *E. coli* mutant strain

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Peptidyl-tRNA hydrolase (Pth) is an essential protein in *E. coli* capable of releasing tRNA from peptidyl-tRNA by cleaving the ester bond in-between. This process allows the cell to maintain the amino acylable-tRNA pool for new rounds of translation. Pth (Ts) *E. coli* mutant strain presents a temperature sensitive phenotype due to a point mutation in the gene *pth*. An increase in temperature causes Pth concentration to fall, therefore accumulation of peptidyl-tRNAs in the cytoplasm, cease of protein synthesis, and cell death.
Use of ribosome profiling technique [1] allows us to get a closer view of the mRNA sequences during translation and how Pth deficit affects protein synthesis. Pth (Ts) *E. coli* mutant strain was grown at 30°C then heat-shocked at 43°C. Samples were taken at different times after the heat-shock (HS) and footprints were isolated from monosomes and polysomes. Library generation of the footprints allowed the massive sequencing of the data, which was later analyzed bioinformatically.
First results indicate an increase of sequences correlated with time after the HS in all samples. Interestingly, a distribution analysis of fragments by size (nt) revealed an abundance of fragments of 35 nt in size in all the samples. This suggests that fragments were true footprints. Along this, genes that are classified as predicted highly expressed (PHX) [2] are visible in all our samples with an increase in sequences as temperature and time increased. A variety of results are still pending, like TE (Translation Efficiency), site P codons of stalled ribosomes during translation, etc.

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Cloning, purification and production of polyclonal antibodies for the detection of Bap adhesin in enterohemorrhagic *Escherichia coli*

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Enterohemorrhagic *E. coli* (EHEC) O157:H7 is a pathogen that causes diarrhea, hemorrhagic colitis and hemolytic uremic syndrome in humans (HUS). This bacterium has the ability to adhere and to colonize the human colon using a large repertoire of fimbrial and non-fimbrial adhesins. EHEC induces the formation of attaching and effacing lesions (A/E) on the mucosal epithelium that destroys the microvilli and reorganizes the cytoskeleton of the enterocytes, generating the formation of a pedestal-like structure cupping individual bacteria. Finally, EHEC produces Shiga toxin (Stx) which is responsible for causing HUS. An important attribute of many pathogenic bacteria is their ability to adhere and form biofilms on biological and non-biological surfaces, this is due in part to a multitude of surface proteins that mediate interactions between bacteria and surfaces. In this sense, *S. aureus* is an important pathogen because produces biofilms on tissues and medical devices, increasing its virulence and resistance to antimicrobial treatments. The ability to form biofilms is due in part to the production of a high-molecular weight protein called Bap (Biofilm-Associated protein) that determines the multiadhesive ability in the strains that express it. In the genome of the EHEC Sakai and EDL933 strains, as well as in other pathogenic *E. coli*, is present an *orf* (Z0615) with sequence similarities to *bap*, however is not known if it participates in the adherence and pathogenesis of *E. coli.* The aim of this work was the generation of polyclonal antibodies to detect Bap expression in EHEC and to determine its importance in the development of the disease. For this, a fragment of the *orfZ0615* was cloned into pBAD-myc-his A, the recombinant protein Bap (4696-4826)-6XH was purified by affinity chromatography, and inoculated in New Zealand rabbits. The antibodies were evaluated by western blotting to determinate Bap expression on total protein extracts of EHEC grown under conditions of induction (DMEM medium/37°C) and repression of virulence genes (medium LB/37°C). The results obtained indicate that Bap is expressed when EHEC is cultured in DMEM/37°C while in LB medium it was not detected. Such data support the importance of Bap in the pathogenicity mechanism of EHEC. In addition, antibodies generated for this work serve as a tool for the detection of this protein in other pathogenic *E. coli* in order to understand the role of Bap in the mechanism of biofilm formation, adherence and colonization.

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IDENTIFICATION OF GENES INVOLVED IN MEMBRANE LIPID HYDROXYLATION IN Burkholderia cenocepacia J2315.

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Burkholderia cenocepacia J2315 is a gram-negative, non-sporulating, aerobic and non-fermenting bacillus recognized as an opportunistic pathogen for plants and humans. It has a special predilection for the lung in patients with Cystic Fibrosis (CF) or with chronic granulomatous disease. Conventional antibiotics are no longer as effective against this bacterium (Yasmina Araque, et al, 2008). An interesting characteristic of this strain is its polar membrane composition, among them phosphatidylethanolamine (PE) and ornithine lipids (OLs), as well as the hydroxylated derivatives of these, mainly; 2-OH-OLs (OLs hydroxylated in the C2 of the esterified acyl group), NL1 (OLs hydroxylated in the C2 of the amidified acyl group), NL2 (OLs hydroxylated in the C2 of both acyl groups) and 2-OH-PE (PE hydroxylated in the C2 of the acyl esterified in position *sn*-2). PE is a membrane lipid present in eukaryotic and prokaryotic membranes, unlike OLs that are limited to eubacteria. The abundance of these hydroxylated lipid species is increased in stress situations, implying that the strain probably uses them as part of a survival strategy. Specific membrane lipids also have been reported to have an important role in pathogenicity. However, the functions of these modified lipids in *Burkholderia cenocepacia* J2315 are still not known exactly and some of the genes responsible for their formation are unknown. Therefore, an aim of this project is to identify these unknown genes. An heterologous expression screening was carried out transferring a bank of cosmids of this strain to *Bordetella* sp, a bacteria forming OLs, but not able to hydroxylate them. *Bordetella* transconjugants presenting the hydroxylated ornithine lipid were identified, the cosmids isolated and subsequently sequenced. Within the cosmid sequence, three candidate genes were found: *BCAL0884, BCAL0870, BCAL2208*. These will be studied in more detail to identify the gene responsible for the hydroxylation of OL. Furthermore, a chemical mutant library was constructed and two mutants presenting a decrease in the production of PE-OH lipid were isolated. The genomes of these mutants were sequenced to identify nucleotide polymorphisms in comparison to the wildtype.

Studies of microbial communities associated to the root of *Typha spp.* exposed to a mixture of diclofenac and naproxen in a horizontal wetland of subsurface Flow

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Non-steroidal anti-inflammatories are pharmaceuticals of high consumption worldwide, they have been detected in wastewater, for example diclofenac and naproxen have been quantified at concentrations of 54 and 1.5 ng / L respectively. Treatment methods to remove these contaminants are inefficient, for that reason alternatives of low cost and greater efficiency are necessary. Constructed wetlands are a promising alternative for the degradation of pollutants, where plants and associated microbial communities play a key role in degradation of these compounds (Ibekwe et al., 2007; Ligi et al., 2014). However, studies are still limited, so detailed knowledge about microbial communities are required. In this project the objective was to identify bacterial consortium associated to the root of Typha spp., which growth in contact with a mixture of diclofenac and naproxen in a constructed wetland. First, wetland was installed, using gravel as a support material and *Typha spp* like vegetation, later a synthetic solution of diclofenac and naproxen was added to the system in continuous flow (3:5 mg/L). After 100 days of operation, plants (n=9) were collected, and roots were separated. 1 g of root was homogenized with sterile distilled water and serial dilutions were carried out, 10 µL of each dilution were plated on minimum agar M9 supplemented with diclofenac and naproxen as a carbon source, incubated for 48 h and 28 °C. Colonies were count and classified by morphotype, molecular identification, tolerance tests and biochemical characterization of the bacterial isolates were performed.

Analysis of the effluent flow show that diclofenac is removed by 75 %, and naproxen by 78 %. Moreover, data show that diclofenac and naproxen did not affect development of Typha spp. Regarding to bacterial isolates, 208 colonies were identified and grouped on 14 morphotypes, which tolerate 30 mg/L of diclofenac and 50 mg/L of naproxen. The biochemical and molecular characterization of these isolates can give information about their possible role in pharmaceutical degradation

Safety of *Pseudomonas* spp. and *Bacillus* spp. strains that inhibit the growth of *Fusarium* spp. and promote maize growth.

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Bioinoculants are one of the sustainable and ecological strategies to increase the agricultural production worldwide; in this way, in addition to evaluate the promotion of plant growth and inhibition of plant pathogens, it is necessary to carry out tests on the safety of these microorganisms. *Pseudomonas* sp. CM-CNRG 50, *Pseudomonas* sp. CM-CNRG 57, *Bacillus* sp. CM-CNRG 435 and *Bacillus* sp. CM-CNRG 436, are potential strains for the development of bioinoculants for their ability to promote maize plant growth and/or inhibit the growth of phytopathogenic strains of the species complexes of *Fusarium fujikuroi*, *F. oxysporum*, *F. incarnatum-equiseti* and *F. sambucinum*, with a proportion of 9-75%; however, the safety of the four strains has not yet been evaluated. For these reasons, the objective of the present work was to evaluate the four bacterial strains with the basic microbiological tests of DNAase, hydrolysis of gelatin, lipases, amylases, proteases, cellulases and coagulase, following standard methodologies. As well as their effect on maize plants of 7 days of growth, inoculated with 5 μL of a suspension at 10^6 CFU, visual damage, height, root length, dry weight and chlorophyll concentration of the plant were evaluated 5 days after inoculation, non-inoculated plants were considered as control treatment. The negative results obtained in all the microbiological tests, and non-significant difference between inoculated plant evaluations and control treatment suggest that the four bacterial strains are safety to humans, animals and plants, data have also to be confirmed with toxicity tests, but this was the first approach to consider the development of a bioproduct from the four bacterial strains evaluated for biological control and promotion of growth in maize crop.

PHYSIOLOGY AND METABOLISM

XLI National Meeting of the Mexican Association of Microbiology (AMM)
VI Meeting of Biochemistry and Molecular Biology of Bacteria (BBMB)

Oaxaca, Oax. October 27 - 31, 2019.

Functional analysis of the aminoacid sequence involved in localization of the hybrid protein CdgB of *Azospirillum brazilense* Sp 245

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Over the last decades, a second messenger called (3´5´) guanosine-monophosphate-cyclic (di-GMPc) has been in the focus of researchers because this molecule is key in signaling systems, it regulates different bacterial process such as mobility, virulence, exopolysaccharide production, biofilm formation and cellular cycle. Enzymes with diguanylate cyclase carry out the synthesis of this second messenger while phosphodiesterase proteins accomplish its degradation.
It has been showed that increasing or decreasing the intracellular levels of this second messenger carries out regulation of bacterial activities and it is believed that for a fast and efficient communication, the existence of bacterial microcompartments (BMC) is important. These microcompartments are organelles composed entirely of proteins that promote specific metabolic processes by encapsulating and collocating enzymes with their substrates and cofactors, so that cell concentrations of di-GMPc can be regulated by enzymes located at specific sites.
Genome analysis of *Azospirilum brasilense* Sp245 has demonstrated the existence of 35 proteins involved in the metabolism of this second messenger. Here in we analyze a hybrid protein, which was named CdgB, that posses a transmembrane censor domain called MHYT and GGDEF-EAL domains in tandem. Due to recent data of MHYT domains, the objective of this study was to determine and analyze the signal peptide involved in translocation of CdgB hybrid protein in the cytoplasmic membrane of *A. brasilense* Sp245.
Previous work in our laboratory showed a polar location of CdgB by using a translational fusion of *cdgB* gene with the gene that codifies for the enhanced green protein gene fluorescent (eGFP). Through bioinformatics, the presence of putative signal peptide.
In order to determine the functionality of this putative signal peptide, two different translational fusions using the eGFP gene were constructed: in the first one, we removed a sequence composed of 16 aminoacids in the amino terminal region of CdgB protein, while a complete deletion of MHYT domain was performed in the second one.
Using confocal microscopy it was determined that the mutant without MHYT domain was delocalized in the cytoplasm, while the mutant lacking the putative leader peptide did not show a marked delocalization. These results were corroborated by Western Blot using membrane and cytoplasmic samples of both mutants.

An exploration of tRNA-utilizing enzymes using EvoMining reveals novel antibiotic biosynthetic gene clusters in *Actinobacteria*

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Abstract:

Natural products (NPs) are an important source of bioactive agents for drug development. Research on bacterial NPs has been reinvigorated in the last decade due to the popularization of genomics and public concern on the widespread multi-antibiotic-resistant pathogens. The use of NP genome mining approaches has made clear that most organisms have a greater capability for NP synthesis than what can be usually detected under standard laboratory conditions due to the cryptic nature of genes encoding pathways. Historically, tRNA-utilizing enzymes (tRUEs) were considered exclusive to ribosome-dependent protein synthesis and critical primary metabolism processes, such as targeted protein degradation and peptidoglycan synthesis. However, recently, it has been reported that tRUEs paralogs have been recruited by specialized metabolism. Here we show that, after a non-conventional genome mining approach called EvoMining, tRUE expansions related to conserved NP biosynthetic pathways in many streptomycetes, including the widely studied laboratory strains *Streptomyces lividans* 66 and *Streptomyces coelicolor* A3(2), can be identified. These two strains were found to encode a cryptic BGC containing an alanyl-phosphatidylglycerol synthetase tRUE with similarity to the MprF family. Expression induction of the BGC in optimized N-limited conditions allowed the identification of a new metabolite with antibiotic activity. According to EvoMining predictions, NP clusters associated with tRUEs may be more abundant than previously appreciated, extending the exploration of NP chemical space beyond well-known BGC classes.

Ureolytic bacteria as Geological Agents: Their role in metal carbonates formation in mine tailings

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The formation of mineral carbonates by microorganisms occurs in different ecological niches such as microbialites, stromatolites and halo-alkaline sediments. Due to the presence of charged chemical groups on cell surface and diverse metabolic capabilities of bacteria, they are able to interact intimately with metal ions present in their environment inducing the formation of minerals. In the present study the ability of ureolytic bacteria isolated from Mg-Ca-rich alkaline mine tailings to produce metal carbonates has been investigated under *in vitro* and *in situ* conditions. Based on the 16S rDNA sequence analysis, these isolates were identified as belonging to the genera *Sporosarcina, Bacillus, Oceanobacillus* and *Staphylococcus*. *In vitro* assays show that they able to form $CaCO_3$ as calcite, vaterite and aragonite in high concentrations of Ca^{2+}. However, when Ca^{2+} was replaced by other metal ions, they precipitate carbonates of Pb^{2+} (Cerussite), Cd^{2+} (otavite), Mg^{2+} (hydromagnesite), Mn^{2+} (rhodochrosite), Sr^{2+} (Strontianite), Ba^{2+} (whiterite), Zn^{2+} (hydrocycite) and Mg^{2+}-Ca^{2+} (magnesium calcite and dolomite), confirmed using a combination of Scanning Electron Microscopy (SEM) coupled to an Energy Dispersive Spectroscopy (EDS) devise and X-Ray Diffraction (XRD) techniques. Some of these minerals are present in the mine tailings from which they were isolated. Additionally, when these strains were inoculated in mine tailings, they produced calcite, vaterite, smithsonite and cerussite *in situ*, which led to formation of rigid structures like a biocement with a high concentration of carbonates. These results show that ureolytic bacteria act as a nucleation site for formation of metal carbonates with diverse microstructure and chemical composition, so that many minerals present in mine tailings could be from biogenic origin.

Changes observed in insulin and IL-15 levels in patients with pulmonary tuberculosis with or without type 2 diabetes mellitus.

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Tuberculosis (TB) is an infectious disease, which is a global health problem. Its incidence, however, may vary if a risk factor such as diabetes mellitus (DM) is present. Some evidences suggest that the pro-inflammatory state present in the diabetic patient could favor the development or reactivation of TB. In this context, cytokines such as interleukin-15 (IL-15) or IFN-gamma (IFN-γ) seem to have a relevant role, since intervening to infection control, but also because they can modify the action of insulin, altering the levels of glucose in the plasma. Therefore, the objective of this work was to determine insulin levels and determine their probable association with IL-15 levels and IFN-gamma, in patients with pulmonary TB (PTB) with or without type 2 DM (DM2). This was an analytical cross-sectional study, where insulin and levels IL-15, IFN-gamma, as well other cytokines involved with the immune response in patients with tuberculosis (IL-4, IL-10, and TNF-alpha) were determined. Were evaluated 4 groups: 1) patients with PTB; 2) patients with TBP-DM2; 3) household contacts with DM2 but without PTB (H-DM2); and, 4) healthy household contacts (H-C) (n = 20 in each group). The medians and the interquartile ranges were determined, and the Shapiro-Wilk test was used to determine the normal results. To identify differences between groups, the Kruskal-Wallis test was used, a $P < 0.05$ was considered statistically significant. Our results did not show differences in the levels of IL-4, IL-10 or TNF-alpha in any of groups. In comparison, insulin levels were different and showed a positive association with IL-15 in the TBP and CS-DM2 groups ($P < 0.05$). In contrast, this association was lower in the TBP-DM2 and CS groups ($P > 0.05$). Regarding IFN-gamma levels, these did not show a clear association with insulin levels, but significant differences were found in insulin levels between the TBP vs. TBP-DM2 groups and between TBP-DM2 and CS-DM2 vs CS ($P < 0.05$). In conclusions, our results indicate a positive association between insulin levels and IL-15 only in PTB or H-DM2 patients. In comparison, we did not find a similar association between IFN-gamma with insulin levels. The foregoing suggests that similar studies are necessary to improve our knowledge about what is happening in the patient with TBP and DM2.

Presentación: Cartel
Área: Interacción patógeno-hospedero

FUNCTIONAL IDENTIFICATION OF TWO OPERON RELATED TO THE USE OF SPECIFIC CARBOHYDRATES OF TISSUES OF ANIMAL ORIGIN, IN *Avibacterium paragallinarum*

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Avibacterium paragallinarum (AVPG) is a Gram-negative bacterium that belongs to Pasteurellaceae family, pathogenic for birds mainly in poultry. The bacterium produces a respiratory disease known as infectious coryza. This disease is detected by typical symptoms in the animals such as sneezing, swelling of the head, anorexia and diarrhea. In survivors there are weight loss and diminished egg production, causing important economic losses. Several virulence factors have been identified in AVPG, some of them have been deduced through experimental research and others through the analysis of genomic sequences. The result from these analyzes has contributed to deduce the probable existence of two enzymes termed Chondroitin lyase I and II (*chly*I, *chly*II). Looking for *chly* in GenBank database for genomic. Primers to amplify *chly* sequences helped to confirm the full gene incidence. The AVPG bacterial growth with chondroitine supplement showed better development measured by changes in optical density and colony unit count. Bioinformatics and experimental evidences suggest that Chondroitin lyases are virulence factors to promote bacterial development in the host, organs colonization and tissue destruction and contribute to evade immune response. Using the best mexican AVPG characterized strain at the genomic level, AVPG 2015, the two *chly* operons were established. The *chly* coding sequences were amplified and two *chly* constructs (pBV-SCTa and pBV-SCTb) were made with pBluescript II KS(-) vector in *E. coli* in strain M15. This bacterial heterologous system developed to analyze chondroitin lyase enzymes production should be helpful to evaluate the contribution of each enzyme to modify the infectious coryza severity and to evaluate immunogenicity response mediated by Chly enzymes in animal models.

The cytotoxicity of *Thymus vulgaris* in eukaryotic cells

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Introduction:
At present there have been different studies that highlight the antibacterial activity of different substances of plant origin. For example, the strong inhibitory effect of the essential oil of *T. vulgaris* has been reported, acting on *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, *Proteus vulgaris*, *Vibrio* sp, *Listeria monocytogenes*. The essential oil of *T. vulgaris* is mainly composed of thymol, terpinen, p-cimeno, carvacrol and linalool. However, little is known about the cytotoxicity of *T. vulgaris* essential oil in eukaryotic cells. In the present study, the cytotoxicity of *T. vulgaris* in eukaryotic cells was studied.

Objective:
To determinate the cytotoxicity of *T. vulgaris* in eukaryotic cells.

Methodology:
The cytotoxic activity of *T. vulgaris* essential oil was determinated using an erythrocyte suspension and adding different concentrations of essential oil. The samples were incubated 10 min at room temperature and then an aliquot was taken and a trypan blue staining was performed. The effect of *T. vulgaris* was also tested in *Candida albicans* and *Saccharomyces cerevisiae*, under the previous test conditions.

Results:
The results showed that *T. vulgaris* essential oil produced an alteration in the morphology of erythrocyte cells and a decrease in the number of them. A similar effect occurred in cells of *S. cerevisiae.* The *C. albicans* cells showed more resistance to essential oil of *T. vulgaris*. In general, eukaryotic cells used in these assays supported higher concentrations of *T. vulgaris* compared to results obtained in other assays using bacteria (previous studies).

Conclusion:
T. vulgaris showed a cytotoxic effect on the eukaryotic cells tested, measured by changes in morphology and cell viability.

Mutation and phenotypic study of the gene AMK58_RS02950 that encodes a proposed hybrid protein (GGDEF-EAL) of *Azospirillum brasilense* Sp7.

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Agriculture's area has been planned to use sustainable alternatives to crops development and growth, one of these is the use of some bacteria, such as those that belong to the *Azospirillum*'s genus, as bio-fertilizers, in which the specie *Azospirillum brasilense* is one of the most studied. *A. brasilense* is an alphaproteobacteria that has been described as a PGPR (**P**lant **G**rowth **P**romoting **R**hizobacteria), is a nitrogen fixing, Gram Negative bacterium, which is able to form cysts. This is a morphological transition that the bacterium differentiates in response to environmental conditions, contributing to shelter in soil and protection to survive under harsh conditions. It was described that one signal for control the encystment involves the production of the second messenger the c-di-GMP. The production and cellular levels of c-di-GMP are controlled by enzymes synthesizing it, and named diguanylate cyclases (DGCs, with this activity provided by their GGDEF domains) and degraded by specific phosphodiesterases (PDEs, with either EAL or HD-GYP domains). These signaling enzymes usually exhibit a modular domain architecture and controlling the levels of c-di-GMP, those levels are important for the bacteria metabolic differentiation.
In 2018 Malinich and Bauer described the transcriptome analysis of *A. brasilense* Sp7 in vegetative and cyst states, indicating that three diguanylate cyclases were differentially regulated. One of them is the presumed diguanylate cyclase encoded for the gene AMK58_RS02950. The bioinformatic analysis from the translational product of this gene shown that the predicted protein contains 794 amino acid residues with two transmembranal domains (TMD) including a sensor Cache-2 domain, and the HAMP, PAS, and DGC-PDE domains, figure 1.

To evaluate the hybrid putative function, we will carry out an insertion mutation with a Kanamycin cassette and we will evaluate the mutant with the wild type strain during the cysts formation. This study will provide a novel understanding of cysts as a non-replicative but still active morphotype of *A. brasilense* and its role in colonization of their host-plants.

Reference

Malinich EA, Bauer CE. 2018. Transcriptome analysis of *Azospirillum brasilense* vegetative and cyst states reveals large-scale alterations in metabolic and replicative gene expression Microb Genom. 2018;4(8). doi: 10.1099/mgen.0.000200.

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Indole-3-acetic acid biosynthesis by the bacterium *Azospirillum brasilense* cultured under a biogas atmosphere allows its beneficial association with microalgae

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Azospirillum spp. are the most studied plant growth promoting bacteria (PGPB) due to their capacity to positively influence the physiology and improving the growth, development and productivity of plants and microalgae. Phytohormone production as cytokinins, gibberellins, Jasmonic acid, brassinosteroids, and auxins is considered the major mechanism involved in microalga and plant development. Indole-3-acetic acid (IAA) has the highest activity among auxins and plays the greatest role among the phytohormones produced by this bacterium. Nonetheless, IAA biosynthesis by *Azospirillum* is negatively or positively regulated by environmental factors and biotic or abiotic stress. Currently, knowledge about positive incidence of *Azospirillum* on agricultural applications is continuously expanding. Nevertheless, the use of this bacterium is an emerging field in environmental and ecological topics for mitigating environmental problems or improving biotechnological processes based on microalgae, such as wastewater bioremediation and CO_2 fixation. Today, microalgae represent an environmentally sustainable biotechnology strategy to capture CO_2 from biogas – a gaseous effluent from the anaerobic digestion of organic waste, which is considered an important energy source since it is composed mainly of methane (CH_4; 55–75%) and CO_2 (20–35%) – and simultaneously performs CH_4 upgrading because CO_2 content reduces the calorific value of CH_4 preventing meeting the specifications of a fuel gas. Nevertheless, high CO_2 and CH_4 concentration from biogas can be detrimental for several microalgae and bacteria. Therefore, this study assessed the effect of biogas on IAA biosynthesis by *Azospirillum brasilense*, as well as the impact of this bacterium during growth and CO_2 fixation from biogas by *Chlorella vulgaris* and *Scenedesmus obliquus*. IAA production by *A. brasilense* was quantified by HPLC, whilst the expression of the *ipdC* gene was determintated by RNA isolation, cDNA synthesis and quantitative Real Time PCR. The CO_2 fixation by microalgae was analyzed by gas chromatography. The results demonstrated that *A. brasilense* showed better growth capacity and biomass production cultured under biogas with respect to control (air); IAA biosynthesis was positively regulated in this bacterium mainly induced by the acidic pH and anaerobic conditions produced by the biogas atmosphere; although the *ipdC* gene level expression was low under the stressful condition generated by biogas. Similarly, this bacterium was able to establish its beneficial association co-immobilized with *C. vulgaris* and *S. obliquus* inducing higher cell density and CO_2 fixation under biogas. Overall, these findings broaden research in the field of *Azospirillum*–microalga interactions and incidence of *Azospirillum* in environmental and ecological topics besides supporting the uses of PGPB to enhance biotechnological strategies for biogas upgrading.

iorA gene is involved in IAA biosynthesis in *A. brasilense* Sp7

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SUMMARY

Indole-3-acetic acid (IAA) is a phytohormone that induces positive phototropism of the plant, development of secondary roots and root hairs, yield increment, among other responses. *Azospirillum brasilense* is an alpha-proteobacteria that synthetizes IAA, 90% is synthesized by the indole pyruvate decarboxylase pathway (IPyA), through the following reactions: tryptophan → indole-3-pyruvate→ indole-3-acetaldehyde→indole-3-acetic acid. Remaining 10% can be synthesized by other tryptophan-dependent pathways, but the specific genes that participates in the residual IAA production, have not been identified yet.

Previous researches proposed that indole pyruvate ferredoxin oxidoreductase gene (*ior* or *iorA*) could participate in IAA biosynthesis in rhizobacterias as *Rhizobium tropici* and *Bradyrhizobium japonicum*, converting indole-3-pyruvate to indole-3-coenzime A (an intermediary of IAA biosynthesis, in bacterias lacking IPyA genes). A transcriptome analysis of *A. brasilense* Sp245 revealed a broad transcriptional response to the auxin IAA, furthermore, an *iorA* gene increased its transcription about 17 times in a *ipdC* mutant when IAA was added to the growth medium.

We performed bioinformatic analyzes searching domains in the protein sequence of *iorA*, and we obtained three-dimensional models obtained by homology. These models have structural similitudes to other oxidoreductases mainly with the pyruvate ferredoxin oxidoreductase from *Desulfovibrio africanus*, this allowed us to infer the functionality of each domain and the possible enzymatic function of putative iorA protein coded by *iorA*.

Starting from a wild type strain of *A. brasilense* Sp7, we made a mutant of *iorA* gene, by inserting the mCherry protein gene and a kanamycin cassette in ORF region. We measured the IAA produced by the wild-type and the mutant strain using HPLC, both strains were growth in k-malate medium with 100 ug/ml of tryptophan, we founded 67% decrease in IAA biosynthesis at 16 hours, at 48 hours the IAA biosynthesis decreased 7.5% compared to the wild strain.

Participation of *iorA* gene was confirmed by complementing the *iorA* gene in trans, using the plasmid pJB3Tc20, a wide host range vector with a low copy number. Two versions of the plasmid were obtained, with a different transcriptional regulation. The first through plasmid lacZ promoter (pJBiorA-DP), and the other by wild-type *iorA* gene promoter (pJBiorA-SP).

Complementation in the IAA biosynthesis was obtained at 36 hours using both plasmids: pJBiorA-DP and pJBiorA-SP, difference in IAA biosynthesis was not found using one or another plasmid carrying the *iorA* gene, so *iorA* could participate in IAA biosynthesis in *A. brasilense* Sp7 using indole-3-pyruvate as substrate.

The effect of different carbon sources in *Kluyveromyces marxianus* growth kinetics.

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Yeasts are microorganisms highly used in the manufacture of different fermented foods such as wine, beer, bread and various dairy products. Currently we know about a great diversity of yeasts, being *Saccharomyces cerevisiae* one of the most studied species but not the only one. Yeasts are microorganisms of high interest since they have a high growth rate and high biomass yields can be achieved. *Kluyveromyces marxianus* is a species with a high biotechnological potential since it has been proven to be thermotolerant, with high growth rates and with high metabolic plasticity to utilize a wide variety of substrates [1], these properties can be exploited in the fermentation of industrial wastes to obtain biomass, secondary metabolites and the treatment of these same wastes; Thus transforming an industrial by-product that would normally be discarded into products of high commercial value.

This study aims to analyze the relationship between biomass production and substrate concentration using different carbon sources for PK1 strain of *Kluyveromyces marxianus*, seeking its subsequent industrial application in the fermentation of different by-products of the dairy industry. The microbial growth kinetics will be carried out comparing biomass production with respect to different carbon sources (glucose, galactose and lactose) at different concentrations (1, 2.5, 5 and 10% w/v), the O.D. (600 nm) will be measured every 4 h for a 24-hour period, final dry weight yield will be determined and carbohydrate consumption rates will be obtained through HPLC. The O.D. will we correlated to CFU through a calibration curve.

The information obtained will be applied in industrial processes optimization.

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Physicochemical analysis of four mixed cultures prepared with different strain combinations

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Sumary

Introduction. Jocoque is a fermented dairy product, with a fresh acid flavor, which is consumed in some regions of our country. In Durango, most of jocoques production, is not standardized. The objetive was, to evaluate the capacity of fermentation in batch, de 4 mixed lactic cultures, to developa starter culture.

Metodology. The strains used were, **ZC101 (A)**, **ZM103 (B)**, **ZM104 (C)**, **ZM105 (D)**, were prepared according to the Simplex Centroide experimental design for mixtures. They are inoculated at a concentration of $1X10^8$CFU/mL. and incubated at 37ºC/48 h., using 10%reconstituted milk, Nido brand. Th variable as e type and concentration of strains were used as independent variables and pH, acidity, density and consistency as response variables.

Results. The idependent variables significantly affected the old physicochemical parameters of the crops, with the exception of density. Over time, the pH decreased, the acidity increased and the consistency improved. The process generated a high concentration of acidity, through the Embden-Meyerhof-Parnas route (Rodríguez *et al*., 2011), very important for the optimization of quality product. The combination **ACD**, generated the highest acid concentration in acidity. (0.90% Ac. Láctico).

Conclusions. Nowadays, it is proven that mixed crops propmote the optimization of product quality characteristics, such as the improvement of consistency. **ACD (ZC101/ZM104/ZM105)** presented the best technological properties for the development of a starter culture.

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Bioprospecting for Actinobacterias isolated from tropical soils with antimicrobial properties

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Actinobacteria are a ubiquitous and heterogeneous group of Gram-positive bacteria. They are widely distributed in many habitats and are characterized by the production of a high diversity of secondary metabolites with antimicrobial and pharmacological qualities, which is why they have contributed with a great variety of antibiotics for the control of many infectious diseases[1]. Therefore, we have focused on the search for Actinobacteria in unexplored environments such as tropical rainforests of Tabasco, given the need to find antimicrobial compounds for the treatment of multiresistant microorganisms. Three of rainforests (Coconá, La Florida and Puyacatengo) were selected by their low anthropogenic activity. The isolation was performed by serial dilutions and spread plate method on five different cultures media, and the typical Actinobacteria colonies were isolated. A total of 172 culturables Actinobacteria were isolated and primary screening was performed by cross-streak assay in ISP-2 medium, against ATCC type strains, three bacteria *S. aureus* (25923), *B. subtilis* (6639), *E. coli* (25922) and one yeast *C. albicans* (10234), of which 133 isolation (77%) showed antimicrobial activity against at least one of the tested strains. The bacteria *B. subtilis* and *S. aureus* were more sensitive to metabolites produced by Actinobacteria strains (61% and 54 respectively), followed by *C. albicans* (35%) and the lowest activity rate was found against *E. coli* (23%). A secondary screening was performed by well diffusion agar cylindres method with 43 Actinobacteria selected because of their broad-spectrum activity, these were spread and incubated for 15 days in six different culture media (ISP-2, ISP-5, ISP-7, BHI, Peptone-starch (PS) and Casein-starch (CS). Stainless steel cylinders were placed over agar plates. Then, the plates were overlaid with agar containing an overnight culture of reference strains adjusted at $DO_{600\ nm}$ 0.06-0.09. The cylinders were removed when the overlay was solidified, and were placed cylinders of 7mm of each Actinobacteria in different cultural media. The Plates were incubated at 30°C for 24 h and inhibition diameter zones were measured. The most effective culture medium was PS where the largest inhibition zone was recorded for *B. Subtilis* (30.1 mm), *S. aureus* (25.5 mm) and *E. coli* (12.6 mm). Interestingly, the greatest of inhibition zone was of 33 mm for *C. albicans*. In conclusion, our findings suggest that the unexplored soil tropical a high percentage of Actinobacteria, particularly from Coconá and La Florida show strong antimicrobial activity, so these sites are an interesting source in the search for Actinobacteria for the production of new metabolites capable of inhibiting a large group pathogenic microorganisms and the PS medium is the best option to obtain a better expression of those metabolites.

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Seeding public goods is essential for maintaining cooperation in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa has a wide variety of quorum sensing-controlled virulence factors such as hydrogen cyanide, exoprotease, pyocyanin, etc. Exoprotease secretion is a cooperative behavior that is susceptible to social exploitation by quorum sensing-mutants (cheaters) that do not invest in the exoprotease production but assimilate the amino acids and peptides derived by protein hydrolysis, hence, these exoprotease-less mutants have higher fitness when compared to WT individuals. In sequential cultures with protein as sole carbon source, these social cheaters can reach an equilibrium with the WT individuals but also have the potential to collapse the growth of the whole population by over exploitation of the cooperators, causing a tragedy of the commons. However, this phenomenon has not been described in recently isolated clinical strains but only in reference strains such as PAO1 or PA14. Furthermore, the importance of the initial amount of public goods (exoprotease) that comes with the inoculum in each subculture has not been evaluated in cheater emergence and later population collapses. In this work, using the reference strain PA14 and a clinical isolate from a burn patient, we find that exoprotease-less mutants emerge from 3 to 5 daily passes in M9 medium with 0.25% sodium caseinate as sole carbon source. We also demonstrate the importance of the initial amount of public goods (exoprotease) by doing sequential subcultures in M9 medium with 0.25% sodium caseinate with a) washed inoculums with 0.9% NaCl, in which all populations collapsed after 7 daily passes in agreement with an abrupt proliferation of cheaters; b) washed inoculums with daily-added exogenous protease after the 5th pass, in which growth was reestablished as WT individuals replaced the cheater population; and c) washed inoculums in media complemented with 0.025% casamino acids, in which no protease-less individuals nor population collapses were registered after 9 daily passes. Our results demonstrate that the initial amount of public goods (exoprotease) that comes with the inoculum in each sequential culture is essential for maintaining population growth and that eliminating the exoprotease in the inoculum leads to rapid proliferation of protease-less mutants followed by population collapses. Therefore, our results suggest that sequential washes should be combined with public goods inhibitors to more effectively combat *P. aeruginosa* infections.

Engineering a biosynthetic pathway for the production of novel bioactive diterpenoids

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Keywords: Combinatorial biosynthesis; *Streptomyces*; Structural biology; Synthetic biological circuit.

The labdane-related diterpenoids (LRDs) are a large group of natural products with a broad range of biological activities that are divided into four subgroups from bicyclic to pentacyclic LRDs skeletons (C_{20}). They are synthesized through two consecutive reactions catalyzed by class II and I diterpene synthases (DTSs) using geranylgeranyl pyrophosphate (GGPP) as a substrate. Recently, we have determined the first X-ray crystal structure of a bicyclic LRD (*E*)-biformene synthase, LrdC, a class I DTS which belongs to a cryptic gene cluster (*lrd*ABC) encoded in the genome sequence of *Streptomyces* sp. K155, a streptomycete isolated from the soil of Valle de Chalco. Functional and structural analysis showed that the *lrd*ABC gene cluster is involved in the biosynthesis of (*E*)-biformene, as well as the LrdA enzyme synthesizes GGPP, class II DTS LrdB synthesizes copalyl pyrophosphate (CPP) from GGPP, and LrdC synthesizes (*E*)-biformene from CPP. Notably, a conserved pair of aromatic residues Phe^{189}/Tyr^{317} in LrdC were also proposed to play a critical role in controlling the (*E*)-biformene synthesis, but the conserved Phe^{189} residue was speculated to prevent cyclization beyond the bicyclic LRD skeleton. In this work, several synthetic biological circuits regulated under the constitutive ribosomal promoter pRpsJ from *S. avermitilis*, were designed and integrated into an engineered *Streptomyces* host *S. coelicolor* M1152. Thus, while two synthetic biological circuits codified the *lrd*ABC$_{F189A}$ and *lrd*ABC$_{Y317W}$ gene clusters with two site-specific mutants of LrdC, the other two *lrd*ABDC$_{F189A}$ and *lrd*ABDC$_{Y317W}$ gene clusters contain a gene of a cytochrome P450 (LrdD enzyme) to further modify the LRD hydrocarbon skeleton by incorporating different functional groups. Together, this work provides a rational combinatorial biosynthesis for the production of novel LRDs through reprogramming the LrdC enzyme, as well as by using a tailoring enzyme to decorate the final LRD natural product and thereby increasing the structural complexity.

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Characterization of PGPR isolated from rhizospheric soils of *Agave angustifolia*

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Some of the most important and representative products from Mexico are the products produced from agave, such as tequila, mezcal and bacanora. The quality parameters from this products depends of the agave's characteristics and mature grade, one limitation of agave crop is the time which the plant reaches the mature state to be process, aproximately seven years old. This investigation has as aim to isolation and characterization of Plant Growth Promoting Rhizobacteria (PGPR) from rhizospheric soils of *Agave angustifolia* with the purpose of use them as biofertilizers in agave cultivation, hoping to reduce the time in which the crop reaches maturity.

Rhizospheric soil samples were collected from small *Agave angustifolia* plants, the rizospheric soil was obtained by shaking method and subsequent brushing of remaining root system soil, bacteria were isolated from the rhizospheric soil samples by serial dilution technique on starch agar plates (SA), agar for phosphate solubilizers (PSA), Czapek DOX agar (CA), Czapek DOX Agar suplemented with carboxymethylcellulose, all of them incubated at room temperature for 72 – 96 h. After incubation period, metabolic activities were evaluated on correspondent agar, strains that showed the activity corresponding to the agar where they were isolated were evaluated on the other agars more Congo red agar.

A total of twenty-seven strains were isolated, all of them showed at least one metabolic characteristic of interest, one strain showed a interesting potential, it has the four metabolic capabilities: amylolytic activity, phosphate solubilizing, cellulolytic and denitrification activities. Four strains showed three activities and the rest showed one or two metabolic capabilities, the biochemical characterization of the strains is not concluded, however, we are positive for owing the potencial that the strains have.

In conclusion, four strains that show a set of plant growth promotion activities, such as amylolytic activity, phosphate solubilizing, cellulolytic and denitrification activities, have been isolated. Actually we are evaluating the synthesis of vegetal hormonas by the strains.

Antimicrobial metabolites from fungal strains isolated from agroindustrial waste products

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The increase in antimicrobial resistance of pathogenic bacteria to commonly used antibiotics has led to a global public health crisis. There emergence of multidrug resistant bacteria is a challenge for therapeutic measures, and the synthesis or identification of new antimicrobial compounds is time consuming and expensive. The search for compounds with antibiotic capacities usually turns to plant extracts or microbial metabolites. The objective of this research was to isolate fungal strains from food waste residues, and to test their cell-free supernatant for antimicrobial capacity against multidrug resistant bacterial isolates and against strains from culture collection (ATCC). Samples from food waste residues, included spent coffee grounds, pomegranate seeds and rind, and were inoculated in Potato Dextrose Agar and Sabouraud Agar plates. After incubation, colonies were re-inoculated until a pure culture was obtained. Fungal strains were partially characterized by colonial morphology and microscopic examination. The strains were inoculated in 50 mL of Yeast Extract Broth in 250 EM flasks and incubated at 25°C with agitation (125 rpm) for one week. Biomass was separated by filtration. The cell-free supernatant was filter-sterilized and tested for antimicrobial capacity by placing a drop of the extract on top of an inoculated plate. Five ATCC strains were tested (*Bacillus cereus* 11778, *Escherichia coli* 35218, *Listeria monocytogenes* 19114, *Salmonella* Typhimorium 14028 and *S. aureus* 25923), as well as six multidrug resistant bacterial strains isolated from patients with urinary tract infections (three *E. coli*, one *Enterococcus faecalis*, two *Pseudomonas aeruginosa*). A total of 16 fungal strains were isolated and were initially identified as *Aspergillus*, *Penicillium*, *Trichoderma*, and *Geotrichum*. Results of the antimicrobial capacity demonstrated that five of the cell-free supernatant had partial or complete inhibition against some of the bacterial strains tested. Two extracts showed partial inhibition against the *P. aeruginosa* strains, while one extract had inhibition of the *P. aeruginosa* strains and partial inhibition of *Salmonella*. Another extract showed inhibition against *B. cereus* and *E. faecalis* and one supernatant inhibited the growth of the two *P. aeruginosa* strains, as well as *E. coli*, *Salmonella* and *B. cereus*. Further characterization of the strains and the metabolites present in the cell-free supernatant will be carried out, but results up to the moment are promising, because of the antimicrobial efficiency against multidrug resistant clinical isolates.

Antioxidant peptides from whey fermentation and bacteriocin production by *Enterococcus faecium* strains

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Lactic Acid Bacteria (LAB) are important microorganisms related to fermented foods that can produce proteinaceous compounds with different bioactivities. Bacteriocinns are included among those bioactive molecules, which are antimicrobial peptides that are synthetized ribosomally; on the other hand, LAB can produce bioactive peptides as by-products of protein hydrolysis with antioxidant properties. The aim of the present work was to partially characterize the antimicrobial (bacteriocins) and antioxidant (bioactive peptides) compounds obtained from 6 strains of *Enterococcus faecium* (47T, 224sa, 238sa, 239sa, 251T and 61vl) isolated from the manufacturing process of Chihuahua cheese. The production of bacteriocins and antioxidant peptides was done in Man-Rogosa-Sharpe (MRS) and whey milk (10%) respectively. After incubation of LAB in growth media (24h, 140 rpm, 30 ºC), the cell free supernatants (CFS) were obtained by centrifugation (10000 x*g*, 20 min) then neutralized (pH 6.5, NaOH 3M). In the case of MRS, the CFS was filtrated (0.22 µm) for sterilization, and in the case of peptides from whey, the CFS was pasteurized (60 ºC, 30 min). The resistance of CFS were tested after treatment with proteolytic enzymes (Protease and trypsin), solvents 10% (ethanol and acetone), pH (2 and 12) and shelf life (1 year, 4 ºC), however only solvents and pH were tested for the antioxidant peptides. The antimicrobial activity was measured against *Listeria monocytogenes* by agar diffusion assay and DPPH/ABTS radicals were used to measured antioxidant activity. The percentage of activity reduction was calculated based in the initial and final activity after treatments. When the CFS was treated with proteolytic enzymes, the antimicrobial capacity and the antioxidant activity were reduced by more than 90%. Regarding the shelf life test, the CFS of the strains 47T, 238sa and 239sa maintain almost 50% of the activity after one year of storage. Solvents and pH were the treatment that less affected the antimicrobial and antioxidant activity of CFS. The resistance and sensibility of the bioactive compounds present in the CFS are a confirmation of their chemical nature. The characterization of the bioactive compounds provide valuable information for the possible use of these compounds in the food industry.

Proteins of *Helicobacter pylori*, which scavenge iron from human sources

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Abstract

Helicobacter pylori is a gram-negative spiral bacterial that has been associated with peptic ulcers, gastritis, duodenitis and it is believed to be a causative agent of gastric cancer. Human sources such as lactoferrin, haem and haemoglobin can support the *H. pylori* growth. However, it is not still fully understood how the iron acquisition process occurs. An *in silico* analysis has shown that the *H. pylori* genome has a family of genes coding for three outer membrane proteins regulated by iron (FrpB). Two of them: FrpB1 and FrpB2 were purified as recombinant proteins and their haem- or haemoglobin-binding capability was demonstrated. Nevertheless, the last protein of the family (FrpB3) has not been investigated. In this work, FrpB3 was purified by haem-affinity chromatography and its capacity of haem-binding was analyzed. This protein was identified by mass spectrometry as FrpB3. The analysis by 3D model showed that it is structurally conserved because it has the typical barrel structure, which is inserted in the membrane. Moreover, the motifs necessary for Hb-binding were identified. However, the data cannot explain how these proteins are regulated, for this reason their respective expression was quantified by real time technique under different human iron sources. We observed that FrpB1 was overexpressed with haem, while FrpB2 was induced in presence of haem and also haemoglobin. In the case of FrpB3, this was overexpressed with haemoglobin. We propose that *H. pylori* secretes proteins in order to withstand the extreme environment present in the stomach. Our overall results represent the effort to explain the importance of iron acquisition. Perhaps iron helps the bacterium to resist the acidic environment of the human stomach and this mechanism is vital for *H. pylori* during the infection process.

Efecto sobre el crecimiento de células de las líneas celulares de cáncer HCT15, MDA-MB231, MCF-7, PC3, HeLa y HEP-G2 de metabolitos secundarios producidos por Actinobacterias.

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Las actinobacterias corresponden a un grupo de bacterias de distribución cosmopolita. Los representantes de este grupo de bacterias son Actinomyces, Arthrobacter, Corynebacterium, Frankia, Micrococcus, Micromonospora, Mycobacterium, Nocardia, Propionibacterium y Streptomyces. Las actinobacterias participan de forma significativa en el reciclaje de materia orgánica del suelo. El cáncer se define como un proceso de crecimiento y diseminación incontrolados de células, el cáncer puede aparecer prácticamente en cualquier lugar del cuerpo. Comúnmente las células crecen, se dividen para formar nuevas células conforme el cuerpo las va requiriendo, cuando las células sanas envejecen o son dañadas, mueren, y son reemplazadas por células nuevas. Sin embargo, cuando existe cáncer, este proceso de pasos ordenados se descontrola. Conforme las células se vuelven cada vez más anormales, las células viejas o dañadas sobreviven en lugar de morir y nuevas células se forman aun cuando estas no sean necesarias, dichas células se dividen incontroladamente y suelen formar masas denominadas tumores, estos pueden ser benignos o malignos. Cuando se habla de tumor maligno, se habla de cáncer. El cáncer es la principal causa de muerte a nivel mundial y en 2015 provocó 8.8 millones de defunciones, de acuerdo con el INEGI. Las terapias utilizadas convencionalmente incluyen la quimioterapia, radioterapia, inmunoterapia o bien la cirugía. El tratamiento con distintos tipos de fármacos puede tener diversos efectos secundarios adversos debido a su falta de especificidad, a causa del desconocimiento de las dianas afectadas, esto hace que los compuestos no discriminen entre células afectadas y células sanas. Por lo anterior es importante realizar estudios sobre alternativas terapéuticas, determinando la bioactividad y biofuncionalidad de bacterias para su uso probable como agentes farmacéuticos y la búsqueda de nuevos compuestos antitumorales específicos. La metodología se basa en el crecimiento de actinobacterias ssp en medio de cultivo convencional, obtención de metabolitos secundarios y determinación del efecto sobre líneas celulares de cáncer HCT15, MDA-MB231, MCF-7, PC3, HeLa y HEP-G2. La diversidad metabólica y fisiológica de las actinobacterias las convierte en un grupo con objetivo biomédico y biotecnológico debido a su alta producción de enzimas extracelulares y metabolitos bioactivos. La mayor parte de productos farmacéuticos obtenidos por la fermentación microbiana son los metabolitos secundarios. Los metabolitos secundarios pueden desempeñar papeles tales como antibióticos, antiparasitarios, antifúngicos, antivirales, biorreguladores, inóforos, toxinas y marcadores de especificación intra e interespecífica. Los antibióticos antitumorales que tienen como objetivo el DNA poseen actividad microbiana y tienen la función común de ser agentes tóxicos que dañan el DNA, estas sustancias son producidas por Actinobacterias, principalmente Streptomyces.

Participation of phosphatases in the solubilizing activity of phosphates in *Gluconacetobacter diazotrophicus* Pal5

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Phosphorus (P) is an essential nutrient for biological growth. Absence of this element in the soil could limit the plant development. A greater part of soil phosphorus is in the form of insoluble inorganics and organics phosphates, in order to increase the availability of phosphorus for plants, large amount of fertilizer are being applied to soil, so, the importance of microorganisms which have the capacity to solubilize phosphorus. *G. diazotrophicus* is an endophytic bacterium consider a PGPB due to the characteristics such as benefit plants through different mechanisms among which are the production of plant growth hormones, nitrogen fixation, biological control of pathogens and improving nutrients availability for the plant like the solubilization of inorganic phosphates through the production of phosphatases.

Phosphatases catalyze the hydrolysis of the C-O-P linkage of a wide variety of phosphate esters. They are involved in essential biological functions, including regulation of metabolism, energy conversion, signal transduction and others. Bacterial phosphatases can be classified based on pH optimum (acid, neutral, or alkaline), substrate profile (specific to certain substrates vs. nonspecific), and molecular size (low vs. high molecular weight).

The ability of *G. diazotrophicus* to solubilize P was qualitatively assessed using SRS agar medium. The effect of different parameters like pH (5-7), carbon replacing glucose (xylose, fructose, mannitol, glycerol, lactose and maltose), nitrogen substituting ammonium sulphate (ammonium nitrate, calcium nitrate, sodium nitrate, urea, and L-aspartate), and the effect of different phosphate sources replacing tricalcium phosphate (hydroxyapatite, sodium phosphate and potassium phosphate monobasic) in SRS medium. Phosphatase activity were assayed in liquid SRS medium using p-nitrophenyl phosphate (pNPP), a colour-less substrate that produces a colorimetric end-product p-nitrophenol (pNP) (yellow colour) read to 410nm.

The optimal conditions for the solubilization of phosphates in the SRS medium were established: Dextrose as a carbon source, pH 5, ammonium sulfate as a nitrogen source and tricalcium phosphate as a source of phosphate. Maximum phosphatase production by *Gluconacetobacter diazotrophicus* Pal5 was recorded at 96 h of incubation and decreased upon further incubation up to 192 h. In the 10% PAGE-SDS gel a band of 63kDa approximately is shown and in zymograms assays it shows phosphatase activity revelated by the acidified ammonium molybdate method, which yields coloress bands.

In further studies the phosphatase will be purified and send for sequencing, to know the role it may have in *G. diazotrophicus* Pal5 lifestyle.

The hemolysin of *Gluconacetobacter diazotrophicus*

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Introduction:
The acetic acid bacterium *G. diazotrophicus* is a great ally in the food industry being a large producer of acetic acid, and in biotechnological applications used as a biofertilizer. However, human cases of bacteremia, peritonitis, lymphadenitis, endocarditis and postoperative pneumonia, associated with acetic acid bacteria have been reported. In this work, some evidence is presented regarding the possible pathogenic role of *G. diazotrophicus* due to the hemolytic capacity of this bacterium.

Objective:
To demostre the presence of a hemolysin of *G. diazotrophicus* as a possible virulence factor in the bacteria.

Methodology:
The haemolytic activity of *G. diazotrophicus* was analyzed by observing the growth on blood agar plates. The haemolytic activity also was determinated by measuring the concentration of hemoglobin released from an erythrocyte suspension (spectrophotometrically assay). The reaction mixtures were incubated at 30°C for 0, 10, 20, 30 and 40 minutes. For each sample, protein and hemoglobin concentrations were quantified. A protein test was then carried out which was carried out according to the methodology described by Lowry method. *E. coli* was used as a positive control and an erythrocyte suspension as a negative control.

Results:
G. diazotrophicus produced an alpha-hemolysis when it grown on blood agar plates. While the haemolytic activity measured spectrophotometrically showed hemoglobin release in tests conducted at different times.

Conclusion:
G. diazotrophicus had the ability to produce hemolysis.

The participation of the pyruvate carboxylase and phosphoenolpyruvate carboxylase enzymes in the aerobic metabolism in *Rhizobium phaseoli* CIAT652

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Oxaloacetate (OAA) plays an important role in the TCA cycle and for the biosynthesis of a variety of cellular compounds. Some microorganisms are able to synthesize OAA during growth on glucose via either of the enzymes pyruvate carboxylase (PYC) or phosphoenolpyruvate carboxylase (PPC). Pyruvate carboxylase (PYC), is a member of the biotin-dependent enzyme family, that catalyzes the ATP-dependent carboxylation of pyruvate to oxaloacetate. PYC has been found in a wide variety of prokaryotes and eukaryotes. The PEP carboxylase enzyme is present in plants and some types of bacteria, but not in fungi, animals or humans. Similar to pyruvate carboxylase, PEP carboxylase restores oxaloacetate in the TCA cycle.

Genetic and biochemical studies have demonstrated the importance of several tricarboxylic acid (TCA) cycle enzymes in allowing the establishment or maintenance of an effective nitrogen-fixing symbiosis. Rhizobia use a variety of anaplerotic pathways to maintain TCA cycle activity under different growth conditions; and some of these reactions are also required in symbiosis. For instance, *R. etli pyc* mutants are unable to grow in minimal medium with pyruvate as a sole carbon source. However, during serial cultivation in minimal medium containing 30 mM succinate, the supplementation with biotin is enough to restore the growth rate in the wild-type strain but not in the mutant. This result indicates that PYC is necessary for the growth of *R. etli* under these conditions.

Interestingly, analysis of the *Rhizobium phaseoli* CIAT652 genome suggest us that in this bacterium exist one ORF for each enzyme. In order to define its functionality and its possible participation in the TCA cycle we carried out the mutagenesis of both genes separately and also constructed a double mutant. We analyzed the effect on the growth of these mutants when they are cultivated in aerobic conditions in minimal medium with different carbon sources in the presence or not of biotin.

This work was supported by grant PAPIIT IN201617 from DGAPA-UNAM.

Biochemical characterization of two proteins involved in the metabolism of polyhydroxybutyrate (PHB) in *Azotobacter vinelandii*

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Polyhydroxybutyrate (PHB) is a natural polyester synthesized by *Azotobacter vinelandii*, as a carbon and energy reserve. PHB accumulates intracellularly as polymer granules when there is abundance of the carbon source, but it is degraded (mobilized) when the carbon source is depleted. This mobilization of PHB is carried out by intracellular enzymes PHB depolymerases, which are bound to the PHB granules. Other proteins associated with the PHB granules called phasins, constitute the main protein component of the granules, although they have no catalytic activity in the metabolism of PHB.

In the genome of *A. vinelandii* we've identified seven putative genes for PHB depolymerases. Among them, the product of the gene *avin_34710*, has 33% identity with PHB depolymerases already characterized from other organisms. In addition, our analysis of the proteins associated with the PHB granules of *A. vinelandii* by mass spectrometry revealed the presence of a new protein, which has no similarity with any other known protein, and whose gene (*avin_34720*) is located immediately downstream from the putative PHB depolymerase gene *avin_34710*.

Our objective was to characterize these two proteins: Avin34710 and Avin34720. For Avin34710 we analyzed its PHB depolymerase activity *in vitro* and *in vivo*; while for Avin34720, we studied its role in the metabolism of PHB *in vivo*, because previous studies reported that some phasins can act by modulating activity of PHB synthases or PHB depolymerases. Biochemical characterization *in vivo* was performed by heterologous expression of *avin_34710* and *avin_34720* in an *E. coli* strain able to produce PHB and by the construction and analysis of an *A. vinelandii* mutant lacking the *avin_34710* gene.

When we characterized the activity of these proteins expressed in *E. coli*, we observed that Avin34710 degraded the PHB produced, while Avin34720 caused a higher accumulation of PHB. The purified Avin34710 protein showed PHB depolymerizing activity *in vitro*, while the corresponding *A. vinelandii* mutant presented more whitish phenotype compared to the wild type strain (WT), caused by a higher PHB content. The quantification showed an increased specific production of PHB at 72 h of culture (74% higher than the wild type). The results obtained in this work show that Avin34710 is a PHB depolymerase that, like the previously characterized enzyme Avin03910, participates in the degradation of PHB in *A. vinelandii* and suggest that Avin34720 could be activating the synthesis of PHB.

Purification of the recombinant Fur_{1248} protein of *Gluconacetobacter diazotrophicus* Pal5 strain: Factors that affect the oligomerization state

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Introduction: *Gluconacetobacter diazotrophicus* Pal5 is an endophyte microorganism, belongs to the plant growth promoting bacteria (BPCV) group. Require the participation of transition metal ions such as iron that play essential role in the structure and function of macromolecules (oxide-reduction enzymes, Krebs cycle, electron transport chain, biological nitrogen fixation, etc.). However, high intracellular concentration of Fe^{2+} ions can cause the increase of highly reactive oxygen species (H_2O_2, O_2•, HO•) harmful to the cell, there must be a fine regulation to maintain intracellular iron homeostasis. These are regulated by the Fur protein transcriptional regulator, the basic fold of the protein consists of two domains: an N-terminal DNA binding and a C-terminal dimerization domain. Bertalan *et al*. (2009) complete genome sequence of the sugarcane nitrogen fixing endophyte and find two *fur*-like genes, GDI_1398 and GDI_1248. In this research, we study the factors that affect the oligomerization state of the purified recombinant protein $Fur_{1248}60$ of *G*. *diazotrophicus* Pal5. **Methods**: The fur_{1248} gene was cloned into a pEXP5-CT expression vector, generating the plasmid pEXP5-CT::$fur_{1248}60$. After the sequencing of the gene and its analysis by the BLAST program, pEXP-5CT::$fur_{1248}60$ was transformed into the *E. coli* strain BL21 pLys(pEXP5-CT::$fur_{1248}60$), the optimal expression conditions of the recombinant Fur_{1248}^{60} protein were 0.1mM IPTG, 37ºC, 175 rpm for 2 hours, the estimate molecular weight was of 18.4 KDa, this was confirmed by immunodetection with anti-His monoclonal antibodies. Recombinant $Fur_{1248}60$ was purified by affinity chromatography Ni-NTA. **Results and discusion**: A fraction was obtained of 344 µg/mL, which was used to perform the oligomerization tests with ionic strength (NaCl 10 to 500mM), divalent metals ions (Fe^{2+}, Zn^{2+}, Co^{2+}, Mn^{2+} and Ca^{2+}, 1mM) and reducing and oxidants (DTT, β-mercaptoethanol and H_2O_2) agents. The data obtained suggest that the $Fur_{1248}60$ recombinant protein of *G*. *diazotrophicus* Pal5 presents different behavior in the state of oligomerization against various proven effectors. **Conclusion**: This state responds to dynamic changes in its quaternary structure after treatment with the mentioned effectors, thus suggesting able to influence their function.

Flow cytometry assessment of membrane integrity of lactic acid bacteria maintained under preservation

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Lactic acid bacteria (LAB) comprises a group of bacteria metabolic and physiologically related. In general terms, LAB are Gram positive (+), negative to catalase and oxidase activities, lacking of cytochromes, aero- and acid-tolerant, fastidious, regularly non-sporeforming and non-respiratory cocci, coccobacilli or rods. Preservation *ex situ* and maintenance of these bacteria, imply to keep them intact in the *long term,* (phenotypic- and genetically). This study proposes and validate a protocol to preserve and assess integrity of LAB. Eight strains of LAB (including *Lactobacillus plantarum, L. curvatus and Leuconostoc mesenteroides)*, previously genotyped at species level, were included in the preservation assay. Bacterial suspensions were prepared using fresh cells of each strain and thyoglycolate broth without dextrose and without indicator (Difco™), at an approximately density of 1 x 10^9 CFU ml^{-1} according to the McFarland standard. Briefly, a 500 µl aliquot of each cell suspension was deposited into 2 ml cryo-tubes and mixed with 500 µl of 20 % sterile glycerol by vortex. Cryo-tubes were storage at -20 °C for 2 hours and then preserved at -80 °C for 180 days. After that, cryo-preserved bacterial suspensions were thawed at room temperature and membrane potential was measured by flow cytometry using the commercial kit BacLight™ B34950 according to the procedure suggested by the manufacturer; meanwhile, kinetics parameters were obtained by growing each strain in MRS broth through optical density. Results showed that, after 180 days of cryo-preservation, *Lactobacillus curvatus* maintained its membrane potential in a good level (89 % of active cells), similarly to *Lactobacillus plantarum* that exhibited an 86.5 % of active cells at membrane level after preservation; meanwhile 78.9 of preserved cells of *Leuconostoc mesenteroides* preserved their membrane potential. Kinetics of all preserved and recovered strains corresponded to a normal kinetics for LAB, keeping normal adaptation times (Lag phase, 4 h) and Log phases (9 h average), before to achieved stationary phase. Preservation proposed protocol resulted in a good alternative to long-term preservation that allow preserve both viability and metabolism of LAB; besides, the use of flow cytometry allowed assess, quickly and accurate, the membrane integrity of bacteria.

GENOMICS AND EVOLUTION

XLI National Meeting of the Mexican Association of Microbiology (AMM)
VI Meeting of Biochemistry and Molecular Biology of Bacteria (BBMB)

Oaxaca, Oax. October 27 - 31, 2019.

Nodules of *Phaseolus vulgaris*: the unexpected place for *Rhizobium* conjugation

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Rhizobium etli CFN42 is a Gram-negative bacterium, with great agricultural importance due to its symbiotic association with *Phaseolus vulgaris*, through the formation of nodules. The genome of *R. etli* CFN42 consists of one chromosome and six large plasmids. Among these, pRet42a has been identified as the conjugative plasmid, and is transferred at a high frequency (10^{-2}) in laboratory conditions. The expression of the transfer genes is regulated by a "*quorum sensing*" system that includes a *traI* gene which codes for a homoserin lactone (HSL) and two transcriptional regulators (TraR and CinR). Here, using confocal microscopy and flow cytometry, we show that pRet42a transfers on the root's surface, and unexpectedly, inside the nodules, where it also promotes transfer of the symbiotic plasmid. Conjugation was restricted on the plant surface by: 1) placing the QS *traI* regulator under the promoter of the nitrogenase gene which is only expressed inside the nodules, and 2) inhibiting the QS transcriptional induction of transfer genes with a *traM* antiactivator on an unstable vector that is maintained on the plant surface and lost inside the symbiotic structures. In both experiments transconjugants were still generated inside the nodules, conclusively confirming the occurrence of conjugation in these structures. This uncovers a novel function for nodules, as a protected niche where bacteria can diversify due to genetic exchange, including symbiotic and non-symbiotic information.

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Structural genomics for nonribosomal peptide synthetases analysis in symbiotic bacteria

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Photorhabdus and *Xenorhabdus* are Gram-negative bacteria wich are symbionts of soil nematode of the families *Heterorhabditidae* and *Steinernematidae*, respectively. Members of this bacteria have a complex life cycle, it mantains a mutualistic simbiosis with soil nematodes, and on the other hand it is highly pathogenic toward a variety of insect species. *Photorhabdus* and *Xenorhabdus* colonize the gut of the infective juvenile stage (IJ) of the nematode, the IJ infect larvae of insect and once inside the bacteria are liberate from the gut of the IJ in to the insects hemolymph. Later the bacteria replicate and rapidly establish a lethal septicemia in the host by the production of a board range of diferent secondary metabolites and toxins.

Many of these toxins and secondary metabolites or natural products (NP) play a key role in support a complex life cycle, specifically in diferent facets of the nematode development, for example antimicrobial peptides for defense or in insect immune system. Hence, the NPs produced by some bacteria are esencial for many simbioses an have a increasing number of described functions, highlighting for their versatility of biosynthesis, mainly those produced by non-ribosomal peptidosintetases (NRPSs). Curiously has been identified in the genome of both genera (Photorhabdus and Xenorhabdus) that code for a considerable number of NRPSs, comparable to Streptomyces.

This NRPSs are a large multi-enzime complex using thiotrmplate mechanisms and they use peptide bond formation to build larger molecules from diferent building blocks, not restricted to 20 preoteogenic amino acids. Moreover these non-ribosomalpeptides (NRP) display unique structural elements like D, N or C methylated, glycosylated or phosphorylated amino acids. Therefore it is important to analyze the NRPSs and its products to identify possible peptides with antimicrobial activity or molecules involved in the symbiosis, pathogenicity or regulation of the metabolism of these bacteria.

For this we analyzed the biosynthetic potential (with antiSMASH) of 51 strains with different lifestyles to determine if bacteria with other lifestyles have the biosintetic potential of NRPSs. Subsequently, using the antiSMASH results, a network analysis was performed using BiG-SCAPE, an open source software to determine if the groups of biosynthetic genes present in the 51 strains were related to each other and to the MiBiG database (**B**iosynthetic **G**ene **C**luster characterized experimentally) at the level of sequence identity. Later, with the analysis of networks, BGCs were identified that are candidates to perform mutagenesis and determine their possible role in the symbiosis or as an antimicrobial peptide.

Finally, as a conclusion, the strains of Photorhabdus and xenorhabdus were identified as those with a greater biosynthetic potential in the production of NRPSs, even greater than that of Streptomyces strains, and most of the BGC identified in this analysis have no relationship at the level of identity of sequence with other BGC characterized experimentally.

Genomic analysis of *Rhizobium* prophages.

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Bacteriophages or phages, are virus that infect and reproduce within bacterial cells. They kill the bacteria or could enter in lysogeny. In this last case, the phage genome integrates into the bacterial chromosome as prophage. The prophages may contribute to the host fitness by expression of toxins, antibiotic resistance genes, surface proteins, among other traits. Under stress conditions prophage can be induced to a lytic cycle. The dynamic of lysis-lysogeny is poorly known in bacterial communities. Previous work by our group has shown lytic phages able to infect the nitrogen-fixing symbiotic bacteria *Rhizobium etli*. However, we failed to demonstrate prophage induction with mitomycin C in more than 150 *R. etli* strains. A hypothesis congruent with this result is the absence of complete prophages in the *R. etli* genome. Alternatively, mitomycin could be the incorrect prophage inductor. To obtain evidence for the first hypothesis, we performed a bioinformatics search in the genomes of this bacterial genus already reported in the Genbank.

We downloaded 504 *Rhizobium* genomes deposited in the Genbank, and performed prophage predictions using PHASTER (1). The program uses GC content, presence of repeated elements, and reference phages to make the predictions. It classified the predictions in three prophage groups: intact, questionable, and incomplete. A high proportion of questionable and incomplete prophages was obtained (> 1000 genome segments) in about a half of the 504 genomes. In contrast, 320 intact prophages were predicted in 216 genomes. These predicted prophages consist of regions of about 20 and 40 kb in length. To understand the distribution of intact prophages in *Rhizobium* species, we constructed a bacterial phylogenetic tree using 33 conserved ribosomal proteins. Most of the prophage predictions cover great part of the *Rhizobium* species. However, there were less intact prophages predicted for strains of *R. etli* and *R. phaseoli.*

Analysis of the gene content of the predicted prophages indicates that they lack of key lytic and lysogenic genes. This finding could explain the failure to experimentally induce prophages in these species, and also implies the limitations of prophage predictions based on general parameters.

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Intra- and inter-plasmid regulation of conjugative transfer in *Sinorhizobium*

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Rhizobia are nitrogen-fixing symbionts of plants. Their genomes frequently contain large plasmids, some of which are able to perform conjugative transfer. There is evidence suggesting that conjugative transfer (CT) has impacted on the diversity of rhizobial strains. *Sinorhizobium fredii* GR64 was isolated from bean nodules in Granada, Spain. This strain contains 3 plasmids in addition to the chromosome: pSfr64a (183 Kb), the symbiotic plasmid (pSym) pSfr64b (460 Kb), and megaplasmid pSfr64c (>1000 Kb). pSfr64a and pSfr64b contain a complete set of transfer genes. Also, they contain quorum-sensing (QS) regulatory genes: *traRa* - *traIa* on pSfr64a, *traRb* - *traIb* on pSfr64b, and *ngrI* - *ngrR* on the chromosome. Although both pSfr64a and the pSym pSfr64b contain a complete set of transfer genes, CT of each plasmid depends on the presence of the other. In order to further understand the mechanisms that cause this phenomenon, we analyzed mutants in the regulatory genes of both plasmids, and determined their expression in the wild type and mutant backgrounds, by transcriptional fusions with reporter genes and by qRT-PCR.
We suggest that NgrI, or the product of another gene, regulated by NgrI, forms a complex with TraRa for the induction of the pSym transfer genes. We observed that in a derivative containg only a cloned *traRa* gene (lacking the rest of the plasmid), the pSym can perform CT, but if a mutation is introduced in the chromosomal *ngrI* gene, the strain looses its transfer ability.
In general, our results indicate that a regulatory circuit is formed between both plasmids, demonstrating that pSfr64a and the symbiotic plasmid depend on each other for conjugative transfer, suggesting that, in addition to the external signals, the "cytoplasmic environment" may pose a barrier.

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Unveiling the elusive nature of *Clavibacter michiganensis* subsp. *michiganensis* pathogenicity after a functional phylogenomics approach

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Abstract:

Clavibacter michiganensis subsp. *michiganensis* (*Cmm*) is the main cause of Bacterial Canker in tomato (*Solanum lycopersicum*), a disease that causes great losses in greenhouse production worldwide. This plant endophytic bacterium can switch from a commensal lifestyle to highly pathogenic forms, depending on the Genotype (G) x Environment (E) x Phenotype (P) relationships. Previous work has been done trying to determine specific features that confer its pathogenicity to *Cmm*, but the *formula* remains unclear. *Clavibacter* strains have been isolated from greenhouses throughout the country for a period of eight years. Genome sequencing and phylogenetic reconstructions have shown that the collected strains cluster in at least eight sub-clades, from which we selected the most representative strains according to gene family presence. Phenotypic characterization of the eight selected strains emphasize the enormous diversity within species and remarks the importance of performing studies not only on the reference strain NCPPB382 (who was isolated almost 40 years ago); but also to consider the ever-changing nature of *Clavibacter*. Since *Cmm* invades and proliferates in xylem vessels, causing gradual degradation of vascular tissues, here we investigate the importance of gene families encoding plant cell wall degrading enzymes (PCWDE). Experimental design of a protocol for massive mutagenesis in newly isolated strains from Mexican facilities is under development. These tools will lead us to characterize the differential expression and functional profiles of these selected 'model' sub-clade specific strains, to determine the role of specific PCWDE genes towards host colonization as well as other functional traits.

Bioinformatic analysis of aerobic carbon monoxide dehydrogenase from *Streptomyces thermocarboxydus*

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Carbon monoxide (CO) dehydrogenases (CODHs) are important enzymes in the CO metabolism of diverse microorganisms either in aerobic or anaerobic conditions. The enzymatic CO oxidation supplies the microorganism with reducing equivalents that are channeled into a respiratory chain. By using a *Streptomyces thermocarboxydu*s strain isolated from arid soil samples and based on the PATRIC and RUST databases, we wondered whether this microbe may have a carbon monoxide dehydrogenase to allow it to use carbon monoxide as carbon source. The results revealed the presence of an aerobic type carbon monoxide dehydrogenase complex in *S. thermocarboxydus*. Moreover, the strain showed the presence of genes also coding for the anaerobic CO dehydrogenase/acetyl CoA synthase complex. Interestingly, the CODH active site was present in the *coxL* gene, which was divided into two other groups i.e. *coxL* type I and *coxL* type II. We observed that *S. thermocarboxydus* has the *cox*L type II gene encoding a protein with the AYRGAGR sequence based on PATRIC and RUST databases. The phylogenetic tree for *cox*L was constructed based on the Neighbour joining method using Mega 7.0 version. This is the first report about a *cox*L type II in *S. thermocarboxydus*, an enzyme with strong potential for decreasing the CO pollution levels from the environment.

Keywords: Carbon monoxide, *Streptomyces thermocarboxydu*s, PATRIC, RUST, and Phylogenetic tree

Translation efficiency and codon usage bias among prokaryotes

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Introduction

The genetic code is formed by a set of molecular relationships to translate information encoded in nucleotide sequences into proteins. The principle of these relationships lies in the codons, corresponding to the main 20 amino acids that make up proteins. This code is redundant, that is, there are synonymous codons codifying for the same amino acid. However, the frequency of these synonymous codons varies between genes and genomes, a phenomenon called codon usage bias (CUB). This variation is explained by the balance between genetic drift and natural selection. When natural selection is responsible for CUB in prokaryotes the translational elongation efficiency is improved. This may have implications on the lifestyle of the organisms, e.g. cells showing larger CUB tend to show shorter generation times; this, in turn, allows these organisms to colonize specific habitats. But, why is the translation efficiency, due to CUB, being selected? In this work we aimed to answer this question at genome and gene function level, improving and developing new strategies.

Materials and Methods

We built a dataset of 210 prokaryotic genomes (including bacteria and archaea) with metrics for CUB under selection, per gene and genome. To quantify CUB under selection per genome we used a Pearson correlation between two metrics. The first one measures the effective number of codons per gene (Nc') and the other one measures the coadaptation of the tRNAs of a genome with the codons of a gene (tAI). Then, we explored the relationship between minimal generation time and CUB. Finally, we applied a gene set enrichment analysis to explore possible cellular process under CUB selection.

Results

We noticed that CUB under selection is linked to the evolutionary history, and exploring the data, we found that some phyla/subphyla tend to have larger CUB than others: Gammaproteobacteria, Firmicutes, Actinobacteria, and Betaproteobacteria. We found a significant correlation between CUB under selection and minimal generation time, but, surprisingly, it is not so strong ($rho=0.47$, $p.value<0.001$). Finally, we observed that canonical biological processes and some specific processes linked to specific lifestyles have genes with high levels of CUB (i.e. photosynthesis, methanogenesis).

Conclusions

We concluded that translation efficiency by CUB is under selection because it is important to maintain several fundamental cellular functions in some organisms depending on their evolutionary history. Generation time could be an important factor that leads to selection but is not determinant. It may conceivably be that some organisms have other strategies to improve their elongation efficiency or that the elongation step is not as important as the initiation step for protein translation.

Evolutionary and Transmission Dynamics of a highly prevalent Acinetobacter baumannii lineage (ST 758) in Mexico.

Lucía Graña-Miraglia y Santiago Castillo-Ramírez

Hospital-aquired infections (HAIs) triggered by the rapid spread of antibiotic resistance, are one of the major global public health challenges nowadays. Acinetobacter baumannii (AB) is a HAIs associated pathogen that has became one of the most frequently isolated organism in nosocomial infections around the world. The success of AB is due mainly to an extraordinary ability to develop resistance to antibiotics, mediated by all the major resistance mechanism known in bacteria. Moreover, AB outbreaks are very difficult to eradicate due to its endurance in dry surfaces which can last up to 5 months.

Even though a big effort is being made to study AB mexican populations, the first genome sequence of a Mexican MDR-AB isolate was reported quite recently. Next generation sequencing (NGS) offers the advantage of high resolution geontyping and has shown an incomparable epidemiological power, including outbreak and transmission routes analysis.

Here, we used a population-genomics approach to analyse the evolutionary dynamics of gene content over a very short period of time and from a single population. In order to do this, we sampled several strains (all from the same lineage) from two tertiary hospital in a developing country (Mexico). To put this lineage in the context of the global population of A. baumannii, we have also incorporated publicly available genomes to create one of the most inclusive data sets for this bacterium, as far as different lineages (STs) are concerned.

We found evidence of diversification of the lineage and of transmission among the facilities in Mexico City. Also, the isolates showed slight differences in their resistant profiles, and the acquisition of a new OXA-23 family allele that could be directly linked to the expansion of this lineage. Gene content variability was shown to be a major source of genetic variation that takes place much faster than the accumulation of de novo mutations in bacterial populations at very early stages of diversification. This is the first time a NGS approach is used to analyze transmission dynamics of a prevalent Mexican AB lineage.

Identification of the gene involved in biosynthesis of polysaccharide produced by *Lactobacillus hilgardii* WKGMX in water kefir

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Water kefir is a fermented beverage consumed around the world and produced by water kefir grains. The beverage is conformed by a consortium of bacteria and yeast associated to a polysaccharide grains produced by *Lactobacillus hilgardii*. Axenically, *L. hilgardii* isolated from water kefir has only been reported associated to a viscous polysaccharide and *L. hilgardii* WKGMX isolated from Mexican water kefir is associated to a hard-gelled polysaccharide with similar morphology as water kefir grains. The aim of this study was to sequence and annotate *L. hilgardii* WKGMX genome, make a comparative genomics analysis with previously reported strains of the same species isolated from different environments and identify *in silico* the gene that are involved in the polysaccharide production. *L. hilgardii* WKGMX genome library was constructed and sequenced using the Illumina HiSeq 2000 platform, obtained 1148250 reads with a length between 35-301 and 39% GC. Raw reads quality control assessment was performed by FastQC and filtered using Trimmomatic. Filtered Illumina reads were assembled using SPAdes. The final draft assembly contained 349 contigs, totaling 2.916 Mbp in size. Phylogenomic tree was performed and revealed that *L. hilgardii* WKGMX were clustered in the clade of *L. hilgardii*, nucleotide comparison using ANI revealed that *L. hilgardii* WKGMX have 99.9% of similarity with strain type. A Markov Hidden Model was used to find the *gtf*C gene that encodes for a glucosyltransferase, is in the complementary chain and has a size of 3713 bp. Glucosyltransferase enzyme was modeled using the Swiss-model server by homology modeling, resulting model has a 67% of similarity with the crystallized model. *L. hilgardii* polysaccharide is the most important part of water kefir consortium because is the microbiota hauler to keep and start the water kefir fermentation.

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Metagenomic analyses uncover the differential effect of azide treatment on bacterial community structure by enriching a specific Cyanobacteria present in a saline-alkaline environmental sample.

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Introduction. Isabel Island is the upper part of an emerging volcano. It is located 32 km from Nayarit's coast. Its crater has a a saline-soda lake fed by rainfall that is strongly affected by evaporation. A large community of birds contributes widely with nutrients from their excretions [1].

Sodium azide has been widely used as preservative [2,3], due to its capacity to inhibite heme-containing enzymes [4]. The aim of this work was to assess the differential effect of adding sodium azide on the structure and composition of the bacterial community present in water samples of the Isabel Island's saline-alkaline lake. Metataxonomic and metagenomic (shotgun sequencing) approaches were used to address changes in the prokaryotic community.

Methods. Three water samples were collected in duplicate. On-site, sodium azide (0.015% w/v) was added to one of the samples set (WA) , the other set were left untreated (WU). Extracted metagenomic DNA from WA and WU were used for PCR-amplifications of 16S rRNA gene (V1-V3) [5]. Pyrosequencing was carried out. Bioinformatics analyses were performed using MOTHUR 1.33 [6]. Selected qualified reads were assessed with UCHIME [7]. Further assignment of OTUs was computed using Silva database [8]. Diversity metrics were calculated (quantity of OTUs, Shannon diversity index, Simpson reciprocal diversity, Pielou's eveness index, and Chao1 estimator for species richness). Principal Component Analyses (PCA) were performed with STAMP [9]. Miseq protocol was carried out to WA and WU at the FISABIO Sequencing and Bioinformatics Service (Valencia, Spain). Quality assessment was performed by using Prinseq-lite [10]. Quality pair-end reads from each library (WA and WU) were compared to selected bacterial genomes using BlastN algorithm [11]. Whole clean reads were multiply matched to the highest scoring genomes of representative genera. Data were plotted using Mummer 3.0 for whole genomes [11]. Selected genome regions were plotted and visualized using Geneious Pro software (Biomatters Ltd, New Zealand) [12].

Results. Electrophoretic analysis gave the first clue of an effect on WA. Amplicon bands for WA samples are broader and about 50 bp smaller than those of WU.

Pyrosequencing show that OTUs richness, diversity and evenness estimators were smaller in WU. WA and WU shared the same 5 major taxa at class level. Families in the bacterial communities changed; in WU, bacterial community was dominated by γ-Proteobacteria (86% of Halomonadaceae). On the other hand, WA pointed to cyanobacteria (Synechococcaceae) as dominant (53.5%). PCA at genera level, separated WA from WU samples along the axis PC1 accounting for 95.6% of the variability, while PC2 explained 3.8% of variability and was affecting mainly WU samples. Information obtained via pyrosequencing confirmed differences between WU's and WA's bacterial communities. The genome of *Halomonas campaniensis* strain LS21 recruited the most reads in WU library. Meanwhile, in WA's library the most recruited reads matched to *Synechococcus* sp. RS9917. The sequence-hit abundance for *H. campanensis* in WU was 5 times higher than WA's. The opposite happened with the hit recruitment by *Synechococcus* RS9917, resulting in 13-fold higher in the WA versus WU library.

Conclusions. The combined effect of treating samples with sodium azide and keeping them in darkness has proven effective on the selective enrichment of a cyanobacterial group. This approach may allow the complete (or nearly complete) genome sequencing of cyanobacterial strains from metagenomic DNA of different origins. The expected azide/darkness-driven enrichment of Cyanobacteria in the samples can be evaluated by a PCR targeting the V1-V3 variable regions of the 16S rRNA gene, prior submitting for shotgun sequencing.

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The essential genes of *Pseudomonas aeruginosa* are well conserved in *Azotobacter vinelandii*.

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Pseudomonas aeruginosa is a highly adaptable bacterium that thrives in a broad range of ecological niches, and in humans it is an important opportunistic pathogen in compromised individuals. On the other hand, *Azotobacter vinelandii* is able to fix nitrogen and cyst-forming, soil bacterium. It has been reported that *A. vinelandii* is very closely related to *P. aeruginosa* even though these bacteria have different biological characteristics. The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level and high-throughput genomic approaches such as transposon sequencing have been used to identify essential genes. Until now has been defined that the essential genes are in the core genome, however, it has been postulated that the essential genes not always are part of the core genome. Several studies have proposed a certain amount of essential genes in *P. aeruginosa* with several approaches and in several culture conditions. Moreover several works consider that essential genes are variable among species and among strains and non-orthologous gene displacement (NOGD) is considered as a cause of variability among essential genes.

The proteomes we used in this work were retrieved from NCBI: *P. aeruginosa* PAO1 NC_002516, *P. aeruginosa* PA14 NC_008463 and *A. vinelandii* DJ CP001157. The 352 general essential genes of *P. aeruginosa* were searched by sequence and synteny in the genome/proteome of *A. vinelandii*.

In this work we evaluate the conservation of those candidates for essential genes of *P. aeruginosa* proposed previously in *A. vinelandii* with bioinformatic approaches. We show that 342 of the 352 general essential genes are in *P. aeruginosa* are conserved in *A. vinelandii*, a nitrogen-fixing, cyst-forming, soil bacterium. Moreover, two out of the eight general *P. aeruginosa* genes that are absent in *A. vinelandii* (*nadE* and PA0092) are not essential genes, since Tn-insertion mutants have been isolated in the PAO1 strain. These results support the hypothesis of *A. vinelandii* having a polyphyletic origin with a Pseudomonads genomic backbone, and are a challenge to the accepted theory of bacterial evolution.

Transcriptome analysis of a conditional knockdown mutant in an essential gene participating in cell division and cell polarity in *Rhizobium etli* CFN42

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Rhizobium etli CFN42 is a nitrogen-fixing bean symbiont with a genome composed of a chromosome and six large plasmids (p42a-p42f). Previous work in our laboratory identified two novel essential genes on p42e (RHE_PE00001 and RHE_PE00024), which cannot be eliminated unless we provide wild-type copies *in trans*. RHE_PE00001 is a hypothetical protein with a DUF1612 domain (domain of unknown function) and a helix-turn-helix motif. RHE_PE00024 is a sensor histidine/kinase hybrid protein, participating in a two-component signal pathway. However, the response-regulator protein and the target genes for the signal pathway are as yet unknown. Both genes are highly conserved within the Rhizobiales.

In order to determine their functions, we implemented conditional knockdown (cKD) system, where expression of the corresponding gene is under the control of a cumate-regulated promoter. A cKD mutant in gene RHE_PE00024 (cKD24) revealed a striking variation in cell morphology. In the absence of cumate, 63% of the population is comprised by round cells, the remaining of the cells display the normal bacillary form.

The round cells in cDK24 are able to divide, albeit very slowly. Its offspring can be round (62%), bacillary (34.5%) and even branched (3.5%) cells. On the other hand, bacillary cells of cKD24 exhibit loss of polarity and its offspring can generate both bacilli (79.3%) and round cells (20.7%). These results strongly suggest that the decrease in the expression of RHE-PE00024 elicit problems in cell division and polarity establishment.

In order to clarify better the function of RHE-PE00024, we undertook an RNA-seq analysis of cKD24 in absence of cumate. The analysis showed 919 differentially expressed genes, of which 479 are up-regulated, and 440 are down-regulated. Notably, several genes that participate in cell cycle regulation in alpha-proteobacteria are differentially expressed, including the master regulator of cell division *ctrA*, and *popJ*, that participate in the determination of polarity and asymmetric division. Furthermore, a substantial part of the genes that participate in the peptidoglycan synthesis pathway showed a decrease in expression levels. Finally, we found several genes that can participate in cell envelope formation, such as a L-D transpeptidase, a lytic murein transglycosylase, and genes involved in the glycerophospholipid synthesis pathway. These data indicates the importance of RHE-PE00024 as a regulator in cell division, polarity and cell envelope formation in *Rhizobium etli.*

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A detailed analysis of the sets of essential genes in *Pseudomonas aeruginosa* strains PAO1 and PA14.

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Pseudomonas aeruginosa is a highly adaptable bacterium that thrives in a broad range of ecological niches, and in humans it is an important opportunistic pathogen in compromised individuals. The increased availability of genome sequences has provided the basis for comprehensive understanding of organisms at the molecular level and high-throughput genomic approaches such as transposon sequencing have been used to identify essential genes. In order to understand phylogenetically the genomic information it has been classified in three major classes. The core genome, which includes all genes responsible for the basic aspects of the biology of a species and its major phenotypic traits. By contrast, dispensable genes or accessory genome, contribute to the species diversity and might encode supplementary biochemical pathways and functions that are not essential for bacterial growth but which confer selective advantages, such as adaptation to different niches, antibiotic resistance, or colonization of a new host, is acquired by horizontal gene transfer (HGT). Given that these genes are not necessary for survival or maintenance of the species, they can also be deleted from the genome. The pan-genome is the global gene repertoire of a bacterial “species”: core genome + dispensable genome. Until now has been defined that the essential genes are in the core genome, however, it has been postulated that the essential genes not always are part of the core genome. Several studies have proposed a certain amount of essential genes in *P. aeruginosa* with several approaches and in several culture conditions. Moreover several works consider that essential genes are variable among species and among strains and non-orthologous gene displacement (NOGD) is considered as a cause of variability among essential genes. In this work we evaluate the conservation of those candidates for essential genes of *P. aeruginosa* proposed in different studies in the strains PAO1 and PA14 with bioinformatic approaches. With our analysis we propose that the conservation of essential genes is greater than expected in both strains and that the postulation of candidate essential genes based on high-throughput genomic approaches must be carefully reviewed and confirmed with other approaches as deletion of single genes. In addition, no examples of NOGD were found.

Stability of Drug Resistance in Bacterial Population.

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Most of our understanding of drug resistance evolution is based on wet-lab experiments performed in constant environmental conditions. But natural environments are heterogeneous, both in time and in space, and expose bacterial populations to a dynamic range of antimicrobial concentrations. In this work we address the following fundamental question: ¿how does evolutionary history impinges upon the stability of drug resistance? To answer this question we will use experimental evolution of *Escherichia coli* MG1655 to a beta-lactam antibiotic to obtain bacterial populations with the same phenotype (drug resistance), but with diverging evolutionary histories. In particular, we will perform serial dilution experiments with increasing concentrations of antibiotics using different selection regimes: high and mild selection (phase 1). For both treatments, we selected the populations with the highest level of resistance, we carry out the serial transfers in drug-free environments for eight days and at the same time we measure the level of resistance of each population with a dose-response curve (phase 2). Finally, we readapted these bacterial populations to the same concentrations of antibiotic than phase 1 (phase 3).

As expected, populations under strong selective pressure have an accelerated rate of adaptation compared to the mild selection regimen. Also, fitness costs associated with resistance were enhanced in the high-selection regime, suggesting that bacterial populations exposed to high antibiotic concentrations evolve highly-specific, but costly mechanisms, while intermediate drug dosages select for generic, and more stable resistance mechanisms. However, when the antibiotic is removed from the environment we observe that under the mild-selection regime the resistance is more stable maybe for the mechanism of resistance acquired and in the populations re-evolved increased their rate of adaptation and acquired resistance as fast as in strong-selection.

"Transcriptional expression and phylogenetic analysis of outer membrane protein genes (OMP) *alpA* and *alpB in Helicobacter pylori*"

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Helicobacter pylori has several virulence factors that promote colonization and persistence of infection, among the most representative virulence factors of *H. pylori* are the outer membrane proteins (OMP), which facilitate initial colonization, persistence of Infection and release of more virulence factors to host epithelial cells. Among the proteins that belong to the OMP family are adhesins, whose main function is to adhere to the human gastric epithelium. In this work the gene expression of two *alpA* and *alpB* adhesins was analyzed, using the real-time PCR technique of the planktonic phase clinics and during biofilm formation; On the other hand, evolutionary relationships are determined through a phylogenetic analysis of study adhesions in clinical and reference clinics of *H. pylori*. The sequences were aligned and evaluated to obtain the best evolutionary model and generate phylogenetic trees.
A statistically significant high-expression was observed in both genes, in the planktonic phase, in comparison, with the biofilm phase ; on the other hand, when analyzing the study strains no relationships were found considering the pathological origin of the strains, however, the results seem to respond to a grouping possibly by geographical origin.

In silico approach of potential like binding proteins of Cry *Bacillus thuringiensis* in mammals

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Abstract.

Crystalline (Cry) toxins produced by *Bacillus thuringiensis* are a multigenic family of more than 500 proteins, is strong immunogen, as well as potent carrier and adjuvant. The mechanism of immunogenicity and adjuvanticity until now remains poorly understood . However, in vivo studies using the mouse model has been proposed to occur via receptor-like molecules. Indeed it has been suggested that Cry1Ac toxin is interacting through Toll-like receptors. In general Cry toxins are formed by three domains Domain I, formed by a bundle of alfa –seven helix, domain II or the binding domain formed by anti-parallel beta-sheet and the domain III , formed also by a sandwich of beta-pledged sheets, domain which has been showed protects the fragment of proteolysis but also could participate in the binding to the receptor, that are iimmersed on the microvellosiity of the epithelial intestine. Iin the present work we aimed to approoach the study of the potential like binding proteins in mammals through an *in silico* analysis (Uniprot gene, Mega 7, Protein blast, NCBI, HGNC/,HUGO), From the data, it is tempting to think that one of the potential candidates might be the cadherins E, present in the different epithelial tissues. It is noteworthy the percent of identity and the alredy reported predicted Ca2+ binding sites (DQD, DGED, DEYD or DPD) are common with insect cadherins (MW around 220 Kda), suggesting that there is ra conservation in the structure-function of receptor like molecules between different species and that potentially we propose are exerting a key role in the Cry1 action on the host response.

Environmentally-driven gene content convergence and the *Bacillus* phylogeny

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Members of the *Bacillus* genus have been isolated from a variety of environments. However, the relationship between their potential metabolism and the niche from which these bacteria have been isolated has not been extensively studied. The existence of a monophyletic aquatic *Bacillus* group, composed of members isolated from both marine and fresh water, has been previously proposed. Here, we present a phylogenetic / phylogenomic analysis to investigate the potential relationship between the environment from which these organisms have been isolated and their evolutionary origins. To test the phyletic origins of aquatic *Bacillus*, we performed phylogenetic reconstructions with 16S rRNA genes, with a collection of concatenated proteins from a set of marker proteins, as well as with concatenated protein sequences that we detected as core proteins in our genome samples. The phylogenetic reconstructions showed that *Bacillus* strains classified as aquatic have evolutionary origins in different lineages. Although we observed the presence of a clade consisting exclusively of aquatic *Bacillus*, it was not composed of the same strains previously reported. To test for potential environmental effects on the genetic content of these bacteria, we classified the proteins encoded in the genomes of our *Bacillus* sample into functional categories. The functional categories were found by matching these proteins to known families or domains. The classification was followed by hierarchical clustering. In contrast to the phylogenetic results, clustering based on functional categories resulted in groups more compatible with the environments from which the organisms were isolated. This evidence suggests a detectable environmental influence on bacterial genetic content, despite their different evolutionary origins. In conclusion, our results suggest that aquatic *Bacillus* species have polyphyletic origins, but exhibit convergence at the level of gene content.

Characterization of local adaptations in the genus Virgibacillus through pangenomic analysis

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Extremophilic microorganisms are excellent models to look for molecular mechanisms underlying particular adaptations. In many cases, phylogenetically related microorganisms differ more across environments than across taxa. These adaptations can be recognized through functional diversification led by environmental factors. Particularly, the genus *Virgibacillus* comprises gram-positive halophilic bacterial strains which genes could reveal osmoadaptation mechanisms and also those genes involved in various unique metabolic pathways. In this work, we performed a pangenomic and functional analysis of the genus *Virgibacillus* with 34 genome samples available in public databases. Likewise, a new *Virgibacillus* genome recently isolated from a site with high salinity in Cuatro Ciénegas, Coahuila was assembled and incorporated to the analysis. We obtained the pangenome and performed the functional analysis of the exclusive genes. Furthermore, we gathered information from the environmental variables corresponding to the sampling sites of each reported organism. Additionally, we built a phylogeny based on all the genomes to understand the relationship between its functions, environment and evolutionary history. From the pangenomic analysis, we got a total of 20,430 genes in three fractions; 794 core genes (4%), 7,795 shared genes (38%) and 11,841 exclusive genes (58%). Furthermore, the exclusive genes of each individual (singletons) were functionally characterized, which represent potential local adaptations to the environment and will provide relevant information on the ecology and evolution of this genus.

Genomic analysis reveals genetic markers that can be used for the specific identification of *Campylobacter fetus*, an important livestock pathogen

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Campylobacter genus groups Gram-negative, micro-aerophilic and curve-shaped epsilonproteobacteria. Nowadays, there are 32 recognized *Campylobacter* species, which can infect different hosts. *C. fetus* is a pathogenic species for humans and livestock. In cattle and sheep, *C. fetus* normally inhabits the gastrointestinal tract, but it can produce genital campylobacteriosis, an illness associated with intertility and abortion; therefore, *C. fetus* is a veterinary pathogen of great economic importance. A timely diagnosis is key for the proper treatment of any pathogen. To date, there are several phenotypic tests and molecular typing methods available to identify *C. fetus*; however, the bulk of them are impractical since require bacterial cultures and are time-consuming or laborious. Hence, a rapid and easy-to-use method is necessary for the identification of *C. fetus*. At present, the developed sequencing technologies have permitted to know the whole-genome sequence of many bacteria, including *Campylobacter* spp. On another hand, there are distinct bioinformatic tools that can be used to compare the whole-genome sequence of different bacteria, which can lead to the identification of species-specific nucleotide sequences (genetic markers). The discovery of species-specific genetic markers is crucial for the development of molecular methods for the rapid and easy diagnosis of pathogenic bacteria, based for example in the application of techniques such as PCR on clinical samples.

In this work, we aimed to identify genetic markers for *C. fetus*. Firstly, we determined the pan-genome of *Campylobacter* spp., by using the open-source software GET_HOMOLOGUES and 237 publicly available genomes spanning 21 species of the *Campylobacter* genus. Homologous clusters were generated using three clustering algorithms: BDBH (BiDirectional Best-Hit), COGtriangles (Clusters of Orthologous Groups) and OrthoMCL (Ortho Markov Cluster), which revealed the cloud (7,976 gene clusters), shell (3,282 gene clusters), soft-core (806 gene clusters) and core (261 gene clusters) genome of *Campylobacter*, as well as species-specific genes. For *C. fetus*, we found 103 potential specific genes (based on nucleotide sequences), including genes annoted as hypothetical proteins (57), genes encoding virulence factors (5), secretion systems factors (4), membrane proteins (3), among others. Blastn analyses, with default parameters, confirmed the presence only in *C. fetus* of some tested specific-genes. Thus, some of the *C. fetus* specific-genes identified in our study have the potential to be used as genetic markers for diagnosis of this bateria.

Gut microbiome from *Goepherus berlandieri* tortoises contain nitrogen fixing *Klebsiella variicola*

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In termite guts there are nitrogen-fixing bacteria that help insects feed on wood which is nitrogen-poor. Similarly, other animals which feed on plants may benefit from nitrogen fixation in guts. Here we report that herbivorous tortoises that feed on cacti contain nitrogen-fixing *Klebsiella variicola* in their feces. Acetylene reduction activity to measure nitrogenase was detected in feces from seven adult tortoises. From feces, nitrogen-fixing bacteria were cultured in a nitrogen-free medium under microaerobic conditions. Bacteria recovered were identified as *Klebsiella variicola* using 16rRNA gene sequences, and all showed acetylene reduction activity. Under nitrogen-free conditions and microaerobiosis, an exo-metabolomic analysis revealed that a nitrogen-fixing *K. variicola* isolated from tortoises was capable of excreting alanine. This is remarkable as rhizobia may excrete alanine as bacteroids and this would avoid ammonium toxicity. Additionally, a metagenome was obtained from *Goepherus berlandieri* feces. The most abundant bacteria in the tortoises gut were clostridia as in other reptiles. In the metagenome the only nitrogenase *nif* genes obtained corresponded to *K. variicola*. The *K. variicola* genome recovered from the feces metagenome showed that it contained fewer virulence genes than those from *K. variicola* strains that have been reported previously. We discovered and named *K. variicola* in 2004 from plant and clinical isolates in Mexico and since then these have been reported in many countries and their virulence determinants and antibiotic resistance genes have been analyzed from many genomes available.

Cophylogenetic analysis suggests cospeciation between scorpions and their Mollicutes symbionts

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Scorpions are ancient arachnids which originated around 430 million years ago and colonized most landscapes on Earth. They use venom to capture their prey, as well as for protection against predators. Some species are very poisonous to humans, but the components of their venom are being studied to be used as antibiotics, against immunological diseases or as bioinsecticides. Despite its importance, there is a lack of knowledge on scorpions' symbiotic bacteria which could participate in nutrient uptake and synthesis, toxin production or degradation and protection against pathogens. It is the aim of this work to study the presence of these symbionts in different groups of scorpions.

We had previously described two new Mollicutes lineages in the guts of *Centruroides limpidus* (Buthidae) and *Vaejovis smithi* (Vaejovidae), Scorpion Group 1 (SG1) and a scorpion Mycoplasma clade (SMC) (Bolaños *et al*., 2016). In this work, we analyzed the presence of these bacteria in 23 scorpion morphospecies by sequencing 16S rRNA PCR products obtained with directed primers. We found SG1 only in Vaejovidae, however SMC was present in some Vaejovidae and Buthidae species. Particularly, 43% of *Vaejovis smithi* individuals had both symbionts. We did not find these bacteria in scorpions from the families Carboctonidae, Euscorpiidae and Diplocentridae.

Additionally, we analyzed whether a cospeciation process could have occurred between scorpions and these bacteria. For this purpose, we also sequenced scorpion genes (CO1, 16S rRNA and 18S rRNA) and compared the hosts and symbiont phylogenies. These analyses suggested that both, SG1 and SMC may have cospeciated with their hosts. However, a host switch of SG1 may have occurred and some scorpions may have lost their symbionts (Bolaños et al., 2019). Cospeciation may indicate bacterial vertically transmitted from mother to offspring. A metagenome analysis is under analysis to help us understand the symbiont role in scorpions.

Bolaños *et al.* (2016). Environmental Microbiology 18(5): 1364-1378. doi: 10.1111/1462-2920.12939
Bolaños *et al.* (2019). PLoS One 14(1): e0209588. doi: 10.1371/journal.pone.0209588

Whole genome sequencing of *Mycolicibacter kumamotonensis*: in search of structural and functional characteristics of this potentially pathogenic microorganism.

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Mycolicibacter kumamotonensis (*Mk*) is a slow-growing non-tuberculous mycobacterium (NTM), that was first described in Japan in 2006. The cellular and molecular factors involved in the host-pathogen relationship of *Mk* are poorly known and its genome has not been completely sequenced yet. Therefore, the objective of the present work was to sequence the whole genome of *Mk* in order to describe some new structural characteristics that allow us to further understand its pathogen-host relationship. *Mk* CTS7247 genomic DNA was purified using standard protocols and sequenced by the NextSeq500 automated system of the Illumina platform. Sequence quality analysis was performed using the FastQC v0.11.8 and One codex software, then the *de novo* assembly of the sequences were performed using the Velvet v1.2.10 tool. Phylogenomic analysis (where sequences of genomes belonging to different members of the *Mycobacteriaceae* Family were included) using the VAMPhyRE program, generated a virtual genomic footprint that allowed us to make a phylogenomic tree using the MEGAX program. Subsequently, the sequences assembly files were analyzed by the MAUVE and BRIG tools in order to show the presence, absence, truncation and variation of those sequences. In parallel, the notation of genes was performed using the RAST server, which identified some putative genes that code for *M. tuberculosis* virulence factors homologues. The sequencing results on the Illumina platform indicated: 1) a depth of 350, 2) a total of 135 contigs, and 3) a genome size of 4,643,668 bp with a 67% content of G+C. In addition, an N50 value of 41,141 and an L50 value of 35 were determined. According to the phylogenetic tree obtained, the closest neighbor was *Mycolicibacter sinensis,* whose genome was used to perform multiple alignments with the MAUVE tool to verify the proximity between both species. When performing the genome notation on the RAST platform, a percentage of functional gene allocation coverage of 77% was obtained with 914 genes assigned. Using the BRIG tool, some putative genes related to virulence were identified, such as *rpf*B, *fad*D21, *hsp*X, *fts*Z, Rv2660c, *mmp*L7, *dos*R, *tgs*1, *lip*Y, Rv3312A, *gro*EL, *ksh*B and *esax*A. The identification of those virulence genes, previously reported for *M. tuberculosis,* in an NTM suggests that most mycobacteria possibly share similar infection mechanisms. We are currently investigating some additional structural and functional features that may contribute to the pathogenesis of this mycobacterium species.

Genomic Diversity of Bacteriophages Associated to Rhizobium a Nitrogen-Fixing Bacteria

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Bacterial viruses or bacteriophages are the most diverse and abundant biological entities on earth. They play a major role in bacterial ecology and evolution, by facilitating horizontal gene transfer and controlling the density of bacterial populations. Much of the diversity and ecology of bacteriophage remains unexplored. There are few studies dedicated to analyze the diversity of phages associated with rhizobial bacteria (1, 2). The Genbank contains only 37 complete genome sequences of *Rhizobium* phages compared with the reports of *E. coli* or *Pseudomonas* (660 and 498 phage genomes respectively). Therefore, is our interest to extend the knowledge about the genomic diversity of the phages that infect *Rhizobium*.

In this work, we isolated phages from soil using the enrichment protocol implemented in a high throughput 96 wells format (3). The phages were isolated from soil of common bean (*P.vulgaris*) agriculture plots from Central Mexico (Tepoztlán, Yautepec and Xoxocotla), and the Northwest Argentina (Salta and Chicoana). We isolated a total of 297 phages by their lytic properties on different sets of *Rhizobium* strains. We obtained the genome sequence of 127 of these phages. Phage genomes have a wide range of length, from 4.8 to 207 Kb, most common length between 40-70 Kb. Whole genome average nucleotide identity (ANIm) comparisons clustered the phage genomes in 18 phage genomic types (PGTs), whereas 22 phage genomes were unique. Most of the phages are Caudovirales; 31 belong to the Myoviridae family, 40 to the Podoviridae, and 28 to the Siphoviridae family. There were 28 small genome phages assigned to the Microviridae family. They represent a new phylogenetic branch within Microviridae. This study uncovers part of the diversity of rhizobial phages expanding the number of known phages. However, a more complete picture of such diversity will come by employing metagenomic approaches, which is our perspective and next objective.

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Study of enzymatic promiscuity at the enzyme level, family and metabolic pathway, and its role in genomic mining of natural products

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Abstract:

An enzyme is promiscuous when catalyzes more than one chemical reaction. Promiscuity is part of the evolutionary process providing material for metabolic innovation, as it is required in the synthesis of natural products (NPs). Often, this innovation is mediated by gene expansions because of gene duplications and horizontal gene transfer, which in turn leads to new enzyme families. Here we address promiscuity by distinguishing between promiscuous enzymes and promiscuous families. Then we generalize the concept of enzyme promiscuity to a biosynthetic gene cluster (BGC) responsible for the synthesis of natural products. To infer promiscuity in prokaryotic (pan)genomes we aimed at developing a series of bioinformatics tools: (i) Orthocore, which seeks to understand gene families preserved without expansions in a taxonomic lineage; (ii) EvoMining, directed to detect chemical innovation through the divergence of families from conserved metabolism. To do so, EvoMining finds families that do have extra copies and organizes these variations in possible metabolic destinations; and (iii) CORASON, with a level in organizational complexity higher, and seeks to understand the variation of groups of genes (BGCs). Scytonemin and detoxin BGCs, in Cyanobacteria and *Streprtomyces* respectively, are shown as examples of the production of molecular variations by BGC families. In-depth bioinformatics analyses, which in some cases were validated by experimental metabolomics analyses, will be presented. Overall, our results not only provide an state-of-the-art genome mining platform, but they help to decipher the metabolic origin and fate of enzymes through the course of evolution.

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Two genetic variants of a D6-like plasmid-prophage are associated with specific IncA/C plasmid types in the emerging *Salmonella* Typhimurium ST213 genotype in Mexico

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The *Salmonella enterica* serovar Typhimurium sequence type 213 (ST213) emerged as a predominant genotype in Mexico. In a previous study, ST213 strains were found both in ill and healthy humans, as well as in pork, beef and chicken meat sources, from four representative geographic regions across the country (Sonora, San Luis Potosí, Michoacán and Yucatán). ST213 strains were characterized by the presence of multidrug resistance (MDR) IncA/C plasmids and the lack of the *Salmonella* virulence plasmid (pSTV) (1, 2). The complete genome of ST213 strain YU39 showed a 89 kb plasmid (pYU39_89) containing bacteriophage-related genes (3), suggesting the presence of a lysogenic phage with a plasmid lifestyle, similar to the *Escherichia coli* P1 and D6 prophages.

Here we show that a D6-like plasmid-prophage is present in most of the Mexican ST213 strains. We used the nucleotide sequence of pYU39_89 to design a PCR typing scheme for the detection of D6-like prophages in the Mexican Typhimurium strain collection. Moreover, the complete nucleotide sequences for the D6-like prophages of three additional ST213 strains were determined. Genome comparisons showed two prophage variants: i) a complete prophage, containing homologous sequences for most of the genetic modules described in P1 and D6 phages, which most likely allow for the lytic and lysogenic lifestyles; and ii) an incomplete prophage, lacking a 15 kb region containing morphogenesis genes, suggesting that it is defective.

The tail fiber gene inversion region was the most divergent one between the *E. coli* D6 and the Typhimurium D6-like genomes, suggesting the production of a distinct set of tail fibers, which could be involved in host range preferences. It was intriguing to find a glutaminyl-tRNA synthetase gene (*glnS*) as part of the D6-like prophage variants (p89 or p75) in the Mexican ST213 Typhimurium population and other D6 genomes, which can be regarded as a moron (cargo gene) since it is related to cell host functions more than phage or plasmid functions. Statistically significant associations were found between the two prophage variants (p75 or p89), the type of IncA/C plasmids (I or II) and geographic isolation regions (Sonora, San Luis Potosí, Michoacán and Yucatán), suggesting a genetic structure composed of locally adapted subclones.

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Comparative genomics of bacterial endosymbionts of the Mexican medicinal leeches

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Summary

Endosymbiosis is perhaps the most extreme instance of symbiotic association since both, endosymbiont and host, obtain mutual benefits and share evolutionary histories. Endosymbiotic bacteria have been traditionally studied in sap-feeding and blood-feeding insects, but similar models are found in hematophagous leeches harboring bacteriomes, of the genus *Haementeria*. To date, only the genome of *Haementeria officinalis* endosymbiont (*Providencia siddallii*) has been sequenced; this genome shares features with insect endosymbionts such as increased A-T content and genome reduction, coupled with the loss of functions, but maintaining only those genes implicated on solving nutritional deficiencies (e.g. supplying nutritional vitamins of the B complex, absent in vertebrates blood). In the present study, leeches of two species of *Haementeria* were analyzed: *H. acuecueyetzin* from Tabasco, Mexico and *Haementeria* sp. from Panama (*Ha*. Panama), in order to isolate the bacteriomes and characterize their respective bacterial endosymbionts. Total DNA from bacteriomes were extracted and sequenced using Illumina HiSeq X then, reads were processed and filtered. Reads were assembled using SPAdes 13.3.0 and annotated in the MG-RAST web server. The *H. acuecueyetzin* endosymbiont genome resulted in ~780 Kb size and 23% G+C. Distinct features were showed in Ha. Panama endosymbiont resulting in ~1.2 Mb genome size and 44% G+C content. 16S analyses indicate that endosymbiont of *H. officinalis* and *H. acuecueyetzin* pertain to the genus *Providencia* whereas bacterial endosymbiont of Ha. Panama is related to *Cronobacter*. Ha. Panama endosymbiont genome features suggest a recent association, probably a secondary symbiont substitution. Preliminary annotation of both genomes show a high proportion of metabolic gene functions, highlighting the presence of vitamins of the complex B biosynthetic pathway (suggesting a relevant role of these genes in such mutualism), also information storage and processing genes were substantially represented, cellular process and signaling functions are also found but less abundant. Comparison between *P. siddallii* and the endosymbiont of *H. acuecueyetzin*, and free-living *Providencia* genomes suggest the loss of functions in endosymbiotic genomes that give insight of gene loss process suffered, having confined endosymbionts to intracellular lifestyle by losing genes involved in chemotaxis, motility and signaling, but maintaining an extent of independence as a living cell with genes that make the symbiont able of achieving nearly complete pathways of energetic metabolism and respiration. In addition of B vitamins synthesis, protein degradation could play an important role in this symbiotic association making available amino acids from blood proteins ingested by the leech host. On the other hand, comparative analysis among endosymbiotic *Providencia* and *Cronobacter*-like genomes would shed light on evolutionary convergences in two independent endosymbiotic lineages of a monophyletic group of leeches, by identifying their core-genome.

How to live under strong selection pressures and be successful? The case of *Pseudomonas mendocina* P6115, a bacterium isolated from mine tailings

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Wastes from the mining industry represent a potential source of pollution at different levels. These sites are an extreme environment of anthropogenic nature, with high selection pressures that reduce existing biodiversity. However, such environments can be colonized and allow the proliferation of microorganisms with high genetic potential which could be used for bioremediation by bioaugmentation and acceleration of natural attenuation. *Pseudomonas mendocina* P6115 is a cyanotrophic bacterium isolated from mine tailings from Durango, Mexico, among its phenotypic characteristics are the siderophores production in presence of moderate concentrations of heavy metals, arsenite and arsenate tolerance and the abilities to oxidize arsenite and growth with metal complexes of cyanide as sole nitrogen sources. The aim of this study was to sequence and annotate *P. mendocina* P115 genome and identify *in silico* related genes with importance in the survival of mine tailings environment. *P. mendocina* P6115 genome library was constructed and sequenced using the Illumina HiSeq 2000 platform, obtained 1739183 reads with a length between 35-301 and 62% GC. Raw reads quality control assessment was performed by FastQC and filtered using Trimmomatic. Filtered Illumina reads were assembled using SPAdes. The final draft assembly contained 354 contigs, totaling 4.97 Mbp in size. Phylogenomic tree was performed and revealed that *P. mendocina* P115 were clustered in the clade of *P. mendocina*, nucleotide comparison using ANI revealed that *P. mendocina* P6115 have 87.68% of similarity with strain type. Regarding the genes related to the metabolism in the mine tailings were found resistance genes to cobalt, zinc and cadmium, mercury reductases, genes of tolerance to copper, membrane transporters of nickel, copper and cobalt, as well as genes related to cyanate hydrolysis, nitrogen metabolism (denitrification and assimilation of ammonium) and the genes encoding for a cyanide-insensitive oxidase, in addition to oxidative stress response genes. This genetic potential highlights the importance of *P. mendocina* P6115 as bacterium with the ability to colonize, surviving and bioremediate mine tailings.

Plant Cell Wall Degrading Enzymes diversity of Mexican *Clavibacter michiganensis* subsp. michiganensis strains.

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Abstract:

Clavibacter michiganensis subsp. *michiganensis* (Cmm) is a Gram-positive actinobacterium known as the causal agent of bacterial canker in tomato (*Solanum lycopersicum*). Genes encoding Plant Cell Wall Degrading Enzymes (PCWDE) are known to be important for the development of disease symptoms. However, analyses of Cmm strains isolated around the world show overall different occurrence profiles of genes related to pathogenicity, which might influence their virulence. Furthermore, proteomic and biochemical analyses indicate that the presence of a peptide signal sequence for secretion is essential for the factors encoded in these genes to be able to cause symptoms. An analysis of the diversity of PCWDE has never been done with Mexican strains. We performed a genomic analysis focused on PCWDE present in Cmm strains isolated throughout Mexico in a period of eight years (n=150). A genome database was constructed with data from Mexican Cmm strains and selected *Clavibacter* species. Phylogenetic reconstructions showed that Mexican Cmm strains are clustered within 8 distinct lineages. PCWDE sequences were identified as distinctive feature of most of these clusters, and scanned for the presence of the signal peptide sequence. PCWDE genes were clustered into gene families after a pairwise sequence comparison analysis, and Cmm strains showing different PCWDE occurrence profiles, different between them and with regards to other *Clavibacter* species, were identified. Interestingly, the presence of the signal peptide in genes from the same PCWDE families is not homogenous amongst all the Cmm strains. Our analyses provide relevant information about local strains that might direct future studies of Cmm populations and the tomato bacterial canker in Mexico with functional implications.

HOST PATHOGEN INTERACTION

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Associated bacteria to urethritis in men who have sex with men (MSM)

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Introduction

Urethritis in men is characterized by discharge, dysuria and urethral itching, but it may be also asymptomatic. This pathologie is mainly due to sexually transmitted pathogens (Shahmanesh *et al*., 2009). Urethritis is described as either gonococcal (GU), when *Neisseria gonorrhoeae* is detected, or non-gonococcal urethritis (NGU), when it is not identified. Widespread antimicrobial resistance (AMR) in highly variable strains of *N. gonorrhoeae* has continuously compromised the management and control of gonorrhea. Because of widespread AMR, the World Health Organization (WHO) ranked to *N. gonorrhoeae* as the second priority level of study (Wi *et al*., 2017).

Methods

Urethral exudates were collected from patients being treated at the Clínica Especializada Condesa (CEC) of the Secretaria de salud from Mexico City. Written informed consent was obtained from all participants of the study, in addition to collection of demographic, clinical and risk data from an epidemiological questionnaire. Identification of etiologic agents was realized using MALDI-TOF MS technology. Detection of *Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium* and *Ureaplasma urealyticum* were by PCR. Isolates of *N. gonorrhoeae* were analyzed by disk diffusion test following the CLSI-2018.

Results

In the 72 % (73/102) of the cases of urethritis the etiologic agent was identified, while in 28 % (29/102) the etiology was not known (idiopathic urethritis). The predominant disease found was the GU, accounting for 43 %, the frequency for the NGU was 22 %, and 7 % UG and NGU (coinfection between *N. gonorrhoeae* and another etiologic agent). Twelve different etiologic agents were identified included *N. gonorrhoeae* (50 %) *M. hominis* (5%), *Enterococcus faecalis* (5%), *Escherichia coli* (4%), *U. urealyticum* (4%), *M. genitalium* (4%) among others.
51% of isolates of *N. gonorrhoeae* were resistant to azithromycin, 70 % to penicillin, 89 % to ciprofloxacin, 30 % to cefixime, 25 % ceftriaxone, 8 % cefatoxime and the 100 % to doxycycline and tetracycline.

Conclusion

The most frequent etiologic agents was *N. gonorrhoeae,* 39% of the strains were multiresistant (tetracyclines, fluoroquinolones and third generation cephalosporins).

Effect of metabolites produced by *Bacillus atrophaeus* on the activity of enzymes involved in the defense system of Hass avocado (*Persea Americana*)

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The avocado is one of the crops with great economic importance for Mexico. During 2018, a national production of 2, 186, 376 tons was obtained. However, the production of this fruit can present some phytosanitary problems, such as anthracnose, whose causative agent is the fungus *Colletotrichum gloeosporioides*. The anthracnose disease generates large economic losses. The synthetic fungicides application is the most used method for their control; however, the excessive use of these, cause damage to the environment and the human health, have a long period of degradation and generate resistance by the phytopathogens. It is because that the search for new control alternatives is necessary, such is the case of rhizobacteria PGPR (*Plant Growth-Promoting Rhizobacteria*), highlighting the genus *Bacillus sp*. As biocontrol strategies, *Bacillus* is able to induce the defense system in plants. Recently, it was reported that *B. atrophaeus* produces metabolites capable of reducing the severity and incidence of anthracnose in soursop and avocado fruits.

In this project was evaluated *in vivo* the effect of metabolites produced by *B. atrophaeus.* The severity and incidence of the disease in avocado fruits treated with bacterial supernatant was carried out. Additionally, the activity of enzymes involved in the defense system of the fruit, treated with metabolites and challenged with *C. gloeosporioides*, was evaluated using the methodology proposed by Tian *et al*. 2006, with some modifications. The results obtained were analyzed using an ANOVA with a significance level of 95% and the means were compared using a Fisher LSD test.

The severity of anthracnose disease after the application of bacterial metabolites was reduced by 64.19% in avocado fruits. At the same time, a reduction of 50% was observed in the incidence of the disease. On the other hand, the enzymatic activity of Polyphenol Oxidase (PPO) and Peroxidase (POD) was performed. The avocado fruits treated with bacterial metabolites and challenged with the phytopathogen, showed an increase in the activities of these enzymes (between 1.84 times for PPO and 1.56 times for POD, at day 2 compared to the control), presenting significant differences in comparison with the controls.

Our results suggest that bacterial metabolites could exert an inducing effect on the defense system of the fruit, which could contribute to a greater resistance of the fruit to the disease caused by *C. gloeosporioides*, helping to reduce post-harvest losses of this fruit.

INFECTION OF *Phratylenchus spp*. IN ROOTS COLONIZED BY NATIVE ARBUSCULAR MYCORRHIZAL FUNGI OF *Zea mays* L.

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Pratylenchus spp., known as the "radical lesion nematode" causes considerable damage in agriculture, in contrast to the arbuscular mycorrhizal fungi, which are in symbiosis helping the plants to obtain nutrients that compensate for the loss of the root surface caused by the nematodes. The soil's characteristics influence the colonization percentages of both the nematodes and the arbuscular mycorrhizal fungi. In our work, the percentage of nematode eggs as well as arbuscular mycorrhizal fungi was determined in the roots of plants from nine plots in three phenological stages of corn (V6, flowering and senescence) the percentages were correlated with the different soil conditions of the parcels. The results showed a higher percentage of colonization in the phenological stage V6 by the nematode eggs, while the highest percentage of colonization of mycorrhizal fungi occurred in the flowering stage. The Pearson's correlation coefficient indicated that the percentage of clay and organic matter in the soil affected the percentages of nematode eggs in the roots, for the arbuscular mycorrhizal fungi correlation was found with the following soil characteristics: pH, % total carbonate, phosphorus, Ca, Ca / Mg, Mg / K. It is concluded that both nematodes and mycorrhizal fungi respond to specific soil characteristics, the presence of mycorrhizal fungi being important for the plants to reach maturity.

ARBUSCULAR MYCORRHIZAL FUNGI PROTECT PLANTS OF HEAVY METALS

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The Arbuscular Mycorrhizal Fungi (AMF) offer an effective mechanism of phytoremediation, because during the symbiotic interaction the radical system of the mycorrhizal plants increases and with this the potential to take the heavy metals. The transport of Heavy Metals (MP) through the hyphae of fungi and in addition to the possible existence of transporters in the membrane of the arbuscles, that export heavy metals towards the area of intimate contact between the fungus plasma membrane and the radical cell, can reduce the uptake of MP in the plants. In the present work, different AMFs were propagated in plants corn trap (Zea mays L) in order to obtain enough propagules, both from spores, hyphae and colonized roots, to later be used as protection of MP in plants of agricultural interest, for bioremediation purposes. Four types of inoculum were used: a) *Rhizophagus irregularis* MUCL; b) *Gigaspora gigantea* c) Inoculum of native soil and d) Inoculum of soil contaminated with chromium, under an experimental design that consisted of 10 pots per inoculum. Afterwards, the determination percentage was determined. The results showed that all root samples reported colonization by structures of HMA (hyphae, arbuscles, vesicles, external mycelium and coils), presenting greater percentage of colonization in plants colonized by *Rhizophagus irregularis* MUCL with a percentage of 77%, followed by the *Gigaspora gigantea* inoculum that report a colonization percentage of 72%, and the inoculum from the soil of corn, which reached a percentage of 51.1% compared to inoculum samples of contaminated soil that presented 43.9% even though chromium was present in soil.

The role of different amino acid residues of the SehB antitoxin on the virulence of *Salmonella enterica* serotype Typhimurium

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Abstract. *Salmonella enterica* serotype Typhimurium is the causal agent of gastroenteritis and localized diarrhea in humans. In Balb/c mice, this microorganism causes a systemic infection similar to that produced by *S*. Typhi in humans. *S*. Typhimurium genome codes for SehAB, a type II toxin-antitoxin system, where SehA is the toxin and SehB is the antitoxin. It has been reported that the absence of the SehB antitoxin dramatically decreases the virulence of *S*. Typhimurium in the mouse model. To characterize the SehB antitoxin, amino acids conserved were identified and eight amino acids residues were mutagenized. The effect of the eight point mutations of SehB antitoxin was evaluated *in vivo* on the virulence of *S*. Typhimurium, by transformation of the Δ*sehB* strain with different mutant constructions. Competitive indexes showed that all point mutations of the SehB antitoxin were impaired in the colonization of *S*. Typhimurium in spleen, causing attenuation of the bacterial virulence in mice. These data show the functional relevance of these amino acids in the SehB antitoxin during the pathogenesis of *S*. Typhimurium.

Analysis of the expression of SNAP23 and SNAP25 proteins during infection in macrophages by *Brucella melitensis*.

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Brucellosis is a chronic infectious disease caused by different species of *Brucella* spp; produces infertility, abortion and febrile septicemia in various domestic and wild animals. In humans it produces undulating fever and disability due to which it is listed as one of the most important bacterial zoonoses in the world, according to the FAO (Food and Agriculture Organization), WHO (World Health Organization) and the OIE (Office International des Epizooties). Although it is not a fatal disease, approximately 500,000 new cases are reported annually in humans (1). All species of the genus *Brucella* spp behave as intracellular and extracellular pathogens, a crucial aspect in the pathogenesis of the disease since the ability to invade and survive within phagocytic and non-phagocytic cells allows it to resist the action of defense mechanisms of the host (2), but the mechanisms by which *Brucella* modifies the intracellular environment of the macrophage to avoid its destruction in lysosomes have not been clarified in detail. These processes involve membrane fusion processes which require proteins from the SNARE family that regulate vesicular transit (3). In our research group we hypothesize that various intracellular life pathogens, such as *B. melitensis*, can alter the expression of SNARE proteins such as SNAP23 and SNAP25, to promote their establishment within cells. Macrophages of the THP-1 cell line (previously differentiated with PMA) were infected. Once the macrophages were obtained, they were infected with *Salmonella* serovar Enteritidis ATCC 49214 (as control) at MOI of 100: 1 for 1 hour. After this time, gentamicin cells were added to a concentration of 100 µg / ml to eliminate bacteria that were not phagocytosed. Subsequently, total RNA extraction was performed at 15, 30, 45 and 60 minutes to quantify the expression of the genes of interest using qPCR. It was found that in the case of SNAP23 at 60 minutes after *Salmonella* infection the amount of transcript increases indicating that the presence of the bacteria modifies the expression of the gene. With respect to SNAP25 at 30 and 45 minutes post infection, transcript levels decreased in the presence of *Salmonella*, indicating that bacterial infection interferes with gene expression. These results suggest that *Salmonella* regulates the expression of these proteins which, in turn, may interfere with the secretion of the TNF-alpha proinflammatory cytosine at the same time as phagocytizing the bacteria.

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Induction of *Salmonella* Typhimurium expression of PDL1 on B cells is independent of the mechanisms involved in its persistence

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Salmonella enterica serovar Typhimurium (*S*. Typhimurium) infects and resides into B Lymphocyte (B cells). Our laboratory reported that infected B cells express elevated levels of PDL1 at cell surface, which leads to survival of these cells by blocking CD8 T cytotoxic lymphocyte. Bacterial genes involved in this phenomenon and those molecular mechanisms involved in PDL1 modulation in B cells are still unknown. In this work, we confirmed that three genes of *S*. Typhimurium involved in PDL1 expression in Caco-2 and HT29 epithelial cells are important for PDL1 induction in B cells. These genes are *sseL* and *ssrA* encoded in *Salmonella* pathogenicity Island 2 (SPI-2) and *spvD* encoded in a plasmid. In addition, we also described that *S*. Typhimurium is able to replicate and persist within B cells; since more than 90% of the bacteria that enter persisted after 72 hours post-infection. Similar *S*. typhimurium persistence was observed when B cells were infected with *sseL, ssrA* and *spvD* mutants. In line persistent *Salmonella* subpopulations were not susceptible to Ciprofloxacin and Cefotaxin treatment, antibiotics that inhibit bacteria during replication, and also genes regulating PDL1 induction were not involved in the persistence phenotype. Overall these results suggest that *S*. Typhimurium induction of PDL1 and persistence are due to two independent mechanisms.

Rhizobium rhizogenes to transform *Capsicum annuum* and protection against root pathogens using bacteria or fungus as biocontrol agents

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Transformation with *Rhizobium rhizogenes* has impacts on metabolism and root morphology in plants. Greater number and length in the roots can imply greater explored soil and greater possibility of water reach. Changes in metabolism in plants with transformed roots can also impact the interaction with microorganisms of the rhizosphere. The object of this work is the variability analysis in the response of chili plants with roots transformed independently with three strains of *R. rhizogenes*, in the accumulated biomass and in the response in the interaction with bacteria or fungus biocontrol agents expressed in the protection against pathogens causing root rot, and the expression of a defense gene. Strains K599, ARqual and A4 of *R. rhizogenes* were used to transform in root and generate composite chili plants. 28 days after transformation, root and foliage biomass accumulation were quantified, and the index of plant disease was determined inoculated in root independently with three bacteria and a fungus strains as biocontrol agents, at the same time, after biocontrol agent, *Phytophthora capsici* and *Rhizoctonia solani* pathogens were inoculated. Sesquiterpene cyclase gene induction was also analyzed. The experiments performed *in vitro* and in greenhouse. In *in vitro* assays, dry root and foliage biomass accumulation in plants with transformed roots, in the three cases outstands the treatments when was inoculated with binucleate *Rhizoctonia solani* strain as biocontrol agent; ARqual and K599 surpass to A4 *R. rhizogenes* transformed plants. In the protection against pathogens, the root transformed with any of these three *R. rhizogenes* strains, the inoculation with binucleate *R. solani* avirulent biocontrol agent gave the better results; meanwhile 2A-2B, 2A-10A and 3A-6A bacteria protect with variable results between treatments of *R. rhizogenes* transformed plants. Sesquiterpene cyclase gene induction take place in 12 and 24 hpi in plants transformed with K599 or ARqual *R. rhizogenes*, in interaction with bacteria or fungus biocontrol agents, this situation unseen when the root did not transform. In greenhouse conditions, in the root dry mass accumulation, transformation with ARqual or K599 then inoculated with avirulent binucleate *R. solani* or 2A-2B *Bacillus velezensis* bacteria outstand over the A4 strain of *R. rhizogenes* transformation and other bacterial biocontrol agent inoculation. Root transformation with any of these *R. rhizogenes* strains then the inoculation with bacterial or fungal biocontrol agent, gave in all cases effective protection against the two pathogens, except with A4 *R. rhizogenes* transformation and the 2A-2B and 2A-10A bacterial biocontrol agents. In conclusion, in greenhouse conditions, both these bacteria and fungus strains of biocontrol agents effectively protect against these two tested pathogens in plants transformed with any of these three *R. rhizogenes* strains; bacteria with some variability. The induction of Sesquiterpene cyclase gene as defense response, only in plants with transformed roots and biocontrol agent inoculation.

Distinct phenotypic and genomic characteristics of two Mexican *Pectobacterium carotovorum* strains of the subspecies *brasiliensis*

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Abstract

The soft-rot *Enterobacteriaceae i*s a diverse group of phytopathogens of economically important crops around the globe. *Pectobacterium carotovorum* has the capability to infect a wide range of hosts, with varying infectivity. Strains BF20 and BF45, isolated from Mexican cactus (*Opuntia*) and tobacco crops, respectively, differ in aggressiveness: BF45 produces more severe and rapid symptoms in comparison to BF20, in the laboratory host models celery and broccoli. Quorum sensing upregulated phenotypes related to infectivity such as polysaccharide digestion and swarming motility were expressed at higher level in BF45 suggesting a higher quorum sensing signaling in BF45, whereas biofilm formation was more abundant, and flocculation occurred in BF20. To know more about the differences between these strains we obtained their draft genomes and compared them. We identified and compared genes and gene clusters related to pathogenicity (plant cell wall degrading enzymes, quorum sensing regulation, secretion systems, flagellum and motility and secondary metabolites biosynthetic gene clusters) shared between BF20 and BF45, and the reference genomes of other *Pectobacterium* species, as well as those unique to each of our strains, which could be related to the different aggressiveness of the strains.

Vaccine efficacy of BCG in bovine tuberculosis by monitoring response to ESAT-6 and CFP-10 antigens

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Bovine tuberculosis (BTb) has a direct impact in the productive and reproductive efficiency of dairy cattle, causing major economic losses in agriculture. Nowadays, the control of the disease is based on a test and slaughter strategy, an unaffordable method for undeveloped countries, since economic resources are well below the requirements for removal of the positive cattle herds. Because of this, immunization with the BCG vaccine represent the best control alternative. However, there is the disadvantage that its use can induce reactivity to the tuberculin test in the bovine population to which it is applied. In this regard, ESAT-6 and CFP-10 antigens encoded by the RD1 region present in the species of the *M. tuberculosis* complex but absent in substrains of BCG are considered candidate antigens for differential diagnosis of vaccinated and infected cattle. The objective of the study was to monitor the protection induced by the BCG vaccine by evaluating the response to ESAT-6 and CFP-10 antigens from vaccinated calves in blood-based interferon-gamma (IFN-γ) release assays (IGRAs).Thirty-five calves were vaccinated with BCG-Phipps (10^6 CFU/1.5 ml) and had thirty unvaccinated calves, as a control group. Both groups were monitored for seven months to evaluate IFN-γ production in whole blood cultures stimulated with antigens referred. The IFN-γ released in the cultures supernatants was quantified by sandwich ELISA. T-Student was applied for the comparison of results in the different sampling times. There were no differences in levels of IFN-γ to antigens between groups during the evaluated period. However, an increase towards ESAT-6 was observed in one of the animals vaccinated at day 30 pv. In unvaccinated animals, a high and sustained production was observed for ESAT-6 in two calves, in one from the beginning of the study until day 30, and in the other, from day 7 to day 21 with oscillating values. The levels did not rise again during the rest of the study for none of the groups. The low and intermittent responses to ESAT-6 and CFP-10 may be due to the sensitization by non-tuberculous mycobacteria, several of which also contain the genes that encode these proteins and that could be circulating in the herd, since no vaccinated or infected animal was isolated *M. bovis*. According to the results, BCG vaccine had a protective effect during the assessment period, so its use for disease control would be suitable. There is the disadvantage that its use can induce reactivity to the tuberculin test in the bovine population to which it is applied. In relation to this it has been indicated that the referred antigens are suitable candidates for the differential diagnosis among cattle vaccinated from infected. Mostly, it has been shown that these antigens are important targets of T cells and strongly induces the production of IFN-γ in tuberculous cattle, so its use in diagnostic tests of IFN-γ release improves its sensitivity.

Área: Interacción patógeno-hospedero

Effect of coinfection by *Fasciola hepatica* and *Mycobacterium bovis* on bovine tuberculosis immunodiagnosis

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Parasitic infection by the *Fasciola hepatica* trematode affects the host's cell-mediated immunity (CMI), because a modulation triggered by increased IL-4 and IL-10 cytokines production during infestation. This promotes susceptibility towards infections by intracellular microorganisms such as *Mycobacterium bovis* and it may affect diagnostic tests for this infectious agent based on the celular response. Therefore, the objective of this study was to assess the impact of F. hepatica coinfection on the most commonly used immunodiagnostic bovine tuberculosis (bTB) tests in field conditions in an enzootic area for both diseases, located in Hidalgo State, México. For which, from a dairy herd displaying a 59.2% and 28% prevalence of fascioliasis and bTB, respectively. Sixty-one cows were chosen based on their response towards bTB immunodiagnostic tests of Single Intradermal Comparative Cervical Tuberculin (SICCT), gamma-interferon test (BOVIGAM) and enzyme-linked immunosorbent assay (ELISA), along with the assessment of the F. hepatica parasite load and serodiagnosis by ELISA. Of these cows, three study groups were identified according to test results. Group 1: confected, tested positive for both diseases (n = 22). Group 2: non-parasitized cows positive for bTB tests (n = 13) and Group 3: parasitized cows without tuberculosis (n = 26). In addition, a group of cows kept in fascioliasis - and tuberculosis-free zones were included (Group 4, n = 10). A non-parametric Kruskal-Wallis test and a Dunn test were applied in order to analyze the results by considering groups independence. In cows from the Group 1 (considered as tuberculous), significant differences were observed regarding IFN-γ production but not for antibodies against *M. bovis* or reactivity towards bovine PPD in relation Group 2. While, Groups 1 and 3 did not display differences regarding antibodies against F. hepatica. Differences were observed regarding tuberculosis and *Fasciola* diagnostic tests when both confected and infected groups where compared to controls. According to the results, there were no important differences for the different diagnostic tests used for both diseases between the co-infected group, with those parasitized or tuberculous, and only the production of interferon gamma towards the PPD-bovine showed to be affected in the co-infected group, but not the intradermal tuberculin test, which is also associated with a cell-type immune response. This has implications to establish a diagnosis for this disease and the epidemiologic consequences for its control.

Área: Interacción patógeno-hospedero

Monitoring autophagic flux induced by *Haemophilus influenzae* on HEp-2 cells observed by TEM.

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Haemophilus influenzae is a Gram negative, non-mobile, facultative anaerobic bacillus that requires special factors for growth, such as hemin (factor X) and NAD (factor V). It is found as a normal biota and as a human pathogen. It colonizes mainly the upper respiratory tract and is classified based on the presence of capsular polysaccharides. Autophagy is an intracellular catabolic pathway, in which organelles and cytoplasmic components are recycled through the autophagosome and lysosome. All cells exhibit constitutive autophagy, although at different levels. Autophagy has been implicated in various physiological and pathological conditions such as in the development and progression of neurodegenerative diseases. *H. influenzae* can induce this process that could be used as a mechanism to survive intracellularly after cell penetration.

We analyzed the autophagic flow induced by *H. influenzae* in HEp-2 cells with the use of the inhibitors bafilomycin A1 and wortmannin, by quantifying autophagosomes by TEM in an *in vitro* model of autophagy induction. Strains and supernatants of *H. influenzae* ATCC 33930 and *H. influenzae* ATCC 49766 were used. As a result, double membrane structures (autophagosomes) were observed in HEp-2 cells subjected without inhibitors and with inhibitors; each experiment with positive and negative induction controls. When performing an analysis of the cell area obtained by each sample, It is concluded that *H. influenzae* induces the initial stage of autophagy (which was inhibited with wortmannin, which acts on IPK3 kinase preventing the formation of autophagosomes) and the final stage (bafilomycin A1 has an effect on an ATP-loop thus preventing the union of the autophagosome with the lysosome). The strain that presented a greater effect of autophagic induction was *H. influenzae* ATCC 49766.

Saliva an innate defense in oral cavity: Study of histatin 5 effect in *Streptococcus mutans* morphology and Cystatin C in the immunomodulation of human gingival fibroblasts incubated with *Porphyromonas gingivalis.*

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Abstract

Saliva exerts important protective functions, such as physical cleansing, which acts together with a number of innate and adaptative immunecomponents. It has been identified in the major glandular secretions,including secretory IgA, proline-rich proteins, amylase, statherin, histatins, lysozyme, lactoferrin and lactoperoxidase, which form the key components of the innate defense system in the oral cavity[1]. Antimicrobial peptides (AMPs), a heterogenous group of molecules produced by various tissues, possess a broad-spectrum antimicrobial activity[2]. Histatins are present in the saliva and possess a broad-spectrum of antibacterial activity. Histatin 5 exhibits activity against cariogenic bacteria, but its effect on the morphology of *Streptococcus mutans* remains unstudied. The main cariogenic character of *S. mutans* is its ability to form biofilms in the oral cavity. Oral cavity represents a unique environment for a wide array of microbial species that inhabit the dental plaque and requires multiple types of defenses to prevent infection. On the other hand, it has been described the immunomodulatory properties of AMPs, such as the interruption of host cell membrane altering or inactivating the membrane receptors such as TLR 4. Cystatins family include cystatin C which exhibits cysteine proteases properties that blocks bacterial proteases on the target cell. It is very important to find protective mechanisms against bacteria that cause oral diseases, so we studied the effect of cystatin C on cytokines profile on human gingival fibroblasts incubated with *Porphyromonas.gingivalis.* The ability of cystatin C to modify the proinflammatory profile, could be expressed in periodontal inflammatory lesions.

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Actinobacteria associated with indigenous maize soil from the traditional *milpa* agroecosystem display antagonistic activity against the phytopathogenic fungus *Fusarium graminearum*

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Actinobacteria are a bacterial group known for their ability to produce secondary metabolites and extracellular enzymes. As a result, the search for actinobacterias from unexplored environments has emerged as a feasible option for the discovery of new strains with biotechnological potential. The objective of the present work was the isolation, identification and characterization of actinobacterias strains from samples of milpa soils from El Boxo, Alto Mezquital in the State of Hidalgo, México. Isolation was made using specific growth media. Morphological characterization of the isolated strains was carried out and their biological activity such as fixation of N_2, proteolytic and chitinolytic activity, was evaluated. On the other hand, a phytopathogenic fungus was isolated from maize. Subsequently, *in vitro* challenges were made between actinomycetes and isolated fungus. Finally, molecular identification was carried out by amplification and sequencing of the 16S rRNA gene of actinomycetes strains with antagonistic activity. Thirty-two strains of actinomycetes were isolated. The isolated fungus corresponds to *Fusarium graminearum*. In terms of biological activity, 6 isolates had the capacity to fix N_2, 3 proteolytic activity and 6 chitinolytic activity. On the other hand, 4 strains presented *in vitro* antagonism against *Fusarium graminearum*. Analyses of the 16S rRNA gene revealed that strain BX17 belongs to genus *Amycolatopsis* and strains BX5, BX26 and BX33 belong to genus *Streptomyces*. These results suggest that inhibition of fungal growth may have been due to the production of one or more antifungals and/or the production of chitinase and proteases, characteristics reported for these genera. Furthermore, *Amycolatopsis* BX17 strain displayed antagonistic activity against *F. graminearum* in experiments with maize plants. The present research work allowed the obtaining and identification of 4 actinomycetes strains of the genera *Amycolatopsis* and *Streptomyces* with the potential to be used in biocontrol strategies of one of the main persistent phytopathogenic fungus in the agricultural region of Hidalgo (*Fusarium* graminearum), as well as the potential use of their extracellular enzymes in the industry.

Estudio de la virulencia de hongos de interés médico en *Galleria mellonella*

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ABSTRACT

Nowadays invertebrate study models are used more frequently to evaluate the host-pathogen interaction of many pathogenic fungi for the human, since using this type of organisms the obtained results can be comparable with the ones generated from vertebrate study models, such as mice. Moreover, the time, cost, and ethical regulations are not a major impediment when working with these organisms. *Galleria mellonella,* as a study model, provides a valuable and feasible alternative. The fungus *Candida albicans* is characterized by superficial skin infections of the mucous membranes and in an immunocompromised host can cause systemic infections that threaten life. The cell wall is the first structure of the microorganism that comes in contact with the host, and thus has an important role in adhesion, antigenicity, modulation of host immune response and recognition of this fungus by innate immune cells. Therefore, in this work, we analyze eight strains of the *Candida* genus, which include three mutants of *C. albicans* with defects in the cell wall synthesis (mutations in *OCH1, MNS1,* and *ROT2*) along with the reintegrant control strains, and the wild-type strains of *Candida auris* and *C. albicans*. The results showed that loss of *OCH1* attenuated the *C. albicans* virulence; whereas *C. auris* showed higher virulence than *C. albicans* cells.

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Interaction in the production of biofilm between *Candida kefyr*, *Escherichia coli* and *Streptococcus dysgalactiae* isolated from bovine mastitis.

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Little has been studied about *Candida kefyr* and even less on its interaction with other microorganisms related to infectious processes and biofilm formation as a virulence factor in bovine mastitis. This study was performed to demonstrate the biofilm production and the adhesion capacity of *Candida kefyr*, as well as its interaction with *Escherichia coli* and *Streptococcus dysgalactiae*. Fifty samples of mastitis milk were obtained from Querétaro, México. The yeasts were identified by means of fermentation and assimilation of carbohydrates and by automated methods Vitek 2 Systems (bioMériux). Minimun inhibitory concentrations to Amphotericin, Caspofungin, Fluconazole, Flucytosine, Micofungin and Vaniconazole were determined. Bacterial identification was carried out through traditional biochemical tests and the susceptibility to antibiotic was determined using commercial multi-disc. To determine adhesion capacity, the following inoculums were formed: *C. kefyr*, *C. kefyr* + *E. coli*, *C. kefyr* + *S. dysgalactiae*, using a Cowan *Staphylococcus aureus* strain as positive control. Adherence tube tests were performed: qaualitative, classifying them in negative, 1,2 and 3 and quantitative adherence plate trials, which were stained with 0.1% violet crystal and read with a 492 nm of wavelength. Analysis of Variance and a Tukey test ($p \leq 0.05$) were used, as well as Spearman correlation (≤0.01), to determine the association between the two tests. 9 strains of *C. kefyr* were obtained: 1 as alone isolated agent, 3 in combination with *S. dysgalactiae*, 2 with *E. coli* and 3 with *S. dysgalactiae* an *E. coli.* The 9 strains isolated from *C. kefyr* were sensitive to all the antifungals used in this study. The *S. dysgalactiae* were sensitive only to Cefalotin, Cefatoxime and Cefuroxime, whereas the strains of *E. coli* were sensitive only to Amikacin and Trimetroprim/Sulfamethoxasole. Adherence tube trials were positive in all cases. In adherence plate tests only six *C. kefyr* isolates showed a singnificant difference ($p \leq 0.05$). As for the cases of combinations made between *C. kefyr* + *S. dysgalactiae* and *C. kefyr* + *E. coli* all of them had statistical differences with the negative control and with those that only contained *C. kefyr*. The correlations between the quantitative and qualitative tests had a Rho Spearman value of 0.85. This is the first *C. kefyr* isolation report from bovine clinical mastitis in Mexico, as well as the biofilm production and its bacterial interaction.

Immune response of vaccinated calves against bovine tuberculosis defined *Mycobacterium bovis* antigens

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Cell-mediated immunity (CMI) against Bovine tuberculosis (TBb) represents the most important mechanism of acquired immunity against mycobacterial infections. Therefore, it is important to determine the pattern of cytokine secretion under different vaccination schemes in order to identify the best vaccines to be used for TBb control. The objective was to determine cytokine expression in cattle vaccinated with BCG or with Culture Filtrate Protein Extract (CFPE) obtained from *M. bovis* AN5 during the post-vaccination and post-challenge periods. The cytokines Th1 evaluated were IFN-γ and IL-2, and Th2, IL-4 and IL-10. For this purpose, 24 Holstein-Friesian cows of 6 months of age, negative to the commonly used TBb diagnostic tests, such as comparative cervical tuberculin test (CCTT), IFN-γ, ELISA and PCR from samples of nasal exudates. These animals were divided in four groups (6 animals/group). The first unvaccinated control group, the second group was vaccinated with BCG, the third vaccinated with the CFPE, and the fourth group received a treatment with recombinant IFN-γ prior to vaccination with CFPE. The cellular immune response during the post-vaccination period was evaluated by measuring the IFN-γ levels produced in whole blood cultures stimulated with bovine PPD and avian PPD at different post-vaccination times. The evaluation of cytokine expression was made by RT-PCR, using specific initiators for each cytokine and a positive internal control (β-actine). For which peripheral blood mononuclear cells (PBMC) were obtained by gradient of Ficoll-Hypaque, adjusted to $5x10^6$ cells/ml for *in vitro* cultured in RPMI 1640 supplemented with 5% FBS and stimulated with bovine PPD, Dip-z, complex 45-47, as positive control with Concanavaline A (Con A) and without stimulus. The expression of cytokines was represented as the percentage of the relative intensity of the bands corresponding to the amplification products in relation to an internal positive control using LabsWorks 4.0 software. The BCG vaccinated group showed high levels of IFN-γ production compared to the rest of the groups. The expression of this cytokine by RT-PCR, was significant with the different specific antigens used, in greater proportion than IL-2, during the whole experiment; the preferred response pattern was Th1. Especially after the challenge in the IFN-+CFPE vaccinated group, however it was not the predominant type of response, as there was also a Th2 response. With respect to the degree of protection evaluating lesions, BCG and CFPE vaccine could be considered as highly viable alternatives against TBb, used in the control of this disease.

Área: Interacción patógeno-hospedero

Antigenic recognition in vaccinated calves with BCG, or with protein extract of *Mycobacterium bovis*

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Bovine tuberculosis (bBT) continues being highly prevalent in dairy herds in the central zone of the country. This situation has encouraged research into the development of better diagnostic techniques and other control alternatives based on vaccination. Therefore, the objective of the study was to compare the antigenic recognition profile to the secretion proteins *Mycobacterium bovis* of calves vaccinated against bTB either with BCG vaccine or with the Culture Filtrate Protein Extract (CFPE). A collection of sera obtained from calves immunized with different doses of BCG vaccine ($1x10^4$ and $1x10^6$) were used, as well as calf sera immunized with CFPE at different post-vaccination sampling times to perform an analysis of antigenic recognition by immunotransferences. Sera positive naturally infected bovine positives to the comparative tuberculin test, ELISA and BOVIGAM were used to compare antigenic recognition among vaccinated, tuberculous and healthy non-reactor groups. The results were analyzed by Ji-square to determine the recognition frequencies observed in time among treated groups and evaluated antigens. According to it, there were differences in the number of antigens recognized as the intensity of their recognition, depending on the time for the different vaccinated groups in function of time. At the onset of vaccines application, 40 % of calves showed reactivity to proteins of: 60, 50-55, 45-47 and 32 (Ag 85) kDa. In subsequent sampling, vaccinated and control calves exhibited low reactivity to 216, 20, 132, 26, 23, 10 and 6 kDa antigens. Prior to vaccination, the animals exhibited recognition towards several proteins of *M. bovis* CFPE, despite the fact that this time was negative to the different diagnostic tests used for their selection, which indicates a possible sensitization by taxonomically related bacteria. The analysis of recognition for the group of tuberculous animals showed an intense response to the CFPE antigens for all the animals of the group, which was characterized by a higher number of recognized antigens in relation to the response registered for the group of negative animals. The response in the reactor group was greater to the antigens of 6, 10, 22, 23, 32, 35, 38, 45-47 and 50-55 kDa, outstanding reactivity towards the proteins of 35, 23, 22, 10 and 6 kD with a reactivity ranging from 80 to 100%. Thus, According to the results, antigens 38, 26, 23, 19, 10 and 6 kDa can be useful for diagnosis and allow differentiation between vaccinated and infected animals in the event that the control of the disease can be achieved through the application of vaccines. Therefore, this approach, used in conjunction with conventional control policies, also requires the development of vaccine compatible diagnostic tests to distinguish infected from vaccinated animals (DIVA).

Área: Interacción patógeno-hospedero

In silico characterization of *Mycobacterium tuberculosis* PE_PGRS18 protein and its immunogenicity

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Mycobacterium tuberculosis enters to the host by air and passes to the lower respiratory tract where the innate immune response is initiated. If the bacillus is not eliminated, the adaptive cellular immune response mediated by CD4+ and CD8+ T lymphocytes is activated. These lymphocytes recognize peptide antigens presented by the Major Histocompatibility Complex (CMH) molecules, then secreting cytokines that activates the macrophage, which in turn secretes microbicidal substances. *M. tuberculosis* can adapt to the bactericidal environment within the macrophage by the expression of virulence genes that encode for proteins found in the cell wall, such as the subfamily PE_PGRS. We found that the *pe_pgrs18* gene of *M. tuberculosis* strains from Michoacán is highly polymorphic. Their cell wall localization, exposed to extracellular environment, make important to look for peptides in the PE_PGRS18 protein that bind to CMH class I and II molecules, to analyze whether this gene is capable of inducing both a cellular and humoral immune response. These peptides could be tested in the future for inclusion in a tuberculosis vaccine or for early identification of the disease. In this work we analyzed the *pe_pgrs18* gene sequences from 46 *M. tuberculosis* strains from Michoacán. The PE_PGRS18 protein from H37Rv reference strain and the *M. tuberculosis* study strains, was obtained by *in silico* translation with the Mega program and characterized with PredictProtein, Signal-IP and Computer pI/Mw. The epitopes binding CMH class I and class II molecules, were identified with the NetMHCpan and the NetMHCIIpan servers, respectively. The PE_PGRS18 protein has loops (43.11%), beta sheets (40.04%), alpha helices (16.85%), a signal sequence at position 36-56 aa, an isoelectric point of 4.27, a molecular weight of 42074.79 Da and 17 protein binding regions. The protein is membrane-integral, 78.56% hydrophobic and lacks disulfide bonds. 4,953 epitopes of high affinity to CD8+ lymphocytes were identified, the A_6802 allele being the one that binds to a greater number of epitopes, in addition to 1,024 high affinity alleles to CD4+ T lymphocytes, the DRB_0407 allele being the one that binds to a greater number of epitopes. 684 mutations were identified, of which 407 stimulate and 278 evade the immune response mediated by CD4 + and CD8 + T cells. We conclude that changes in the sequence of the *pe_pgrs18* gene that codes for the PE_PGRS18 protein are capable of causing some peptides to be recognized by the CMH molecules, activating the adaptive immune response mediated by CD 4+ T lymphocytes, and with a higher frequency the response mediated by CD8 + T lymphocytes.

Curli, a fitness factor of uropathogenic *Escherichia coli*

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Curli, fimbriae widely distributed in uropathogenic *Escherichia coli* (UPEC), are involved in adhesion to human bladder cell surfaces and biofilm development. The role of UPEC curli was evaluated in a murine model of urinary tract infection. The aim of this study was to establish the role of curli in C57BL/6 mice transurethrally infected with curli-producing and non-curli-producing UPEC strains. We confirmed that curli enhanced UPEC colonization in the urinary tract, resulting in damage in both the bladder and kidney. Intranasal immunization with recombinant CsgA protein protected against the colonization of curli-producing UPEC in the urinary tract. Quantification of cytokines from urinary tract organs showed an increase in interleukin-6 and TNF release in kidneys 48 hours post-infection with curli-producing UPEC. In contrast, mice infected with non-curli-producing UPEC showed the highest release of interleukin-6, -10, and -17A and TNF; curli may hide other fimbriae and LPS, preventing interactions with Toll-like receptors. Intranasal immunization with recombinant FimH and PapG proteins and subsequent infection with this strain were performed; cytokine quantification showed a decrease in the stimulation and release by the uroepithelium. Thus, curli are a virulence factor that enhances colonization in the urinary tract and may be a fitness or persistence factor.

Effect of *Salmonella* Newport internalized in cherry tomatoes in the colonization of the gastrointestinal tract of Balb/c mice

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Introduction

Salmonellosis is a foodborne disease produced by *Salmonella enterica* and it is one of the four main causes of diarrheal diseases worldwide (OMS, 2018). Worldwide, it has been reported that *S*. Newport is one of the most frequent serotypes causing outbreaks by consumption of raw tomato (CDC, 2007). The aim of this study was to evaluate the efficiency of colonization of the BALB/c mice gastrointestinal tract by *S*. Newport serotype, when they are fed with tomatoes contaminated with internalized *Salmonella*.

Material and methods

BALB/c female mice 5 or 6-week-old were administered orally (via an intragastric catheter) with approximately 2.61 $\log^{10}$ of CFU/mL of *S*. Newport or *S*. Typhimurium, suspended in 0.85% physiological saline solution (0.85% SSF), or *S.* Newport recovered from cherry tomatoes post internalization at 0 h (T0 h) and at the third day (T3 d). 24 mice, 6 for each group, were inoculated for 6 continuous days with inoculate prepared the same day of inoculation. The mice were anesthetized with Isoflurane and sacrificed by cervical dislocation on day 7. The gastrointestinal tract was extracted and homogenized with a tissue processor in 5 mL of phosphate buffer. Two aliquots of 1.5 mL of gastrointestinal tract from each individual were taken and centrifuged at 6,000 rpm for 5 min. One mL of the supernatant of each aliquot was taken for made dilutions or direct plated on trypticasein soy agar contained 0.1 g/L of rifampicin to determine colony-forming units CFU. The CFU were reported as Log^{10}CFU/organ.

Results and conclusions

It is well documented that *S*. Typhimurium causes a systemic disease in mice similar to produce by *S*. Typhi in humans (Santos *et al.*, 2001). Burns *et al.,* 2005 reported that 6-week-old BALB/c mice were susceptible to *S*. Typhimurium when the inocula were more than 5 Log^{10} CFU. Our results shown that approximately 2.66 Log ^{10} of *S*. Newport were successful in colonizing the gastrointestinal tract inoculated either in 0.85% SSF or *S*. Newport internalized and recovered from cherry tomato at 0 h or 3 d ($p>0.05$). In contrast, no detectable CFU were recovered from the gastrointestinal tract from mice inoculated with 2.43 Log^{10} of *S*. Typhimurium in 0.85% SSF. Our results suggest that *S*. Newport has a greater efficiency in the colonization of the gastrointestinal tract of BALB/c mice. However more evidence needed in order to support this hypothesis.

C*ampylobacter fetus* induces proinflamatory response in bovine endometrial epithelial cells.

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Introduction. The innate immune system recognizes microbial infections and elicits the immediate response to generate adaptive immunity. Microbial sensing is mediated by pattern-recognition receptors (PRRs). *Campylobacter fetus* causes Bovine Genital Campylobacteriosis which is a reproductive disease that affects livestock, and is transmitted during mating. It is characterized by inflammation that produces infertility and in some rare cases it causes abortions. In this study a primary endometrium epithelial cell cultures were established and challenged with *C. fetus* to determine inflammatory response against these bacteria. **Material and Methods.** The intracellular survival of *C. fetus* was evaluated by gentamicin protection assays at 0, 2 and 4h post-infection (p.i). RNA from epithelial cells was recovered to evaluate IL-1B, IL-8, IFNy and TNF-alpha expression using qRT-PCR by relative quantification. **Results.** The results showed that *C. fetus* induces a rapid proinflamatory response, at 0h post infection IL-1B expression increased to 4.17 folds and IL-8 to 4.26. This expression decreased through time, at 4h p.i. IL-1B expression diminished to 0.86 folds and IL-8 to 1.83. IFNy had a different expression pattern; it increased through time reaching 3.62 fold. Expression of TNF-alpha doesn't have any changes. In order to stablish if bacteria internalization was necessary for cytokine expression, a cytoskeleton inhibition assay was performed and interleukin relative quantification was determinate. The results suggest that internalization of *C. fetus* was necessary to induce a proinflammatory response in endometrial cells. **Conclusions.** The present study examined the proinflammatory response to *C. fetus* in bovine endometrial epithelial cells. The bovine endometrial epithelial cells were able to recognize *C. fetus* resulting in an early proinflammatory response.

Modalidad: Cartel

Área; Interacción patógeno-hospedador

Helicobacter pylori and expression of miR-411-5p, miR-548d-3p and miR-892c-5p in patients with chronic gastritis and gastric cancer

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Gastric cancer is a global health problem and its 5-year survival rate is only 30%. *Helicobacter pylori* (*H. pylori*) is the main risk factor associated with gastric cancer. H. pylori induces changes in the expression of diverse proteins and modifies the expression of microRNAs (miRNAs). The miRNAs are small, non-coding RNAs that post-transcriptionally regulate gene expression and function as oncogenes or tumor suppressors by inducing degradation or inhibiting translation of mRNA. The expression profile of miRNAs from samples of patients with gastric cancer and non-neoplastic gastric mucosa with *H. pylori* infection is different. The aim of this research was analyze the relationship of *H. pylori* and the expression of miR-411-5p, miR-548d-3p and miR-892c-5p in patients with chronic gastritis and gastric cancer. Twenty-one samples of patients with histopathological diagnosis of chronic gastritis and five biopsies of patients with histopathological diagnosis of gastric cancer with their respective adjacent tissue were analyzed. The presence of *H. pylori* was determined by endpoint PCR and the expression of miRNAs was performed by RT-qPCR. There was a greater participation of women with 57.7% (15/26), the average age was 59 years and the general frequency of *H. pylori* was 42.31% (11/26), no significant differences were found in the expression of miR-548d-3p and miR-892c-5p among patients with chronic gastritis compared to those with gastric cancer. The expression of miR-411-5p was significantly different among patients with chronic gastritis and gastric cancer. No differences were found in the expression of miR-411-5p, miR-548d-3p and miR-892c-5p in patients with chronic gastritis and gastric cancer with and without *H. pylori* infection. The results suggest that *H. pylori* does not modify the expression of miR-411-5p, miR-548d-3p and miR-892c-5p in samples from patients with chronic gastritis or gastric cancer.

Chemokine dysregulation during HIV and TB Co-infection.

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Tuberculosis (TB) is one of the ten top causes of death worldwide, and HIV (Human Immunodeficiency Virus) greatly increases the risk to develop TB. The granuloma is the hallmark of TB pathology and is believed to represent immune containment of *Mycobacterium tuberculosis* (*Mtb*) infection at the site of infection. The formation and integrity of the granuloma is regulated by a poorly understood balance of cytokines and chemokines. These chemokines are involved in the recruitment and organization of host immune populations into the granuloma. The granulomas in the lung of human subjects and humanized mice with HIV and TB coinfection are poorly organized compared to those with TB only. The loss of granuloma organization is associated with poor immunity and increased proliferation of *Mtb* bacilli in co-infection. The aim of these ongoing studies is to determine the role of HIV-mediated disturbances of chemokine production in the pathogenesis of HIV/TB co-infection. Our humanized mice model was used for exploratory studies to identify chemokine pathways that were differentially expressed in TB and HIV co-infection. Lung tissue from mice that were non-infected or infected with *Mtb*, HIV, or *Mtb* and HIV was used to isolate RNA and perform high throughput RNA sequencing. We analyze 46 chemokines and 23 receptors of chemokines. Our preliminary differential transcriptome results indicate that *Mtb* infection activates several chemokine pathways important for recruitment of leukocytes to sites of infection such as CXCL1, CXCL5, CCL2 and CCL8, among others. Co-infection with HIV, however, markedly suppressed transcription of many chemokine and chemokine receptor genes, compared to only TB infection. Ongoing studies are focused on validation of results using qPCR and Bioplex ELISA of lung supernatants and multi-color fluorescent microscopy of lung lesions. Long term, these studies may identify the effects of HIV to promote inappropriate transcription, production, or recognition of chemokines as a mechanism for disturbance of the protective TB granuloma structure.

Rescue of the thermosensitive mutation of peptidyl t-RNA hydrolase from *Escherichia coli* by three Pths from *Entamoeba histolytica* modified by directed mutagenesis.

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During translation, ribosomes can be stalled by truncated mRNAs, tRNAs and amino acid starvation, causing the premature dissociation (drop-off) of pep-tRNAs. The accumulation of pep-tRNAs is toxic to the cell. There are different factors that take part in ribosome rescue; peptidyl tRNA hydrolase is an esterase that removes the peptidyl moiety allowing tRNAs to be recharged. In *Escherichia coli*, a single amino acid substitution Gly for Asp at residue 101 confers the Pth thermosensitive phenotype (Ts) to the AA7852 strain. The strain can grow exponentially at 32 °C but if it is shifted to 43 °C (a non-permissive temperature), pep-tRNAs accumulate within ten minutes and cannot be degraded, translation decreases and consequently the cell dies. Our purpose was to know whether the modification of residues K80, K53 and E47 by an aspartic acid (D), in the peptidyl-tRNA hydrolases of *Entamoeba histolytica* PthA, PthB and PthC, increase the efficiency of the rescue of the thermosensitive mutation of *Escherichia coli*. Genomic DNA was extracted from *Entamoeba histolytica* HM1-IMSS trophozoites and PCR reactions were carried out. The amplicons obtained were cloned directly in PROEX-1 and sequenced. Since the Pth-B sequence contains an intron (45 bp), total RNA was extracted and one step RT-PCR was performed with total RNA and the product was cloned into pTOPO-TA. The products were excised with *Eco*RI/*Bam*HI, subcloned into pPROEX-1 and sequenced. The constructions pEhPthA, pEhPthB and pEhPthC were modified by direct mutagenesis. The rescuing efficiency in the thermosensitive strain was compared, measuring growth, at permissive and non-permissive temperatures. The mutant *E. histolytica* Pth enzymes presented peptidyl t-RNA hydrolase activity and rescued the thermosensitive mutation. The order of rescue found was: PthB-mutated> PthB> PthA> PthA-mutated> PthC > PthC-mutated. Three dimensional modeling and evaluation was carried out, in order to better understand the previous findings. In the model structure of *E. coli* wt Pth we observed a channel, which is the place where the tRNA-peptidyl complex is laying and where the catalytic residues are mapped. Comparing the channel structures of different Pths we observed the following: EhPthB's is more open than *E. coli's* Pth, followed by EhPthA's and EhPthC's is narrower.

SELECTION, CHARACTERIZATION AND EVALUATION OF TCR-like DOMAIN ANTIBODIES AGAINST Ag85Bp$_{199-207}$/HLA-A*0201 and ESAT6$_{p82-90}$/HLA-A*0201 OF *Mycobacterium tuberculosis*

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Tuberculosis represents a public health problem worldwide, its eradication is affected for the emergence of drug resistance strains and the inefficiency of the existing vaccine. *Mycobacterium tuberculosis* specific CD8 T cells are activated when their T-cell receptors recognize the specific bacterial peptide/Major Histocompatibility Complex class I present on infected cells surface. Antibodies able to recognize the specific peptide bound to MHC are known as TCR-like and can mimic T cell receptor specificity. **Methodology and Results**: In this work, the selection characterization and evaluation of TCR-like antibodies against Ag85b$_{p199-207}$/HLA-A*0201(C/Ag85b) and ESAT-6$_{p82-90}$/HLA-A*0201(C/ESAT-6), biotinylated complex was carried out through screening of a human single domain- antibodies (sdAb) /phage library. After 3 o 4 rounds of selection, the phage-ELISA allowed us to isolated about 7 and 14 positive clones for each complex respectively. After DNA sequence of the recombinant clones and expression of the sdAb in *Escherichia coli* HB2151 and *E. coli* TG1, only one sdAb for each complex was suitable to be studied. The results demonstrated that the expression of sdAb was successful, because they were identified through SDS-PAGE and Western blot as proteins bands of the expected size of sdAb (~15kDa) that were recognized by an anti-Myc antibody. The sdAb against C/Ag85b was soluble expressed from *E. coli* HB2151 and protein was purified from periplasmic extract by Protein A affinity chromatography. The binding capacity and specificity of this purified sdAb against C/Ag85b complex were evaluated by an immunoassay, using the 16kDa$_{P120-128}$/HLA-A*0201 as non-target complex. **Conclusion:** the strategy for screening and selection of sdAb against HLA-A*0201 *M. tuberculosis* peptides complex, allowed us to isolate different phage-clones that specifically recognized C/Ag85b and C/ESAT-6 and isolate an specific sdAb to C/Ag85b. **Perspectives**: Testing the specificity of sdAb on the surface of T2 cell line.

Área: Interacción patógeno-hospedero

Development of an *in vitro* granuloma model for the study of *Mycobacterium tuberculosis* antigens

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Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb*). Today, TB is the leading cause of death by a single infectious agent. The lack of an efficient vaccine makes difficult to control this disease around the world; therefore, the development of a new vaccine has become into a primordial task. For the development of a new vaccine, it is imperative the study of the immune response towards *Mtb* antigens. Over the years, the use of animal models has been the principal resource for the study of TB. However, animal models can not reply the events of human TB. Therefore, a model that better resembles human TB is needed. During TB, an individual develops granulomas because of the persistence of *Mtb*. These are well organized structures formed by immune cells (lymphocytes and macrophages) and are the hallmark of the host-pathogen interactions in TB. Hence, the study of *Mtb* antigens by using granulomas represents a better way to understand the disease.
The aim of this work is to establish an *in vitro* human granuloma model for the study of immune responses towards *Mtb* antigens. First, we isolated peripheral blood mononuclear cells from healthy/BCG-vaccinated subjects. After, monocytes were isolated by adherence and infected with different doses of *Mycobacterium bovis* BCG Pasteur for four hours, the doses tested were MOI (bacteria-cell) of 10:1, 5:1, 2.5:1, 0.5:1, 0.25:1 and 0.1:1. Next, remaining bacteria was removed by washing the cells and lymphocytes were added to the culture of infected cells. An analysis of optic microscopy was performed for nine days to watch the formation of cellular aggregates that were similar to granulomas. Since day two, we could observe the formation of cellular aggregates. For all the doses, except 0.1:1, all the aggregates were amorphous which agrees with the induction of cell death and the release of bacteria to the extracellular media. Both events were confirmed by a count of cell viability by trypan blue and the count of colony forming units, respectively. With MOI of 0.1 at day seven, spherical aggregates were observed which resembles more a structure like granuloma. Nevertheless, after two days the structure became amorphous and bacteria was released to extracellular media. The above results tell us that we need to evaluate more conditions to get more stable cellular structures that can last longer periods of time so we can proceed to their characterization and application to the study of *Mtb* antigens.

Recombinant enolase from *Haemophilus influenzae*: characterization as a binding protein to collagen, fibronectin and human plasmatic proteins

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Introduction: *Haemophilus influenzae* is a Gram negative bacterium that produces invasive pediatric diseases such as meningitis, epiglottitis, pneumonia, septic arthritis, pericarditis, cellulitis, bacteremia (serotype b). Non-typeable *H. influenzae* (NTHi) strains, are associated with localized infections such as otitis, conjunctivitis, sinusitis, bronchitis, pneumonia; moreover, these strains also cause invasive disease such as meningitis and sepsis in immunocompromised hosts.

Enolase is a metalloenzyme that principally participates in the glycolytic pathway in many organisms. However, this protein is recognized as a moonlighting protein, being able exported to the surface to has a role as adhesin and contribute as a pathogenicity factor.

Objective: Characterize recombinant enolase from NTHi (rENoHi) as a possible adhesin through in vitro and inhibition assays.

Materials and Methods: Far Western-blot assays were performed with purified recombinant enolase from rEnoHi, collagen (Cln), fibronectin (FN) and human plasmatic proteins. 10 µg/ml of plasmatic proteins, Cln I and Cln III were electro transferred to nitrocellulose membrane and incubated with 10 µg/ml of purified rEnoHi; the interaction rEnoHi-plasmatic proteins, Cln and FN, was evidenced by used an anti-His monoclonal antibody or anti-rEnoHi polyclonal antibodies. On the other hand, ELISA tests and bioinformatics analyzes were performed to confirm this interaction. ELISA method was performed to shows the interaction inhibition by using polyclonal anti-rEnoHi antibodies.

Results: rEnoHi presented a significant adhesion level to human plasmatic proteins (where plasminogen is located), CnI I, Cln III and FN. On the other hand, the interaction was decreased when polyclonal anti rEnoHi antibodies were used.

Conclusions: The recombinant enolase of *H. influenzae* has the capacity to interact with human plasma proteins and components of extracellular matrix, having a role as adhesin; this function could play an important role as a pathogenicity factor in the interaction of the bacteria with the host.

Suppression of the Peptidyl tRNA hydrolase (*Ts*) mutation of *Escherichia coli* by Pths A and B of two parasitic protozoa: *Giardia lamblia* and *Trypanosoma cruzi*

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During protein synthesis, genetic information encoded by mRNAs is translated by ribosomes, this process is divided into three stages: initiation, elongation and termination. Sometimes, the peptidyl-tRNA complexes are released from the ribosome before reaching the stop codon and the process stops prematurely. The production of peptidyl-tRNAs is toxic to the cell. The Peptidyl-tRNA hydrolase (Pth) hydrolyses the ester bonds between the peptide and the ribose of tRNA. In *Escherichia coli* the Pth (Ts) mutation determines a thermosensitive phenotype, the mutant strains grow exponentially at 30 °C but at 43 °C they accumulate peptidyl-tRNA, as a consequence protein synthesis stops and the cells die. Furthermore, it has been shown that the three Pth of *Entamoeba histolytica* rescue the pth (Ts) mutation in *Escherichia coli*. Consequently, we wanted to know if the PthA and PthB of the parasites *Giardia lamblia* and *Trypanosoma cruzi* suppress the thermosensitive mutation of *E. coli*. The DNA of both parasites was obtained and the Pth's open reading frames were amplified and cloned into the pPROEX-1 vector and sequenced. AA7852 (thermosensitive) *E. coli* cells were transformed with the constructions. In thermosensitivity tests, it was observed that pGlPthA rescues the mutation but this construction could potentially be toxic to the cells and pGlPthB is inefficient; pTcPthB suppresses the termosensitivity phenotype meanwhile pTcPthA does not. The three-dimensional models fold in globular α / β domains similar to the wild Pth of *E. coli*, but the amino acids observed in the catalytic site are different from those of *E. coli*. However, residues N11, H21 and D94 of the Pth of *E. coli* align with distinct amino acids in PthA *G. lamblia* and PthTcB *T. cruzi*; in both 3D models we observed a cavity that could be the active site.

Mitochondrial antiviral signaling protein (MAVS) expression in MDCK cells infected with Canine Parvovirus.

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Introduction. The innate immune response activation in viral infection is dependent of activation of signaling cascades that allow the production of a wide arsenal of effector molecules that suppress viral replication. Canine parvovirus has developed many strategies to evade the host innate immune response that facilitates its survival. During PVC infection an early association of virions with the mitochondria has been described, which can interfere with the signaling mechanisms in the cell. MAVS and STING (Stimulator of interferon genes) are two important proteins for innate immune response during viral infection, the early association with mitochondria could be a mechanism to interfere with its response. **Material and methods.** In order to describe the mechanisms of immunological interference that allow a successful replication of PVC, in this study the expression of innate immune response genes (MAVS, STING, IFNβ and IFIT1) was evaluated in cells infected with PVC. MDCK cells were infected with a wild type strain of PVC isolated from a dog with parvovirosis. The expression of MAVS, STING, IFNβ and IFIT1 was evaluated in two points during infection assays (day 8 and 11). **Results.** Analysis of kinetic infection showed that first viral gene expressed was NS1 at day 8; VP2 was overexpressed at day 11, this could suggest the beginning of the assembly of virions. Expression of MAVS, STING, IFNβ and IFIT1 during day 8 and 11 post-infection (p.i.) showed a difference ($p < 0.001$). IFNβ expression was observed until day 8 and remains until day 11 p.i. The low expression of IFIT1 at day 8 p.i. was related with IFNβ expression reduction. Administration of exogenous IFN produced an early IFIT1 expression associated with reduction of viral infection, this suggests a possible relation. Expression of MAVS and STING started at day 8 post-infection and increased until day 11 p.i.; MAVS was one of the most expressed genes, this suggests a possible role in the control of PVC infection. **Conclusions**. In this work it was shown that the NS1 protein is overexpressed at day 8 p.i., this suggests an important role in viral replication. IFNβ expression was associated with the high expression of IFIT1 and MAVS. These results showed that MDCK cells are permissive with PVC infection and that IFNβ expression can be associated with MAVS and IFIT production, as controllers of viral infection.

Modalidad: Cartel

Área: Interacción patógeno-hospedador

Non-tuberculous mycobacteria of various environmental origin: their interaction with human macrophages

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Non-tuberculous mycrobacteria (NTM) have taken on importance in the last few years as emerging pathogenic agents due to their great potential for infecting both the immunocompromised as well as the immunocompetent. The pathogenic mechanisms that are present during infection with NTM have been insufficiently characterized as well as their capability of infection and persistence inside human macrophages and the host cell response to said infection. Therefore, the aim of this work was to establish and compare the kinetics of infection and intracelullar multiplication of the various strains of *Mycobacterium. avium*, *M. gordonae* and *M. mucogenicum* inside human macrophages. In order to accomplish this, we used 11 strains of NTM: 4 strains of *M. avium* isolated from water, one from sprouts and two of clinical origin; for *M. gordonae* 3 strains, one from sprouts and two of clinical origin; and 4 strains of *M. mucogenicum,* one isolated from water, one from Mexican sauce (*salsa)*, one from sprouts and one of clinical origin. Human THP-1 macrophages were infected with a 3:1 MOI with each of the aforementioned strains and the kinetics of infection and celullar multiplication were determined. In general, the NTM slow growth strains *M. avium* and *M. gordonae* maintained the integrity of their monolayer, while the fast growing NTM, *M. mucogenicum,* totally destroyed its layer. For one of the *M. avium* strains of clinical origin and the strain from water from homes, the integrity of the monolayer was maintained as much as 80%. In the case of the second strain of *M. avium* of clinical environment origin, it maintained 100% infectivity and destroyed 80% of its monolayer. The strain from the sprouts presented a maximum percentage of infectivity of 60% and completely destroyed its monolayer (48 hpi). In the case of the two strains of clinical origin of *M.gordonae,* the integrity of the monolayer was maintained at 60% (72 hpi) and presented a percentage of infection greater than 85%. On the other hand, the strain isolated from sprouts presented 75% infection and completely destroyed its monolayer (72hip). Finally, the four strains of *M. mucogenicum* destroyed their monolayer at 100% and reached 100% infectivity at 48 hours and 72 hpi. In conclusion, the strains originating from the clinical cases (regardless of mycobaterial species) presented greater persistence inside the macrophages than the strains that originated from sprouts, sauces (*salsas)* or from water.

Molecular toolbox for harnessing plant-microbe interactions with biotechnology potential.

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Microbes serve many functions for host plants, including increasing nutrient availability, promoting plant growth and development, and antagonizing pests and pathogens. Because of these contributions, microbe-based inoculants have been used in agriculture for over a century, and are still considered an ideal alternative for decreasing the use of agrochemicals while maintaining high yields. However, the development of microbial products for agriculture is hindered by our limited knowledge of molecular details of microbe-plant interactions, especially in ecologically complex settings. To address this shortcoming, our research is developing novel strategies to 1) understand plant-microbe interactions at the molecular level with new genetic tools, and 2) understand the molecular dynamics of plant-microbe interactions in complex communities. Here, we present our recent advances in the creation of genetic tools (mutants, fluorescent reporters and gene-overexpressing strains) for understanding agriculturally relevant traits of non-model bacterial species. In the first study system, we discovered the genetic basis of virulence of *Erwinia tracheiphila* (*Et*), a cucurbit pathogen. We found that during infection, *Et* uses exopolysaccharides for survival, Type III effectors for virulence and cellulose-interacting proteins called 'expansins' for systemic colonization. In the second system, we describe cell differentiation processes in the insect pathogen and biocontrol agent *Bacillus thuringiensis* (*Bt*). We found that *Bt* subpopulations for sporulation, biofilm formation, nutrient scavenging and production of Cry insect-toxin proteins can be altered in different fermentation regimes. We have also characterized a repertoire of regulatory protein paralogs that coordinate these collective functions. Such specialization and coordination processes can be exploited for increased yields and efficacy of *Bt*-based biopesticides. Next, we aim to use these molecular tools to understand plant-microbe and insect-microbe interactions within tractable synthetic bacterial communities that mimic the complexity of natural systems. Using fluorescent strains of *Et* and *Bt* as reporters, we are creating a novel framework for high-throughput discovery of emergent functions from bacterial communities with agricultural applications. We expect that this strategy will generate important basic knowledge about microbe-plant interactions in real-life conditions that can be directly applied to develop new commercial products that will be successful in the field.

Induction of autophagy by *Listeria monocytogenes* strains isolated from clinical products.

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L. monocytogenes is a Gram-positive bacillus that causes diseases like meningitis, septicemia, spontaneous abortions and fetal death. *L. monocytogenes* enters to the host cell by phagocytosis, it uses the LLO hemolysin to escape and replicates in the cytoplasm and moves by actin polymerization to reach an adjacent cell. This process happens in case the autophagy is not effective because, if it is, an autophagolysosome is produced, and degrades the bacteria. The aim of this study was to determine if this mechanism is produced by Mexican clinical isolates.

Thirteen strains of *L. monocytogenes* obtained from clinical products of the Hospital de la Mujer from Aguascalientes and of CDMX were identified. HEp-2 cells were propagated in MEM medium (Minimum essential medium), which were mounted in 24-well microplate to the autophagy assay. The LC3B marker (present in autophagosomes) was identified with the rabbit polyclonal primary antibody anti-LC3B Cell Signaling™ and the rabbit goat anti-IgG secondary conjugated to FITC Santa Cruz™ and examined in the Zeiss® epifluorescence microscope Axiophot 1.

The autophagy inducers were Rapamycin and Hanks physiological solution, which were used as positive controls, and MEM and *L. monocytogenes* ATCC 19118 as negative controls. Autophagy induction percentages were calculated by counting 100 total cells, and cells with intense fluorescent green label were considered positive. FITC was used to label the LC3 protein and DAPI to label the nuclei. The assay was performed in duplicate in an incubation time of 4 hours, and a MOI (Multiplicity of Infection) of 25.

The results obtained in these conditions were variable for each strain in a range of 3 to 23% of autophagy positive cells in the first trial. A total of 3 strains exceeded the percentage of cells labeled with LC3 in comparison with positive controls and with the ATCC reference strain (14% of LC3+ cells). In the second trial, the range obtained was 3 to 21% of LC3+ labeled cells, and 6 strains exceeded the percentage of labeled cells obtained by the positive controls and ATCC strain (8%). We conclude that autophagy is an important process that these strains could use as a mechanism to evade the host immune response.

LngA, CstH, and FliC in the ETEC E9034A participate in adherence to intestinal cells HT-29 and LS174T

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Introduction. *Escherichia* coli enterotoxigenica (ETEC) is the etiologic agent responsible for diarrhea in newborns and children under 5 years, as well as in older adults in developing countries, including to Mexico. ETEC expresses several important colonization factors in colonization to the intestinal epithelium. **Objective**. The aim of this work was to generate a double mutation of the *cstH* and *fliC* genes in the strain ETEC E9034A, to determine their effect on the adherence to cell lines LS174T and HT29, both of human intestinal origin. **Methodology**. ETEC E9034AΔ*cstH*Δ*fliC* strain was generated by the inactivation method in a one-step proposed by Datsenko and Wanner in 2000. E9034A, E9034AΔ*lngA*::Km^r, E9034AΔ*fliC*::Cm^r, E9034AΔcstH::Km^r, E9034AΔlngA::Km^{r}Δ*fliC*::Cm^r, E9034AΔ*cstH*::Km^{r}Δ*lngA*::Cm^r strains previously activated in a murine performed were recovered and used to infect on HT29 and LS174T cells. The infection was performed for 4 hours in a partial atmosphere of 5% CO_2. The number of attached bacteria adhered to cell monolayers was quantified by serial dilutions after culturing them on plates with Luria Bertani agar. **Results**. The 7 strains showed a low adhesion profile to the LS174T cell line when compared to the HT-29 cell line. Mutation of the *lngA* and *cstH* genes affected the adhesion profiles significantly to the LS174T cell line when compared to the wild type strain (E9034A); while, the mutant strain in the *fliC* gene did not show a significant difference. E9034AΔ*lngA*Δ*fliC*, E9034AΔ*cstH*Δ*lngA* strains showed a decrease in the number of bacteria adhered to both cell lines, with an adhesion profile similar to strain E9034AΔ*lngA*. Interestingly, E9034AΔ*cstH*Δ*fliC* strain showed a significant reduction in the number of bacteria adhered to both cell lines when compared with the E9034AΔ*lngA*Δ*fliC*, E9034AΔ*cstH*Δ*lngA* strains. **Conclusion.** *lngA*, *cstH* and *fliC* genes in the ETEC E9034A are important in the adhesion process to intestinal cells. The low adhesion observed in the LS174Tn cell line when compared with the HT-29 cell line is possibly due to a high mucus production.

Interferon-gamma-activated macrophages present antigens of *Burkholderia cenocepacia* to T-cells by class I and II major histocompatibility complex molecules

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Abstract. *Burkholderia cenocepacia* is an opportunistic pathogen often associated with fatal pulmonary infections in patients with cystic fibrosis and chronic granulomatous disease. The bacterium survives intracellularly in macrophages within a membrane-bound vacuole (BcCV) that delays acidification and halts maturation into lysosomes. Engulfed exogenous antigens are processed into peptides in a late endosomal compartment (MIIC), which contains class II major histocompatibility complex (MHC) molecules and Rab7. The objective of this study is to identify the ability of infected macrophages with *B. cenocepacia* to present bacterial antigens to T cells. In this study, we used bone marrow derived macrophages, immunofluorescence, flow cytometry, biochemical test and conventional assays for antigen presentation to T-cells. Here, we demonstrate that BcCV and MIIC have overlapping features and interferon-gamma-activated macrophages infected with B. cenocepacia process bacterial peptide antigens that are presented by class II MHC molecules to $CD4^+$ T-cells and by class I MHC molecules to $CD8^+$ T-cells. We also found that infected macrophages release peptides derived from bacterial processing into the extracellular medium, which stabilizes empty class I MHC molecules of bystander cells. Therefore, macrophages infected with *B. cenocepacia* may play an important role in establishing an adaptive immune response against the pathogen.

Isolation and molecular identification of *Pseudomonas sp* from larvae of *Aedes aegypti*, an arbovirus vector.

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Aedes aegypti is the principal vector of important viral diseases including dengue, Chikungunya, Zika and yellow fever, affecting over half the world's population. This mosquito is able to adapt to diverse environments and its dispersion is linked to human traveling. Mosquito control depends mainly on chemical pesticides with high risk to human health and environment and, at present, populations resistant to all main insecticide groups have arose. Microorganisms interact with insects affecting multiple aspects of their biology, including entomopathogenic bacteria and fungi which have been proposed for mosquito control and their study is an important research area. The aim of this work was the isolation of a natural entomopathogenic bacteria that could be considered for mosquito control in the field.

From a naturally infected *A. aegypti* population, bacteria clones were isolated and identified by biochemical and phenotypic characterization using Api®20N, Api20®NE, and API®50 strip kits (Biomerieux, France) and one clone of *Pseudomonas* sp. was isolated which was able to reproduce an infectious syndrome. Bacteria showed 98% similarity in metabolic characteristics with *P. aeruginosa* and little lesser with *P. fluorescens*, but unlike these two species, they can use D-fucose as carbon source. Bacteria is sensitive to ampicillin, chloramphenicol, streptomycin, gentamicin, and tetracycline; but resistant to vancomycin. DNA was obtained from a single colony of *Pseudomonas* sp. and intra-species variability was assessed based on the analysis of the nucleotide sequences using *rpo*D (σ^{70} factor), *rpo*B (RNA polymerase beta subunit), *gyr*B (DNA gyrase β subunit), and additionally the 16S rRNA genes, previously used for phylogenetic studies of *Pseudomonas*. Phylogenetic analysis was performed using Clustal W, Neighbor-Joining method and MEGA4 software. The strain did not show a 100% DNA similarity with any reported *Pseudomonas* sp, which suggests that it is new not reported species.

To characterize the bacteria caused syndrome, infected larvae of the 4th stage were fixed and examined under a scanning electron microscope. We observe that in axenic culture the bacteria are 1 µm average size and do not have flagella. During interaction, the bacteria distribute regularly on the surface of the larvae and generate well developed fimbriae.

Regulation and role in colonization of the *csu*-like fimbrial operon of *Citrobacter rodentium*

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Citrobacter rodentium is an attaching and effacing mouse pathogen that colonizes the intestinal tract and causes colonic hyperplasia. It has been used as a surrogate model for studying the role of virulence-associated factors of human A/E pathogens such as enteropathogenic and enterohemorrhagic *Escherichia coli*. The *C. rodentium* genome contains 19 fimbrial operons, of which only four have been characterized. The objective of this study was to characterize the function and regulation of the *csu*-like fimbrial operon, a putative adhesive pilus of the chaperone-usher family. Mutation of the *C. rodentium* DBS100 *csuA* gene, encoding the major fimbrial subunit, reduced bacterial adherence to HeLa and Caco-2 cells, in 55% and 95%, respectively, and also affected early colonization in the mouse model. Complementation in trans of the *csuA* gene in the DBS100Δ*csuA* strain restored the adherence levels to Caco-2 cells similar to the wild-type strain. *In silico* analysis of the upstream sequence of the *csu*-like operon, predicted a putative promoter, as well as a consensus cyclic AMP receptor protein (CRP) binding site. A transcriptional fusion between the *csu* predicted regulatory region and the promoterless *cat* reporter gene, encoding chloramphenicol acetyltransferase (CAT), showed activity when *C. rodentium* was grown in minimal medium with glycerol (MM-N), but not when glucose was the carbon source. The deletion of the *crp* gene in *C. rodentium* reduced *csu-cat* expression ten-fold in MM-N + glycerol compared to the wild-type strain.

Additionally, we also found that *csu-cat* fusion is better expressed at 37°C in shaken cultures. In the DBS100Δ*rpoS* strain, the CAT specific activity decreased to levels similar to those observed in the DBS100Δ*crp* strain, indicating that RpoS and CRP are required to activate *csu* expression. Furthermore, two putative binding sites for the Leucine-responsive regulatory protein (Lrp), were also predicted upstream of the -35 box. Electrophoretic mobility shift assays (EMSA) showed that purified Lrp directly binds to the putative promoter of the *csu* operon and that this binding is partially inhibited by L-Leucine, but not by L-Glycine. Collectively, these data suggest that the Csu-like fimbria is required during the early stages of the mouse colonization and that Crp, RpoS, and Lrp regulate its expression in response to environmental cues.

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Participation of α5β1/FAK Integrin Pathway in the *Staphylococcus aureus* Internalization Regulated by Fatty Acids in Bovine Mammary Epithelial Cells

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Bovine mastitis is the main disease that affects dairy cattle worldwide causing important economic losses, being *Staphylococcus aureus* the main microorganism associated. *S. aureus* is able to internalize into non-professional phagocytic cells such as bovine mammary epithelial cells (bMEC) through the "zipper" mechanism, which involves the host cell α5β1 integrins and the focal adhesion kinase (FAK). Internalization allows to the bacteria avoid the immunological response and causes chronic and recurrent infections. In the search for control alternatives, our working group has evaluated the immunomodulatory activities of short and medium chain fatty acids (FA) [propionate (NaP), butyrate (NaB), hexanoate (NaH) and octanoate (NaO)], demonstrating that these molecules reduce *S. aureus* internalization into bMEC; however, it is unknown whether their inhibitory activity is related to α5β1/FAK integrin pathway. Therefore, the aim of this study was to determine the FA effects on the α5β1/FAK integrin pathway during *S. aureus* internalization into bMEC. The *S. aureus* strain ATCC 27543 and a bMEC primary culture were used. The α5β1 integrins membrane abundance was evaluated by flow cytometry as well as the gene expression of the subunits α5 and β1, and the adhesion of *S. aureus* in cells treated with FA. The FAK and Rac_1 participation was evaluated interrupting their activity with a pharmacological inhibitor, followed by gentamicin protection assays (infection multiplicity 30: 1). In addition, the activation status of FAK (phosphorylation) and Rac_1 expression were determined by Western blot. The results showed that FA did not modify the integrin β1 subunit gene expression. However, NaP and NaH decreased the membrane receptor abundance (~50%), as well as bacterial adhesion (47% and 50%, respectively); whereas NaB and NaO favored *S. aureus* adhesion (137% and 129%, respectively). Also, results showed that FAK is necessary for bacterial internalization, due that the inhibition of its activity provoked a significant internalization reduction (63%), which was increased in cells treated with FA (73-77%). Likewise, NaH decreased FAK phosphorylation, whereas NaO favors it; however, this was reversed by bacterial stimulus. On the other hand, NaB favors FAK activation but NaP did not modify it. Finally, the data showed that GTPase Rac_1 is necessary for bacteria internalization due that when it was inhibited, its activity was significantly reduced (~71%), this effect was more pronounced in bMEC treated with FA. Also, NaH and NaO significantly inhibit Rac_1 protein determined by western blot (80 and 72%, respectively). In conclusion, the FA differentially inhibit the α5β1/FAK integrin pathway during *S. aureus* internalization reduction in bMEC.

Prolactin and 17β-estradiol induce pro-inflammatory cytokines in bovine mammary epithelial cells inhibiting *Staphylococcus aureus* internalization and modulating epigenetic marks

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Cattle are more susceptible to mastitis during the peripartum, which is characterized by changes in the levels of 17β-estradiol (E2) and prolactin (PRL), compromising the animal innate immune response (IIR). Bovine mammary epithelial cells (bMECs) are target tissue of these hormones and previously we showed that they regulate IIR elements of bMECs affecting the internalization of *Staphylococcus aureus*: PRL induces it (5 ng/ml) and E2 decreases it (50 pg/ml). In spite of both hormones play a relevant role for bovine mammary tissue *in vivo*, their combined effects on the IIR of bMECs during *S. aureus* infection are unknown, as well as the modulation of epigenetic marks, which constitute the main objectives of this work. The results obtained showed that the hormonal combination [PRL (5 ng/ml) and E2 (50 pg/ml)] decreases *S. aureus* internalization (gentamicin protection assay) into bMECs (~50%); which was associated with a reduction in the abundance of integrin α5β1 (~80%) in membrane evaluated by flow cytometry, this receptor is required for bacterial internalization. In addition, the hormonal mixture inhibited p38 and ERK1/2 kinases activation, which was analyzed by flow cytometry. Also, the combination of both hormones in the presence of *S. aureus* reduced the antimicrobial peptide DEFB4 expression (~0.6 fold) respect to infected bMECs, whereas the gene expression of IL-8 was down-regulated (~0.5 fold) with both hormones, but in infected bMECs this expression was up-regulated (~1.3 fold). Respect to the epigenetic marks evaluated we determined by western blot that, H3K9ac was induced by E2 in bMECs (~50%) whereas PRL reduced this mark in infected bMECs (~25%) The hormonal combination reduced this mark (~30%) in non-infected bMECs. The global H3ac was induced by E2 (~40%) in infected bMECs. Furthermore, hormonal combination reduced HDACs enzymatic activity (~25%) at 12 h, which was determined by a fluorogenic assay. By RT-qPCR analysis, we showed that PRL reduced the expression of the genes coding for the enzymes KDM4C and DNMT3A (~60 and 70%, respectively at 12h) in infected bMECs, whereas E2 reduced the expression of only KDM4C 60%) in infected bMECs. In conclusion, the hormonal combination of PRL and E2 modulates elements of the pro-inflammatory response in bMECs, while reducing *S. aureus* internalization and modulates epigenetic marks.

"Role of miR-200a in *Mycobacterium tuberculosis* H37Rv infection"

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Keywords: Infection, *Mycobacterium tuberculosis,* microRNAs, macrophages.

Tuberculosis is an infectious disease that mainly affects the respiratory tract. This pathology is caused by Mycobacterium tuberculosis (Mtb). Alveolar macrophages have a crucial role in the host-pathogen interaction and the outcome of the disease. During intracellular infection, the cell response is regulated by various mechanisms, including the microRNAs (miRNAs). In mice model, the miR-200a, miR-148a, Let7e, and miR-150 are overexpressed in the pulmonary inflammatory infiltrate three days after infection. This study aims to determine if these miRNAs are overexpressed in murine macrophages infected with Mtb and, mainly to evaluate the role of miR-200a in the infection. The murine macrophage cell line J774A.1 were infected with Mtb at MOI 1 and 3, for 24 and 48 hours. After infection, total RNA was purified, and the expression of Let7e, miR-148a, miR-150, and miR200a was evaluated using TaqMan MicroRNA assays. Our results show that after 24 hours of infection (MOI 1), the four miRNAs are underexpressed, while, the miR-200a is underexpressed in macrophages infected with both MOIs. After 48 hours of infection (MOI 3), the miRNAs were overexpressed; being the miR-148a, the most expressed. Subsequently, the macrophages were infected with Mtb and transfected with the mimicry or inhibitor of the miR-200a. After 24 hours of infection, an increase in the number of bacilli was observed in macrophages treated with the miR-200 mimicry, up to 4 times in macrophages infected with MOI 3, while at 48 hours almost twice bacilli were observed concerning control, regardless of MOI. However, in the inhibition assays, it did not show a decrease in CFUs. Our data suggest that overexpression of microRNA-200a in macrophages favors intracellular survival of the bacillus.

Phenotypic characterization of multidrug-resistant *Salmonella enterica* serovar Typhimurium sequence type (ST) 213 strains isolated in Mexico.

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Salmonella enterica serovar Typhimurium is a facultative intracellular pathogen that causes gastroenteritis in humans. The virulence mechanisms that allow *S.* Typhimurium to cause disease have been extensively studied using prototype strains belonging to the ST19 genotype. However, in recent years, the incidence of strains from different genotypes associated with invasive infections or severe gastroenteritis has increased.

Between 2000-2005 an epidemiological surveillance program carried out in Mexico led to the identification of the emergent multidrug-resistant ST213 genotype among Typhimurium isolates from asymptomatic persons, patients with gastroenteritis or systemic infection, retail meat and food-animal intestines. Strains of the ST213 genotype lack the virulence plasmid present in ST19 strains and instead contain IncC plasmids that confer extended-spectrum cephalosporin resistance.

In this study, different ST213 strains isolated from asymptomatic persons or patients with gastroenteritis or systemic infection and reference strains SL1344 and 4/74 of the ST19 genotype, were selected to perform a comparative phenotypic analysis. Features of the physiology of these strains, such as motility in soft agar and biofilm formation on abiotic surfaces by crystal violet staining, were characterized. In addition, *in vitro* invasion and intracellular replication in Caco-2 epithelial cells, THP-1 macrophages and NRK-49F fibroblasts were assessed using aminoglycoside protection assays.

Motility was similar between the ST213 strains and the ST19 reference strains, except for one ST213 strain that was not motile. ST213 strains showed strong biofilm production, while the reference strains, including the non-motile ST213 strain, form moderate to weak biofilms. All strains used in the present study exhibited differences in the range of 3-10-fold in their ability to invade and survive within Caco-2, THP-1 and NRK-49F cells. The degree of invasion or replication inside the host cell was characteristic of each strain.

These findings challenge the possibility of defining a prototype strain for the study of the ST213 genotype but offer an opportunity to better understand the role that the accessory genome or chromosomal specific changes play in the physiology, gene regulation and virulence diversity of *S.* Typhimurium. Therefore, it is important to study each strain in a particular context since the reference strains do not represent the genomic diversity present in populations of clinical or environmental relevant isolates.

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Functional Analysis of the Type VI Secretion Systems in the Opportunistic Pathogen *Enterobacter cloacae*

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Abstract. *Enterobacter cloacae* has emerged as a opportunistic pathogen involved in health care associated infections. Genomes of sequenced *E. cloacae* show genes that code for at least one type VI secretion system (T6SS). The aim of this study was to analyze the expression and function of two T6SS (named T6SS-1 and T6SS-2) in the pathogenesis of *E. cloacae* ATCC 13047. Initially, transcription of both T6SSs was quantified in different culture media, showing that T6SS-1 and T6SS-2 were preferentially expressed in TSB and DMEM, respectively. Two ATPase mutants of each T6SS (*ΔclpV1* and *ΔclpV2*) were generated. *E. cloacae* T6SS-1 was required to kill *E. coli* and others bacteria genus such as *Klebsiella*, *Salmonella* and *Enterobacter* strains as well. The biofilm formation and cell adhesion to HeLa cells was T6SS-2-dependent. Mutants in *clpV1* and *clpV2* were also defective for colonize the mice gut. These results suggest that both T6SSs are involved in the pathogenesis of *E. cloacae* ATCC 13047 and its components have different functions when they interact with other bacteria or eukaryotic cells.

Epidemiological and clinical characteristics of Coronavirus infections in patients treated at INER (November 2013- March 2018)

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Respiratory tract diseases are an important cause of morbidity and mortality, it´s estimated that cause 4 million deaths worldwide per year. Influenza viruses, parainfluenza and respiratory syncytial virus are well-described pathogens as causing respiratory infections. Coronavirus (CoV) are currently considered as important human pathogens, with up to 30% of the upper respiratory infections being responsible in adults and probably also play a role in serious lower respiratory infections in children and adults. Currently six different CoV strains that are known to infect humans are; HCoV-229E, HCoV-OC43, Coronavirus causing Acute Respiratory Syndrome (SARS-CoV), HCoV-NL63, HCoV-HKU1, and a new coronavirus causing Middle Eastern Respiratory Syndrome (MERS-CoV) which arise in the year 2012.

OBJECTIVE: To describe epidemiological and clinical characteristics of Coronavirus infection in patients that were hospitalized at Instituto Nacional de Enfermedades Respiratorias (INER).

MATERIAL AND METHODS: A retrospective study including hospitalized adult patients with CoV positive by PCR. The results were obtained using descriptive statistics; percentages, means ± SD. It was used chi X^2, ANOVA test to compare characteristics between patients and different subtypes of Coronavirus.

RESULTS: We enrolled 36 patients. The mean age was 47.8 years. 25/36 (69%) were men, male-female ratio was 2.2:1. Neumonia was the most common presentation (14/36). CoV was isolated in asthma and COPD exacerbation in 33%, HIV infection (31%) and systemic arterial hypertension (22%). SatO2 per pulse oximetry was 77.7% (SD ± 14.19). 58% presented PaO2 / FiO2 index between 200 and 300 mmHg. There were no significan to the laboratory findings. Theground-glass opacity was the most frequent radiological finding by tomography in 50% of cases. 25% required ventilatory support for 6.42 (SD ± 26.03) days. Hospital staying 17.6 days and mortality less than 10%. The most frecuent serotypes was HCoV-OC43 in 50% of patients (p = 0.24). Most cases occurred during the winter.

CONCLUSION: Coronavirus can be a frecuent cause of pulmonary infections in HIV patients. Otherwise, this study shows there is no increase in the number of cases every two years as literature suggests. HCoV-OC43 is the most prevalent subtype in mexican patients with pymonary infections.

miR-24-3p and miR-142-3p expression and *Helicobacter pylori* infection in patients with gastric pathology

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Introduction. *H. pylori* is the main cause of chronic gastritis, peptic ulcer and gastric adenocarcinoma. The molecular alterations and mechanisms by which *H. pylori* evades the immune response and perpetuates gastric inflammation in infected patients are still unknown. *H. pylori* promotes the expression of microRNAs that participate in the post-transcriptional regulation of genes with key functions in proliferation, apoptosis, cell differentiation and inflammation processes. **Objective.** To analyze the relationship between miR-142-3p and miR-24-3p expression with *Helicobacter pylori* infection and the diagnosis of patients with gastric pathology. **Material and methods.** 29 gastric biopsies from patients with histopathological diagnosis of chronic gastritis or gastric cancer were analyzed. *H. pylori* infection was determined by PCR using DNA from gastric tissue. Total RNA was purified from gastric biopsies and expression of miR-24-3p and miR 142-3p was determined by qRT-PCR. **Results.** The frequency of *H. pylori* infection in patients with chronic gastritis was 50% (10/20) and 55.5% (5/9) in those diagnosed with gastric cancer. The expression of miR-24-3p and miR-142-3p was higher in patients with chronic gastritis compared to patients with gastric cancer ($p < 0.05$). No differences were found in miR-24-3p and miR-142-3p expression between patients with chronic follicular gastritis, chronic chemical gastritis and chronic chemical gastritis with intestinal metaplasia. In patients with chronic gastritis infected with *H. pylori,* miR-24-3p expression diminished and miR-142-3p expression augmented compared to uninfected patients ($p < 0.05$). There was no difference in miR-24-3p and miR-142-3p expression between patients with gastric cancer infected and not infected with *H. pylori.* **Conclusions.** The expression of miR-24-3p and miR-142-3p is higher in patients with chronic gastritis than in patients with gastric cancer, suggesting their participation in the regulation of inflammation in chronic gastritis. The results suggest that *H. pylori* modulates miR-24-3p and miR-142-3p expression in patients with chronic gastritis.

Imaging analysis of the simple and double mutants in *fliC*, *fimH*, and *csgA* genes of uropathogenic *Escherichia coli* using Transmission Electronic Microscopy

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Introduction. The “Hospital Infantil de México Federico Gómez” daily cares immunocompromised pediatric patients, who are susceptible to the acquisition and complication of urinary tract infections (UTI). These infections are the second cause of morbidity in Mexico associated to uropathogenic *Escherichia coli* (UPEC) as the main etiological agent. UPEC assembles several fimbriae such as type 1 and curli, associated with cell bladder adhesion, as wells as, flagella that contribute to the kidneys colonization. The function of these structural appendices as a multifactorial mechanism has not been described in the adherence process associated with UPEC infection. **Justification.** The mutants generation (single and double) with FimH of the type 1 fimbriae, CsgA of curli and FliC of the flagellum, may be of great relevance to understanding the multifactorial adherence process of UPEC. **Aim.** In this study, were generated single and double mutants in the *fliC, fimH*, and *csgA* genes of UPEC CFT073 and evaluated phenotypically by Transmission Electronic Microscopy (TEM). **Material and methods.** The inactivation of the *fliC, fimH* and *csgA* genes in the UPEC strain CFT073 was generated by the Datsenko and Wanner method. The generated mutants were genotypically verified by PCR analysis using specific primers. The absence of the fimbrial and flagellar structures were confirmed by TEM assays when the strains were grown under the next conditions: 1) LB supplemented with dextrose 1% for the expression of the type 1 fimbriae; 2) yeast extract casamino acids supplemented with DMSO 4% for the curli expression; and 3) flagellum, semi-solid LB with agar 0.3 % for flagellum. **Results.** The simple mutants (*fimH*::Km, *csgA*::Km, and *fliC*::Km), and double mutants (*fimH*::Km/*csgA*::Cm and *fimH*::Km/*fliC*::Cm) were confirmed by PCR. UPEC strain CFT073 showed flagella as flexible polar filaments of approximately 10 µm long accord the TEM micrograph analysis. Under expression condition of type 1 fimbriae, CFT073 strain assembled several short, thin and rigid peritrichous structures suggesting the presence of these fimbriae. Additionally, curli fimbriae were observed as coiled fine fibers, added as an amorphous matrix around the bacterial surface when were grown in YESCA-DMSO medium. UPEC Δ*fimH* showed the absence of the fibrillar structures of the type 1 fimbriae, while the curli and flagella expressions were visualized. UPEC ΔcsgA showed the characteristic structure absence of curli associated with the highest flagella expression. Finally, UPEC Δ*fliC* showed the characteristic structure absence of the flagella, without affecting of the curli or the type 1 fimbriae expression. **Conclusions**. The Single and double mutants generated in the *fimH*, *csgA*, and *fliC*, will be employed to understand the multifactorial adherence process in the pathogenesis of UPEC.

Macrophages infection with dormant *Mycobacterium tuberculosis*: transcriptional analysis

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Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* (*Mtb)* and it is known that one-third of the world's population has latent infection. The aim of this work was to analyze the global expression of *Mtb* and 178 human genes during infection of macrophages when the bacilli came from an *in vitro* latency state rich in cholesterol or dextrose. The Wayne and Hayes model was used to obtain bacilli in both latency phases (NRP1 and NRP2) and THP-1 macrophages were infected for 24h and 48h. Mycobacterial and human RNA were obtained and expression was analyzed through a personalized microarray. Finally, the validation was performed using RT-qPCR. It was observed that during infection with bacilli of both latency phases from a dextrose-rich environment, the *wag*31 and *kdp*C genes were overexpressed during early infection compared to infection with active bacilli. In addition, it was observed that 18 genes were overexpressed during infection with bacilli of the NRP1 phase compared to the NRP2 phase. These genes include: *vap*C17, *par*E2, *rel*G, *tgs*4 and Rv2484c.The results shown seem to indicate that the bacilli of the NRP1 phase are pre-adapted to the intracellular microenvironment, which allows them to survive and persist. Furthermore, analysis of the expression of human genes showed that infection with dormant *Mtb* induced overexpression of more genes in THP-1 macrophages than infection with active bacilli.

Effect of a Monoclonal Antibody IgA Anti-Lipid A in the Polymyxin B Sensitivity of Salmonella

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Introduction: Infections of humans with Salmonella remains a global health problem. The presence of O-antigen has been shown to be important to resistance to Polymyxin B since a *S.* Typhimurium mutant devoid of O-antigen shows high susceptibility to this antimicrobial peptide (AMPs).The lipid A of *Salmonella* is phosphorylated at the 1 and 4′ positions and several studies have shown that de-phosphorylation of Lipid A contributes to resistance against AMPs Also strains that exhibit resistance to polymyxin B also display resistance to antimicrobial peptides. Secretory IgA antibodies can play different roles in the immune response to gastrointestinal infection. There are no reports of anti – Lipid A antibodies in the the resistance to polimixin B. Methods: All the experiments were done with two Salmonella Strains: Salmonella enterica serovar Typhymurium (S.Typhimurium) and Salmonella enterica serovar Typhi (S.Typhi).Both strains were used alone or opsonized with the monoclonal anti-lipid A antibody. Results: the growth of both strains was inhibited by polymyxin B but S. Typhi was more sensitive than S. Typhimimurium. The anti-Lipid A antibody- inhibited the effect of Polymyxin B and this was dose-dependent. Conclusion: S. typhimurium was more resistant to Polymyxin B probably because these bacterias have several mechanism to modify the LPS and the anti-lipid A antibody inhibits the effect of Polymyxin B in a doses-dependent form.

FOOD AND CLINICAL MICROBIOLOGY

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Association between virulence profiles and antimicrobial resistance in *Salmonella enterica* strains from Michoacán

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Salmonella enterica is one of the most important etiological agents of foodborn diseases. The genes *rmbA, ssaQ*, and *sip4-F* are involved in the invasion and infection mechanisms of *S. enterica*. Virulotyping is a new scheme of genotyping based on the detection of these virulence-associated genes (Virulence profile or VP). In this work we evaluated the association between the antimicrobial resistance and the PV. The DNA of 134 *S. enterica* strains was extracted by phenol-chloroform method. The *ssaQ, rmbA* and *spi4-F* genes were amplified by PCR and dendograms were constructed according to the presence or absence combinations. Five PV were found: PV1 (*ssaQ, rmbA, spi4-F*), PV2 (*ssaQ, rmbA*), PV3(*rmbA, spi4-F*), PV4(*ssaQ, spi4-F*), PV5(*ssaQ*). The prevalence of PV1 was observed in the Newport, Derby and Panama serotypes; PV2 in the serotypes Mbandaka, Morganii and Muenster; PV3 was observed with greater incidence in Derby and Enteritidis serotypes; PV4 and PV5 were only in Oranienburg and Montevideo serotypes, respectively. In the case of the association of PV with respect to geographical location, the incidence of PV1 was observed in the municipalities of Morelia, Lázaro Cárdenas and Uruapan; PV2 was observed in Morelia and Uruapan; PV3 was observed in José Sixto Verduzco and Morelia; PV4 only Lázaro Cárdenas and PV5 only in José Sixto Verduzco. The analysis of dendograms showed that serotypes Give, Braenderup, Bovismorbificans, Azteca, anatum, Albany, Agona, Adelaide, Havana, Infantis, Lockleaze, London, Muenchen, Newport, Panama, Poona, Saintpaul, Sandiego, Seftenberg, serogrupo B, Serogrupo C1, Serogrupo D, Serogrupo E1, Serogrupo E4, Sintorf, Typhimurium, Vejle, Vismorbifica and Weltrevreden, they are grouped in the same clade; Derby and Enteritidis serotypes are in another; Mbandaka, Morganii and Muenster they are in a clade, being different Oranienburg y Montevideo. The correlation was found between the PV and geographical location there is a correlation between the municipalities La piedad, Jacona, Huetamo, Gabriel Zamora, Ciudad Hidalgo, Churumuco, Apatzingan, Angamacutiro, Angangueo, Los Reyes, Mújica, Ocampo, Patzcuaro, Tlazazalca, Tuzantla, Yurecuaro, Zamora and Zitucuaro; obtaining a certain difference between the municipalities of Lázaro Cardenas, Morelia, Uruapan and Jose Sixto Verduzco being these municipalities that present diversity in PV. A resistance correlation between PV1 was obtained with respect to the following antimicrobials: Amikacin, Ampicillin, Carbenicillin, Chloramphenicol, Sulfamethoxazole/Trimetroprim; PV2 and PV3 resistance to Sulfamethoxazole/Trimetroprim, Nitrofurantoin and Chloramphenicol was observed. For PV4 it was only observed with Carbenicillin and finally in PV5 resistance to Gentamicin, Amikacin, Carbenicillin and Netylmicin was observed.

Detection of *Serratia marcescens* isolates obtained from pediatric patients in a tertiary care hospital in Mexico City.

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Background: *S. marcescens* is an opportunistic pathogen causing hospital acquired-infections and has been associated with hospital outbreaks. It is important for its increasing antimicrobial resistance and it causes a wide range of infections, such as meningitis, pneumonia, sepsis, endocarditis and fasciitis. In Mexico, information about *S. marcescens* causing infections in pediatric patients is limited

Objective. To detect *S. marcescens* isolates obtained from pediatric patients

Material and methods: During a seven years period (2013-2019), we collected 53 S. *marcescens* clinical isolates obtained from patients of Instituto Nacional de Pediatria (INP). Phenotypic detection of extended spectrum beta-lactamases (ESBL) and carbapenemases was performed according to the guidelines of the Clinical and Laboratory Standard Institute (CLSI). The molecular detection of beta-lactamases genes (*bla*$_{CTX-M-1}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-9}$, *bla*$_{CTX-M8/25}$, *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{OXA-48}$ and *bla*$_{GES}$) was determined by PCR and sequencing.

Results: ESBL phenotypic test was positive in three isolates; in one of them *bla*$_{SHV}$ was detected. Only one isolate was resistant to carbapenems; Carba NP and mCIM tests were negative, carbapenemase genes were not found.

Conclusions: The resistance in *S. marcescens* isolates from INP in limited. The frequency of *S. marcescens* with ESBL was 7.7%. A surveillance program will allow to improve detection and infection control.

Osteomyelitis with Panton- Valentine Leukocidin producing *Staphylococcus aureus* strains, experience in a tertiary pediatric hospital in Mexico city.

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Background: *S. aureus* is the most common cause of osteomyelitis in children. Panton-Valentine leucocidin (PVL) is an exotoxin produced by certain *S. aureus* strains, which can be detected in both methicilin sensitive and resistant strains of staphylococci. Pediatric osteomyelitis by PVL producing *S. aureus* constitute a rare, but highly critical event. They are characterized by a rapid course of marked inflammation, worsening under conservative therapy and a high rate of recurrence. No information is available on osteomyelitis and Panton- Valentine leukocidin producing *S. aureus* in Mexico. **Objetive:** The aim of this study was to determine the molecular characterization of *S. aureus* strains isolated in pediatric patients with osteomyelitis and their clinical features in a tertiary hospital in Mexico City. **Methods:** We conducted a prospective study of children admitted for osteomyelitis, between August 2018 and July 2019, at Instituto Nacional de Pediatría. We obtained an informed consent in children under 12 years of age and an informed assent in children over twelve. The confirmation of the *S. aureus* isolates were performed by amplification and analysis of 16S rRNA and *nuc* genes. The *mec*A and *pvl* genes were detected by PCR, the clinical features were obtained at the admission. **Results:** Twenty six patient were included and 14 cases of osteomyelitis were diagnosed, six were due to *S. aureus*; all of them were methicillin susceptible and two were PVL producers. Both patient had a severe initial presentation, with extensive local abscesses and one required three surgical procedures and developed septic pulmonary embolism. The two patients received double antimicrobial treatment and required a long therapy interval. **Conclusions:** Osteomyelitis with Panton Valentine Leukocidin producing *S. aureus* seems to be more severe. In patients with severe osteomyelitis, it is essential to detect the PVL toxin because they require early surgical intervention and prolonged intravenous therapy. Our findings suggest that the severity of the osteomyelitis is linked with PVL production more than with methicilin resistance due to that all our isolates were methicilin susceptible.

Detection of some virulence factors in *Salmonella* sp strains of environmental origin

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Salmonella is one of the most common foodborne pathogens worldwide. A common cause of *Salmonella* infections in humans are associated with ingestion of pork products contaminated with this pathogen, given that our aim was to determine the presence of some virulence factors such as flagella and siderophores production by phenotypic and genotypic studies, as well as the antimicrobial resistance profile in *Salmonella.*

In this study, 40 *Salmonella* strains were isolated from raw pork following the methodology described in the BAM-FDA. The results showed that all tested strains displayed *swimming* and *swarming* motility on the BHI medium with different agar concentration and all of them produced siderophores on the CAS medium. On the other hand, the 30% (12/40) of the strains developed moderate biofilm, the 27.5 % (11/40) of them were unable to develop biofilm and the rest developed a weak one. Due to the antimicrobial susceptibility test revealed that strains were resistant: 67% to cephalotin, 20% nitrofurantoin, 50% to cephotaxime, 95% to pefloxacin, 62% to ceftriaxone, 50 % to chloramphenicol and 100% to ampicillin. Nevertheless, all of them were susceptible to trimethoprim-sulfamethoxazole, gentamicin, netilmicin and amikacin.

Furthermore, two genes encoding virulence factors were detected using PCR amplification. These included *fliC* and *iroB* genes, which were present in all strains.

The Salmonella strains isolated from raw pork showed multiple resistance to antibiotics and several virulence factors

Presence of virulence markers in *Vibrio parahaemolyticus* strains isolated from oysters.

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Vibrio parahaemolyticus is an emerging bacterium and is the main cause of gastroenteritis associated with seafood consumption, it is often isolated from bivalves, shrimp and crabs. The main virulence factors are direct thermostable hemolysin (TDH), hemolysin related to TDH (TRH), among others. There are few data at the national level on the epidemiology of this microorganism, so the objective of this study was to detect virulence factors in environmental strains of V. parahaemolyticus, for this, 30 oyster samples obtained in Mexico City markets were analyzed using the methodology described in the BAM-FDA. This bacterium was isolated in 9 of 30 samples, 27 strains were phenotypically identified as *V. parahaemolyticus* of which 24 were confirmed as *V. parahaemolyticus* by amplifying the pR72H fragment. The 100% (24/24) of the strains showed swarming and swimming movements and siderophores production. In 96% of the strains the *fla* gene coding for flagelin was amplified, as well as the genes involved in biosynthesis and transport of siderophores (*pvuA, pvsA*) in 100% and 45% respectively. The environmental strains isolated in this study have characteristics that confer virulence and represent a risk to the health of the consumer if they consume raw or undercooked fish products which can lead to a picture of gastroenteritis.

Comparison of internalization in cherry tomatoes between *Salmonella* Montevideo, Newport, Saintpaul and non-pathogenic *E. coli*

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Salmonella is one of the pathogenic bacteria that are frequently transmitted through animal or vegetable food. Several reports have shown that plants may be hosts of human pathogens. The vegetable and fruits can be contaminated during harvest, when they come into contact with fertilizers of animals or poultry wastes that are incorrectly treated or cleaned or during their distribution. The aim of this study is to observe whether the pathogenicity factors of *Salmonella* allow differential internalization between the studied serotypes and non-pathogenic *Escherichia coli* in cherry tomatoes.

Strains of *Salmonella* serotypes Montevideo, Newport and Saintpaul, and *E. coli* MC4100 resistant to rifampin were inoculated in cherry tomatoes by immersion promoting the internalization of the bacteria with a temperature difference of 12 °C. The bacteria adhered to the surface of the tomatoes were eliminated using 96% ethanol and sonication. Internalized bacteria were recovered by breaking the fruit in a stomacher using 50 mL of physiological saline solution (PSS). The internalized bacteria were concentrated in 2 mL of PSS, which were used for direct plating on sulfa agar plates with rifampicin at 0.1 mg/mL (SA-Rf) or for making two serial dilutions in PSS and then plating onto SA-Rf. The colony forming units recovered were reported as Log_{10} CFU/mL.

It has been reported that using high concentrations of *Salmonella* in fruits and vegetables, these bacteria can colonize the roots of the plants and then they are distributed to the stems, leaves and fruits. In the case of tomatoes, the stem scar is the area where more bacteria are retained; however, the fruits do not show signs of infection nor differences in appearance or smell. It has also been reported that *S.* Montevideo is best adapted to the interior of tomatoes fruit compared with the other *Salmonella* serotypes studied. Our results show that 3 days post-inoculation, *S.* Saintpaul and *S.* Newport were recovered at a higher concentration from the interior of cherry tomatoes with 4.18 and 4.11 Log_{10} CFU/mL, respectively. In contrast, *S.* Montevideo and *E. coli*, were recovered at 2.03 and 2.08 Log_{10} CFU/mL. The results obtained suggest that there are genetic differences between *Salmonella* serotypes studied that make them more suitable for internalization in tomatoes and the pathogenicity factors present in them do not seem to be determinants in the internalization into tomatoes.

Epidemiological characterization of virulence factors of Pseudomonas spp. from the "Hospital Central Ignacio Morones Prieto" of San Luis Potosí, SLP.

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Pseudomonas aeruginosa is a non-fermenting Gram-negative bacillus, capable of surviving in a wide range of habitats including medical devices.[1] In Mexico, it is the second most prevalent microorganism with 13% of cases of infections in the nosocomies according to the RHOVE (Red Hospitalaria de Vigilancia Epidemiológica by its acronym in Spanish) in 2015.[2]

Pseudomonas aeruginosa infections are difficult to treat due to the presence of various intrinsic, acquired and adaptive resistance mechanisms that can be acting simultaneously.[3] However, antimicrobial resistance does not explain the rates of complications recorded during the first 24-72 hours of a bacteraemia; In these cases, virulence plays a leading role.[4] In this aspect it is known that the adaptive capacity, resistance and repertoire of pathogenicity that this species has is a consequence of an extensive genetic diversity regulated by means of the "Quorum Sensing".[5]

Currently, the investigation of nosocomial pathogens focuses on resistance, leaving aside the virulence factors that play a fundamental role in the development and prognosis of the infection. The present study focuses on the identification of the pathogenicity determinants prevalent in clinical isolates corresponding healthcare-associated infections (HCAIs) of a hospital in San Luis Potosí city. The aim of this work was the phenotypic and molecular characterization of the virulence factors in strains of *Pseudomonas aeruginosa*, from the “Hospital Central Dr. Ignacio Morones Prieto”. Phenotypic tests of soluble enzymatic factors were used for the identification the production of pyoverdine, caseinases and proteinases. In addition, 5 multiplex PCRs were designed for the identification of 16 genes that code for pathogenicity determinants and 2 genes associated with the Quorum Sensing system. Finally, the presence of these genes was associated with the type of infection developed by the patients.

A total of 189 samples were collected from May 2018 to May 2019, medical records were reviewed identifying cases of HCAIs, among this, the cases of bacteremia and intrahospital pneumonia in adults are the most frequent types of infection, reported. A 37% resistance to meropenem and a 16% resistance to ciprofloxacin were identified, which have at least 70% positivity in enzymatic assays, a sign of the expression of virulence factors; For the molecular tests, a high index of genes related to adhesion was found, particularly in respiratory tract infections and side tissues which are vital for a successful infection, in the toxigenic aspect the genes corresponding to the type III secretion system were presented in the less 50% of the cases indicating high toxicity in the strains.

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Molecular identification of actinobacteria with anti-*Candida* activity.

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Actinobacteria phylum is comprised mainly of Gram positive organisms with high G+C content. In this phylum the genus *Streptomyces* is well-known producer of bioactive compounds as antibiotics and antifungals. In this work, five strains that produce antifungals were identified to species level. These actinobacteria strains excrete inhibitors of *Candida* spp. In liquid and solid media. The minimum inhibitory concentrations of the lyophilized supernatants of the strains were estimated. The molecular identification was based in the amplification and sequencing of six housekeeping genes (16S rRNA, *atpD, gyrB, recA, ropB* and *trpB*). For the 16S rRNA amplification were used universal primers (27F and 1492R) and for the *atpD, gyrB, recA, ropB* and *trpB* genes amplification were designed new primers. All the strains (J24, J29, J29 ori 2, 6B and Q) inhibited the growth of *Candida* spp. interfering with the ergosterol biosynthesis. Although a concatenated phylogenetic tree of sis genes was constructed, the species level identification of the strains was not allowed. Probably, all the strains belong to new species of *Streptomyces* genus.

Activity of the ethanolic extract of six medicinal plants on the growth of Gardnerella vaginalis.

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The aim of this study was to determine the effect of crude extracts of plants *Origanum vulgare*, *Calendula officinalis*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Eucalyptus globulus* and *Lavandula officinalis* on the growth of *Gardnerella vaginalis* (GV) recognized as the agent that initiates the process of dysbiosis which results in bacterial vaginosis (BV).
BV is an alteration of the normal vaginal microbiota, by replacing Lactobacillus with facultative anaerobes, highlighting the presence of GV, which initiates the formation of biofilm in the vaginal epithelium thus establishing resistance to therapy with antibiotics.
BV has been one of the most common and recurrent vaginal infections among the population of reproductive age.
Although metronidazole is one of the most effective medications recommended as the first-line treatment, it has various side effects and disease recurrences have been observed.
Aqueous and ethanolic extracts of each plant were obtained and to determine the antibacterial activity to GV ATCC 14018 agar diffusion was performed, from extracts that were positive minimal inhibition concentration (MIC) was determined by dilution microplate and agar diffusion technique.
Marjoram, thyme, lavender and eucalyptus inhibited the growth of G. vaginalis. Marjoram and thyme have a minimum inhibitory concentration of 8 mg / mL, followed by eucalyptus with 16 mg / mL and lavender with a value greater than 64 mg / mL.
With the MICs, the activity of the extracts was observed in clinical biofilm-forming strains, the Biofilm index formation (BFI) was calculated and the reduction percentage was obtained with marjoram, thyme and eucalyptus, of which eucalyptus showed greater activity of the three extracts.
Four of the six selected plants showed growth GV inhibition, three of them also reduced the biofilm formed by what is a promising option to be an alternative treatment to combat BV.

Genotyping of uropathogenic *Escherichia coli* (UPEC) strains isolated from urinary tract infections during pregnancy in Culiacan, Sinaloa.

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Introduction: Uropathogenic *Escherichia coli* (UPEC) is the main etiologic agent involved in urinary tract infections (UTI), the most common infections in humans and one of the most frequent pathological conditions in community and hospital settings. These infections produce about 150 million cases in the world every year. In adults, they predominate in the female sex, more frequently from the onset of sexual activity and in pregnancy, increasing perinatal morbidity and mortality. Around 66% of pregnant women have an asymptomatic UTI; the most common condition is acute pyelonephritis in the second trimester of pregnancy, which increases the risk of premature birth or low birth weight, maternal anemia, hypertension or preeclampsia. On the other hand, UPEC has included virulence factors in its genome that facilitate colonization, growth and persistence in the host urinary tract and, due to its pathogenicity mechanism, could be related to the development of different pathologies during pregnancy. In this work, we determined the prevalence of UTI produced by UPEC in pregnant women. **Material and methods:** a representative sampling of the study population with UTI was performed at the local level, collecting urine samples in sterile containers of pregnant women and applying a pre-coded interview. Samples were transferred to the laboratory and a general urine test and a microscopic examination were performed; subsequently, samples with high bacterial load were cultured in MacConkey and CLED agar to perform biochemical identification. Then, we proceeded to perform DNA extraction from clinical isolates identified as *E. coli* using the thermal shock technique. Finally, to genotype *E. coli* isolates we performed three different PCR protocols: *hlyA* with a conventional PCR; one multiplex PCR for *papC*, *cnf1*, *fimA*, *fyuA*, *vat*, and another one for *iroN*, *KpsMTII*, *iutA* and *agn43*. **Results**: 77 samples were collected from pregnant women, of which 44 (45%) of them were positive for different bacterias. Nine of these (12.04%) were positive for *Escherichia coli* and eight of these were positive for the *iroN* gene, six for *Agn43*, five for *vat*, five for *cnf1*, two for *kpsMTII*, two for *fyuA* and one for *iutA*,. **Conclusion:** UPEC strains isolated from pregnant women have different virulence genes that could be related to complications during birth. Nevertheless, a patient follow-up must be performed to fully associate both factors.

Isolation and identification of bacterial pathogens from yellow tail snapper (*Ocyurus chrysurus*) and whiteleg shrimp (*Penaeus vannamei*)

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Seafood consumption has increased globally during the last years. It has health benefits such as neural, visual, and cognitive development during gestation and infancy, also minimizes the hazard of cardiovascular diseases. However, it is also common the presence of pathogens which can affect human health as well as pathogens that affects fishes and shrimps health resulting in economic losses. Among the principal infectious agents are bacteria. In aquaculture practices, it is important to monitor the presence of pathogens in order to avoid economic losses associated to aquatic animals infections and guarantee human safety. Monitoring, will also help to establish prophylactic and/or therapeutic measures.
In this study we survey the bacterial pathogens from yellow tail snapper and whiteleg shrimp to determine their frequency and their possible health impact on humans and the aquatic animals themselves.
The fish and shrimp samples (12 each one) were collected from the Investigation Unit-UNAM at Sisal, and an aquaculture facility at Dzilam de Bravo, Yucatan, respectively. Samples of gills, skin and gut of yellow tail snapper and gills and gut of whiteleg shrimp were aseptically obtained by dissection and inoculated in tripticase soy broth (TSB) and alkaline peptone water (APA). The broths were incubated for 24 h (TSB) and for 6 h (APA) at 37°C, an inoculum of TSB was streak on Mac Conkey agar, Thiosulfate citrate bile salts (TCBS) with 2% NaCl and Blood agar, and an inoculum of APA on TCBS. The culture plates were incubated for 24 h at 37°C. Each different colony was subcultured in tripticase soy agar with 2% NaCl. The identification was carried out by BD BBL Crystal Identification systems and Vitek MS system.
A total of 54 strains from yellow tail snappers and 50 from the whiteleg shrimps were identified. In descendent frequency order, the species isolated from yellow tail snappers were *Vibrio alginolyticus*, *Vibrio cholerae* y *Photobacterium damselae, Cryosebacterium indologenes*, *Shewanella putrefaciens*, *Bacillus cereus, Pseudomonas aeruginosa, Aeromonas hydrophila* and *Weeksella virosa/Bergeyella zoohelcum.* In the whiteleg shrimps, the species isolated were *Shewanella algae*, *V. alginolyticus,* sporulating gram positive bacilli, *P. damselae, S. putrefaciens, Enterococcus faecalis*, *V. cholerae*, *Vibrio vulnificus*, *Bacillus subtilis*, *A. hydrophila* and *Enterococcus durans.*
The conclusions from this survey are: The more frequently isolated species in snappers was *V. alginolyticus* while in shrimps was *S. algae.* Most of the isolated species habitat is aquatic and they could be pathogens of snappers, and shrimps if the culture conditions are adverse. The found species could also be pathogens for humans.

ATIBACTERIAN EFFECT OF CRUDE EXTRACTS OF **Vaccinium myrtillus** (ARANDAN) ON CLINICAL ISOLATES

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The blueberry is the fruit of a perennial shrub, generally deciduous, native to the northern hemisphere, belonging to the family of Ericaceae, and the genus Vaccinium, its main components are proanthocyanidins, anthocyanins, flavonoids, phenolic acids, quinic acid, malic and citric acid, iridoids, ursolic acid, fructose and other sugars. The objective of this study was to determine the antibacterial activity of aqueous extracts of industrialized blueberry ethanol vs. bulk blueberry on strains of Escherichia coli, Salmonella sp., Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecalis in Mueller-Hinton medium. Blueberry extract was obtained by maceration method with aqueous solvent and ethanol at room temperature, in concentrations of 20%, 40%, 60%, 80% and 100%. The strains were sown in duplicate in 6 Petri boxes of Mueller-Hinton agar, using the Kirby-Bauer technique, placing the five concentrations mentioned in each box, with a positive control group (antibiogram), and a negative control group (distilled water and ethanol); these were incubated at 37±2ºC for 24 hours. The antibacterial activity of the ethanolic and aqueous extracts of the fruit vaccinium myrtillu (blueberry) from two origins (packaging and bulk) was evaluated, generating significant inhibition for the strain of Enterococcus faecalis ATCC 29212. The experimental substances for ethanolic extract sold in bulk at 20% had an average inhibition halos of 10.75 mm, the aqueous extract of blueberry 20% had an average inhibition halos of 8 mm and 40% of 12.5 mm, while for Escherichia coli, Salmonella sp.., Klebsiella pneumoniae, Staphylococcus aureus and Enterococcus faecalis (aqueous bulk and ethanolic package) there was no inhibition in growth, with respect to positive control in any of the evaluated concentrations. It was demonstrated that there is antibacterial activity in vitro of the ethanolic and aqueous extracts, presenting inhibition against the clinical strain of Enterococcus faecalis.

Phylogenetic groups and virulence transfer of *E. coli* isolated from Puebla women with urinary tract infection

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Urinary tract infections (UTIs) in Mexico have a higher incidence in women (77%) than in men (23%). Uropathogenic *Escherichia coli* (UPEC) is the main ethogenic agent of UTI, accounting for 80% of cases. UPEC has unique genomic characteristics acquired through the horizontal transfer of genes, mainly through the process of conjugation, this has allowed it to develop multiple strategies to colonize the urinary tract and cause damage to the host. It has been reported that UPEC belongs mainly to phylogroups B2 and D. In addition, the high prevalence of UPEC has also been related to the increase in antibiotic resistance, mainly due to the spread of extended-spectrum β-lactamases (ESBLs) encoded in plasmids mainly from the IncF group. The objective of this work was to determine the phylogenetic groups and the capacity of UPEC isolated from urine of women with UTI to transfer genes of virulence and/or resistance by conjugation. We analyzed 51 strains donated by the Hospital ISSSTE of Puebla in 2016. The determination of the resistance profiles was performed by Kirby-Bauer, of the phylogenetic groups by Clermont (2013), of virulence and resistance genes by PCR and conjugation by Poe (2018). 39% of the strains belong to the phylogroup B2, followed by the phylogroup D (19%) and the philogroups A and C (16%). The determination of mob (24%) and tra (53%) genes was performed to perform conjugation tests. Ten strains APr Rfs were selected, 70% were conjugative. We analyzed 1-4 transconjugant clones for antibiotic resistance, virulence and ESBL production. Ninety percent (n = 13) of the transconjugants managed to acquire resistance to first, second and third generation cephalosporins and fluoroquinolones, 71% of these strains also transferred the production of BLEETEM, BLEECTX-M and BLEEOXA. In addition, the transconjugant strains acquired virulence genes such as *iucD, satA, satP, iha*, and *cnf*. With these data we can conclude that UPEC, isolated from women with UTI, carry out the process of bacterial conjugation that is considered the most sophisticated, complex and with a greater spectrum of action on the flow of genes between the bacteria. This process surely allows the dissemination of virulence factors and also mechanisms of resistance to antibiotics in the population analyzed.

Antibacterial, phenolic and antioxidant activities of honey from stingless bees

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Honey is a natural food derived from honey bee and most commonly used as a sweetener also is known for its remedial value. Even though there has a study on the potential of honey in treating several health problems, but studies mostly are focusing on Manuka honey compared to the stingless bee honey due to the low production of honey. Previous studies demonstrated that stingless bee honey exhibit antioxidant, anti-inflammatory, anticancer and antimicrobial activities. However, the beneficial of stingless bee honey has been abandoned in modern medicine due to the paucity of systematic scientific studies for supporting its medical properties. The composition of stingless bee honey differs from other species according to some physicochemical parameters.

The antibacterial activity of honey produced by the stingless bees was evaluated against methicillin-resistant *S. aureus* (MRSA), *E. coli* and *S. mutans*.

The phenolic composition, antioxidant capacity and antibacterial activity of seventeen honey samples from four different species of stingless bees were determined in this study.

Evaluation of the antibacterial activity of honey samples was determined using microdilution method and the minimum inhibitory concentrations (MICs) were calculated. The MIC values ranged from 0.78 to 12.5% for the tested microorganisms.

Total phenolic content was measured with the Folin Ciocalteau reagent, while flavonoid content was determined after complex formation with $AlCl_3$. Antioxidant activity was measured by the DPPH methodology. Honey produced by *Scaptotrigona mexicana* had higher phenolic and flavonoid contents than all the others stingless bee honey samples analyzed. Honey from *Melipona becheii*, harvested in 2019, showed the highest DPPH scavenging activity among all the analyzed samples. The results show that stingless bee honey form Mexico has good antioxidant activity besides its nutritional value. The honey of the stingless bees can thus be utilized as antibacterial substances against *S. aureus* (MRSA), *E. coli* and *S. mutans* strains, and this action is probably due to a synergistic action of different components such as hydrogen peroxide, sugars, and other compounds.

The application of natural products is important for the control of bacterial infections. Further studies are warranted to demonstrate that the antimicrobial activity of the honey examined here could be used in clinical applications for the prevention of bacterial infections.

Área: Microbiología de alimentos y clínica

Tipo de presentación: Cartel

Antimicrobial activity from *Lactococcus lactis* 115 isolated from atole agrio, from Villahermosa, Tabasco

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Atole agrio is a Mexican refreshing, non-alcoholic, fermented maize beverage produced in the South eastern states of Mexico: Tabasco, Chiapas and Veracruz. It is made out of non-nixtamalized maize (*Zea mays*), ground and shaped into balls. The microbiota responsible for atole agrio fermentation is complex. Among bacteria, previous studies have identified lactic acid bacteria (LAB), amylolytic lactid acid bacteria and enterobacteria. It is consumed by indigenous people as a refreshing beverage, during religious ceremonies or in case of illness. It is believed to provide health benefits to the people who drink it. In fermented foods, LAB display numerous antimicrobial activities. This is mainly due to the production of bacteriocins and organic acids. The purpose of the present study was to evaluate the optimal conditions for growth and production of antimicrobial substances by *L. lactis* 115 for its subsequent concentration, semi-purification and characterization. The strain used for this study was isolated from atole agrio in Tabasco, México and stored it in 20% glycerol+ MRS medium -70°. *L. lactis* 115 exhibited important antimicrobial activity against some pathogenic microorganisms by the well diffusion assay, being *L. monocytogenes* one of them. Growth kinetics were performed to observe the effect of the carbon source, pH and temperature on the growth and production of the substance with antimicrobial activity in MRS and HSH media. To concentrate and partially purify the substance with antimicrobial activity produced by *L. lactis* 115, precipitation with ammonium sulfate and the adsorption/desorption method were used. SDS-PAGE and a zymogram were used to estimate the molecular mass of the antimicrobial substance. For *L. lactis* 115, the best growth and production conditions of the antimicrobial substance were; glucose as a carbon source, initial pH of 7.5 and 30° C in MRS medium, while in HSH medium were lactose as a carbon source, initial pH of 6.5 and 30°C. With the adsorption/desorption method, most of the contaminating proteins were removed and a higher degree of purification was obtained. The protein with inhibitory activity produced by *L. lactis* 115 has an estimated molecular mass of 10.9 kDa.

Área: Microbiología de alimentos y clínica
Tipo de presentación: Cartel.

Detection of *Trypanosoma cruzi* by municipality of the state of Morelos in donors of the Hospital General Regional No. 01, del Instituto Mexicano del Seguro Social (IMSS) of Cuernavaca, Morelos.

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Introduction: Chagas disease is caused by the protozoan *T. cruzi* who is transmitted to humans by bedbugs of the genus *Triatoma*. Chagas disease has an acute and chronic phase that can cause alterations of the colon, esophagus and cardiomegaly. *T cruzi* can be transmitted to humans by blood transfusion. Therefore, detection in donors is of great importance to stop the chain of contagion.

Objective: To determine the incidence of reactive serology at *T. cruzi* by municipality of the state of Morelos of the donors of the Hospital General Regional No.01 del Instituto Mexicano del Seguro Social (IMSS) of Cuernavaca, Morelos.

Methodology: A retrospective, cross-sectional and descriptive study was conducted at the Blood Bank of H.G.R. No. 01 of the IMSS in Cuernavaca, Morelos. 52,813 units captured in the blood bank from January 2010 to December 2015 were analyzed using the chemiluminescence technique. The incidence and prevalence of *T. cruzi* was determined by municipality.

Results and discussion: Of 52,813 units analyzed, 0.39% (212) presented reactivity to *T. cruzi*. Of which 20% were women and 80% men. The municipalities with the highest incidence were Cuautla 0.11%, Cuernavaca and Jiutepec with 0.07%, Temixco and Emiliano Zapata with 0.02%, Yautepec and Xochitepec with 0.01%, Huitzilac, Temoac, Miacatlan, Tlayacapan, Jonacatepec, Tlaltizapan and Tepoztlan with 0.002%. Other municipalities not belonging to Morelos with 0.015%.

Conclusion: The highest incidence of *T. cruzi* was found in the municipalities with the highest population density such as Cuautla, Cuernavaca and Jiutepec, which correlated with the municipalities that had the most donors. Therefore, it is important to have an epidemiological follow-up of the donor and his community to have a screening of *T. cruzi* among the donor population.

Effect of the association on co-cultivation of *Listeria monocytogenes* with *Bacillus cereus* in the biofilm formation.

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Listeria monocytogenes is a Gram-positive bacillus that causes listeriosis, which is a serious illness caused by consuming food contaminated with these bacteria. This disease shows high relevance in public health. *L. monocytogenes* finds favorable growth conditions on floors, drains and equipment within food industry premises where it can form biofilm. Associations between microorganisms of different species and their effects on biofilm formation have been studied. The biofilm formed by *L. monocytogenes* has importance, due to it is a big challenge to eliminate it. The aim of this study was to determine the effect of *Bacillus cereus* presence (that is a bacterium that could be present in this kind of samples) on the *L. monocytogenes* biofilm formation, as well as determine the composition and viability of this structure.

The biofilm production was determined on a qualitative assay by using the congo red method. A total of 15 strains of *L. monocytogenes* and one strain of *L. innocua* were examined, all of them isolated from corn flour. The results showed that 11% of the strains produced biofilm. In addition, the semiquantitative analysis was carried out by the crystal violet method in monoculture, where 22% of the strains produced biofilm. Mixed culture conditions with *B. cereus* were performed and the co-culture inocula were prepared by mixing suspensions at different ratios: 1:1; 2:1 B/L (*Bacillus/Listeria*); 2:1 L/B (*Listeria/Bacillus*). These assays showed higher biofilm formation using 2:1 L/B ratio.

Finally, the composition assays were carried out through the LIVE/DEAD® Biofilm Viability Kit (Invitrogen ™) by confocal microscopy with 4 strains that were biofilm forming strains (*L. monocytogenes* 66, 69, 70 and *L. innocua* strain). A co-culture with a ratio of 1:1 was used to determine the bacteria viability within the exopolysaccharide (EPS), where viable cells and long bacilli predominated (belonging to the genus *Bacillus*) in co-culture with *L. innocua* and *L. monocytogenes* 70. On the contrary, in *L. monocytogenes* 66 y 69 co-culture, the authors found a biofilm formed by short bacilli in a greater proportion than long bacilli. Together, the findings suggest that the presence of *B. cereus* affects the biofilm formation process of the genus *Listeria*, due to biofilm production decreased considerably when *Bacillus* was added to the culture. In addition, in the biofilm formed living cells prevailed and with a longer incubation time, EPS proportion increased.

Molecular characterization of *Aeromonas* spp. isolated from rainbow trout farms from Michoacán and isolation of bacteriophages for its biological control

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Aeromonas is a bacterial genus commonly found in rainbow trout infections. *Aeromonas* spp. are found in diverse environments including water, and therefore fish are constantly exposed to the bacteria. In the treatment of bacterial diseases in aquaculture the use of antibiotics is common; these are added to the food, which has implications since one of the main signs in fish illness is the lack of appetite, turning antibiotic therapy inefficient. Phagotherapy is the use of bacteriophages (viruses that infect bacteria). The aim of this study was to obtain a collection of bacteriophages for the biocontrol of prevalent strains of *Aeromonas* spp. from rainbow trout farms in Michoacán. Number of farms to sample was determined by a proportional distribution sampling with the support of WinEpi software and CESAMICH data. We collected six live fish (*Oncorhynchus mykiss*) and sediments in each rainbow trout farm and use the techniques for microbial sampling of fish according to Whitman 2004; samples were collected aseptically from liver, spleen and kidney. The primary culture was perfomed in Glutamate Starch Phenol Red (GSP) agar. All Gram-negative, rod shaped, oxidase-positive, yellow colonies on GSP agar were subjected to biochemical characterization. Purified isolates were stored on BHI agar at -75°C with 15% glycerol. For molecular identification of *Aeromonas* spp. isolates a fragment of approximately 820 bp from *rpoD* gene was amplified by PCR and sequenced as described previously (Soler *et al.,* 2004). Phages were isolated from the sediment of the pond obtained at the fish farms by using an enrichment method. In total, *Aeromonas* spp. were isolated from 38 (59%) of rainbow trout farms. The main organs showing isolates were kidney and spleen, with 19 and 17 isolates respectively. The proportion of farms positive to *Aeromonas* spp. in fish and sediment samples was 30% and 41% respectively. We also identified 27 possible farm-related risk variables and 8 worker-related risk variables associated with the presence of *Aeromonas* spp. in 64 fish farms in Michoacán. Five phage isolates were obtained from fish farms. This is the first report of bacteriophages isolation in Mexico that are infective to *Aeromonas* spp. isolated from rainbow trout farms.

Incidence of *Chlamydia trachomatis* infections in medical students of third and fourth semester in a private University

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Introduction: the infection by *Chlamydia trachomatis* represents a quarter of sexually transmitted infections worldwide and, although it is asymptomatic, it can lead to trachoma, lymphogranuloma venereum and hemorrhagic proctitis, among other complications. Diagnosis is usually not made using culture techniques because it is an intracellular bacterium, so it is recommended to use serological diagnostic kits with demonstrated efficiency and accuracy. Recent studies determine that the most vulnerable population are women between 15 and 20 years of age and it is considered that a significant population of students in the second year of university corresponds to that age range, it is relevant to determine the level of incidence of this pathogen in a sample of the student community. **Methodology:** in the present study, third and fourth semester medicine students in a private university were admitted using the following inclusion criteria: being sexually active, not having taken antibiotics in at least 7 days and showing up in an eight-hour fast; those who accepted, signed an informed consent letter. To make the serological diagnosis, the Bio-Chlamydia IgG kit (6001326, MexLab®) was used, which indirectly measures the amount of the IgG and IgA response, to identify the acute and chronic responses, respectively. Samples were obtained by puncturing the ulnar vein of the volunteers, which were centrifuged to recover the serum, placed in a 96-well plate where the manufacturer's instructions were processed, incubated with the solutions to favor the colorimetric reaction of antibody antigen and read in a plate reader of ELISA at 450nm. **Results:** it was determined that of the 22 students (14 women and 8 men) participants, none of whom were acute or recent for *C. trahomatis*; however, 8 (31.18%) tested positive for chronic infections and the rest of the students were negative for the infection. **Conclusion:** the incidence of *C. trahomatis* was detected in 8 participants (31.18%), which is close to the 25% reported in several studies in Latin America. The presence of *C. trahomatis* was detected more frequently in women than in men (8 women and 2 men). It is suggested to continue monitoring the incidence of this and other Sexually Transmitted Diseases (STDs) and to continue educating students about sexual hygiene habits.

Determination of *C. difficile* from diarrheal samples of hospitalized patients during the period July 2018-July 2019

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C. difficile is Gram-positive rod, spore forming, strict anaerobic bacillus that causes intestinal infections following disturbance of the gut´s microbiota as a result of prior antibiotic treatment. The major virulence factors produced by *C. difficile* are Toxin A (TcdA) and Toxin B (TcdB) which contribute to its pathogenicity inducing mucosal inflammation and diarrhea. Some toxigenic *C. difficile* strains, solely or additionally to TcdA and TcdB produce a binary toxin (CDT); *C. difficile* infection (CDI) has become a significant healthcare-associated infection. While in other parts of the world the emerging situation of *C. difficile* infection is clear, in Mexico the prevalence and actual behavior of this disease is unknown, except in some high specialty academic hospitals. Therefore, the purpose of the present work is to determine its presence and characterize it molecularly to know its importance in Mexican hospital environments. *C. difficile* was isolated from diarrheal samples in the cycloserine-cefoxitin fructose and taurocholate agar (TCCFA). The fecal samples were collected and then treated with ethanol for 20 min prior to TCCFA inoculation. Incubation was achieved in an anaerobic atmosphere using a GasPak at 37°C for 48 h. The toxin genes *tcdA* (Toxin A), *tcdB* (Toxin B), *cdtA, cdtB* (Binary Toxin) and *tpi* a species-specific internal fragment of the triose phosphate isomerase housekeeping gene, were amplified using PCR. A total of 44 diarrheal samples from 3 hospitals were collected during July 2018 and July 2019 (Hospital Ángeles Puebla, IMSS the Margarita Puebla and Children's Hospital of México). 52.2% (n=23) of the samples tested positive for the culture, 82.6% of these isolates were positive for the gen *tpi* (n=19), 63.1% *tpi* positive were also positive for *tcdA* and *tcdB* (n=12) and 5.2% were positive to *cdtA* and *cdtB* genes (n=1). The *tpi* negative isolates (n=7) did not carried genes for enterotoxin, cytotoxin, and the binary toxins (*tcdA, tcdB, cdtA, and cdtB*). Toxigenic *C. difficile* and non-toxigenic *C. difficile* were isolated. In Puebla, there is no or little information on the prevalence of *C. difficile* in hospitalized patients suffering from diarrhea related to antibiotics consumption. According to our results, the pathogenic strains isolated are A-positive toxin, B-positive toxin (A+ B+), and this is the toxigenic profile circulating in our environment.

Activity of the ethanolic extract of six medicinal plants on the growth of Gardnerella vaginalis.

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The aim of this study was to determine the effect of crude extracts of plants *Origanum vulgare*, *Calendula officinalis*, *Rosmarinus officinalis*, *Thymus vulgaris*, *Eucalyptus globulus* and *Lavandula officinalis* on the growth of *Gardnerella vaginalis* (GV) recognized as the agent that initiates the process of dysbiosis which results in bacterial vaginosis (BV).
BV is an alteration of the normal vaginal microbiota, by replacing Lactobacillus with facultative anaerobes, highlighting the presence of GV, which initiates the formation of biofilm in the vaginal epithelium thus establishing resistance to therapy with antibiotics.
BV has been one of the most common and recurrent vaginal infections among the population of reproductive age.
Although metronidazole is one of the most effective medications recommended as the first-line treatment, it has various side effects and disease recurrences have been observed.
Aqueous and ethanolic extracts of each plant were obtained and to determine the antibacterial activity to GV ATCC 14018 agar diffusion was performed, from extracts that were positive minimal inhibition concentration (MIC) was determined by dilution microplate and agar diffusion technique.
Marjoram, thyme, lavender and eucalyptus inhibited the growth of G. vaginalis. Marjoram and thyme have a minimum inhibitory concentration of 8 mg / mL, followed by eucalyptus with 16 mg / mL and lavender with a value greater than 64 mg / mL.
With the MICs, the activity of the extracts was observed in clinical biofilm-forming strains, the Biofilm index formation (BFI) was calculated and the reduction percentage was obtained with marjoram, thyme and eucalyptus, of which eucalyptus showed greater activity of the three extracts.
Four of the six selected plants showed growth GV inhibition, three of them also reduced the biofilm formed by what is a promising option to be an alternative treatment to combat BV.

Effect of supernatants of *Streptomyces* sp. strains on ergosterol biosynthesis of *Candida albicans* and *Candida glabrata* strains resistant to fluconazole.

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Candidosis is a systematic infection caused by *Candida albicans* and other emerging *Candida* non-albicans species. These yeasts are opportunist pathogens affecting mainly to immunocompromised patients. The antifungal resistance of *C. albicans* and *C. glabrata* clinical strains has recently increased. Secondary metabolism of actinomycetes continues to have significant potential for the discovery of new antifungals. In this work, the capacity of *Streptomyces* sp. J29 ori2 and *Streptomyces* sp. 6B supernatants to inhibit the ergosterol biosynthesis was evaluated. The antifungal activity of the lyophilized supernatants and phenotype of *Candida* strains were evaluated by M27-A3 standardized method of the Clinical Laboratory Standard Institute. The inhibition of ergosterol biosynthesis was tested through the sterol extraction and quantification and the rescue of growth was assayed by the addition exogenous ergosterol. *Streptomyces* sp. J29 ori2 and *Streptomyces* sp. 6B exhibited minimum inhibitory concentration values of 156 µg/mL and an IC_{70-90} of 78 µg/mL. Ergosterol biosynthesis was reduced to 50% in both *Candida* species and the exogenous ergosterol rescued the growth of the strains in presence of supernatants. Transmission electron microscopy revealed an evident membrane damage. In conclusion, the lyophilized supernatants of *Streptomyces* sp. J29 ori2 and *Streptomyces* sp. 6B do inhibit ergosterol biosynthesis in fluconazole-resistant *C. albicans* and *C. glabrata* strains.

Trimethoprim sulfamethoxazole (SXT) resistance in clinical isolates of *Stenotrophomonas maltophilia*.

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Abstract.

S. maltophilia is a Gram-negative bacilli related with nosocomial infections and intrinsically resistant to diverse antibiotics, but only trimethoprim sulfamethoxazole (SXT) is the option for treatment. Nevertheless, SXT^R strains appeared causing intrahospitallary infection. **Aim.** To establish whether the strains isolated from the last outbreak from 2016-2018 at one Hospital could be related phylogenetically. We performed multi-locus sequencing typing (MLST) on these *S. maltophilia* multidrug-resistant strains. **Material and methods.** We studied 106 clinic isolates identify by Vitek 2 as *S. maltophilia* from November 2016 to October 2018. Additionally we automatically determined antibiotic resistance by Vitek 2. Furthermore, DNA was purified by standard methods and resistance genes *sul*1, *sul*2 and *int*1 were detected by PCR using *S. maltophilia* ATCC 17666 as positive and *Escherichia coli* ATCC 25922 as negative controls. For MLST we amplified *mut*M, *nuo*D, *pps*A, *rec*A, *gua*A, *gap*A and *atp*D. Sequences from PCR amplicons were edited and compared with the Sequence Type data base at https://pubmlst.org/smaltophilia/. **Results.** It was demonstrated that 38.7% (41/106) from blood culture and 23.6% (25/106) from sputum. Many strains were isolated from ICU and Hematology than other services. We found that 10.4% (11/106) of the isolates were SXT resistant. However, all SXT^R isolates were positive to the SXT resistance genes *sul*1, *sul*2 and *int*1. Three out eleven isolates were randomly selected for MLST and their sequences corresponded to the ST84 and ST23 and one is not recorded. In summary, we detected that patients from ICU and Hematology departments were more susceptible to the infection of *S. maltophilia*. Also sputum was the common source for isolation of this bacterial species, which confirms its association as etiologic agent for nasopharyngeal infection. Furthermore it may suspect that one outbreak was cause by *S. maltophila* occurred from February to March 2018. **Conclusions.** Not all strains were phylogenetically related because all three sequences belonged to different sequencing types. We are currently performing further analysis to determine whether the strains without unknown ST may represent a new variety.

Loperamide exerts bactericidal activity against Mycobacterium species

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Abstract

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis*. TB is highly prevalent, characterized by the constant occurrence of drug-resistant cases, and confounded by the incidence of respiratory disease caused by nontuberculous mycobacteria (NTM). NTM are naturally resistant to TB-drugs. In the last decade, there has been a renewed focus on the development of drugs to treat TB, and several compounds are being evaluated in clinical trials. While new drugs in development may appear promising, their efficacy and safety for use in humans remains to be validated, which represents cost and time. An alternative to address the therapeutic challenges in tuberculosis is the repurposing of FDA-approved drugs, particularly as host-directed therapy. Recently, we demonstrated that loperamide is a potent immunomodulator. Loperamide is a phenylpiperidine derivative and is a highly lipophilic peripherally acting μ-opioid receptor agonist commonly used to treat infectious and noninfectious acute and chronic diarrhea. We aimed to evaluate its bactericidal activity against *M. tuberculosis, M. bovis* BCG*, M. terrae* and *M. smegmatis*. Our result showed that loperamide exhibited an inhibitory effect against all mycobacterial species tested, with MICs of 100 and 150 μg/ml. Loperamide killed mycobacteria but not *E. coli* at concentrations suitable for human use. This drug may uses as a novel adjunct therapies that synergize with TB and NTM drugs

Prevalence of *vacA / cagA* genotypes *of Helicobacter pylori* in saliva of mystical asymptotic children 6 to 12 years of age (Palo Blanco Guerrero, Mexico)

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Helicobacter pylori (*H. pylori*) is currently the most prevalent human pathogen and aethiological agent responsible for 80 to 90% of cases of gastritis, ulcer and gastric cancer in adults. Several studies have shown the presence of the bacteria in the oral cavity, especially dental plaque and saliva. *H. pylori* presents pathogenicity factors that allow it to adapt to the environment, producing substances that neutralize gastric acids, forming a kind of protective cloud around it. The virulence factors of *H. pylori*, such as cytotoxins *CagA*, *VacA* and adhesin *BabA2*, are determining factors in establishing tissue damage and are associated with the clinical diagnosis of gastroduodenal diseases.

A total of 111 apparently healthy children were studied, 68 of them were female and 43 were male, aged between 6 and 12, belonging to Daniel Delgadillo Elementary School in Palo Blanco, Guerrero, Mexico. Saliva samples were collected for molecular detection of *H. pylori* by PCR, amplifying a fragment of the *16S rRNA* gene with a molecular weight of 522 bp. Subsequently, the detection of the *vacA* and *cagA* genotypes was performed by means of specific PCR, for the *vacA* genotype two regions were identified, the signal sequence and the middle region, for the *cagA* genotype the constant region and the variable region.

18.9% of the children (11/21) were positive for *H. pylori*. The *vacA* genotype was found in 42.8% (9/21) of the samples, in which at least one of the 2 allelic variants was presented; both the *m1* and the *m2* allele had a 19.05% (4/21); While the *s1* allele was presented in 4.76% (1/21), 57.14% (12/21) of the samples were not typifiable for this genotype. For the genotyping of *cagA* it was found that 47.62% (10/21) of the samples were $cagA^{+}$ and 52.38% (11/21) were $cagA^{-}$. When combining both genotypes, 3 patients were found with the strain *vacA s0m1/*$cagA^{+}$ and one patient with the strain *vacA s0m2/*$cagA^{+}$.

Our study indicates that *H. pylori* is present in apparently healthy children, finding virulent strains with the presence of the $cagA^{+}$ genotype and the *vacA* genotype with at least one of the two alleles. Being able to analyze and identify both the presence of the bacterium and its genotypes, will allow in the future to propose new strategies to prevent its spread.

Characterization of antimicrobial resistance and AIEC phenotype in *Escherichia coli* strains isolated from patients with Inflammatory Bowel Disease in Puebla

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INTRODUCTION: Inflammatory bowel disease (IBD) is defined as a set of diseases characterized by chronic inflammation of the gastrointestinal tract of multifactorial etiology. The exact cause of IBD is unknown; however, genetically susceptible individuals appear to have an immune response of deregulated mucosa to microbiota, resulting in chronic and exacerbated intestinal inflammation.

OBJECTIVE: To identify and characterize antimicrobial resistance and virulence determinants in *Escherichia coli* strains isolated from patients with Inflammatory Bowel Disease

MATERIAL AND METHODS: The search of patients with clinical characteristics of Inflammatory Bowel Disease was carried out, through their informed consent and Endoscopy Specialists support; they gave us bowel biopsies for *E. coli* strains search and isolation, at the same time their biopsies were also sent to pathology for their definitive diagnosis. Three patients were included: two 23 and 34-year-old male patients with Ulcerative colitis histopathological diagnosis and a 61-year-old patient with subsequent diagnosis of Chronic Colitis and Diverticulitis. Once the *E. coli* strains were isolated and identified, they were tested for antimicrobial susceptibility, minimum inhibitory concentration (MIC) for Rifaximin and adhesion and invasion assays in HeLa cells to determine AIEC and DAEC phenotype.

RESULTS: 103 strains were obtained and 37 strains were identified as *E. coli*. The antibiogram showed that 100% were multiresistant, with 5 strains producing Betalactamases, 100% resistant to Tetracycline, Nalidixic Acid and Ciprofloxacin, 93.75% resistant to Trimethoprim/Sulfamethoxazole, Gentamicin and Ampicillin. For Meropenem and Amikacina the sensitivity was 100%. The MIC for Rifaximin of the Chronic Colitis patient strains was 100% resistant, probably because she had been treated with this medication but with inadequate time and dose. Adherence tests were positive for all strains: strains of the first patient with Ulcerative Colitis was 75% and for the second 57.4%, the patient with Chronic Colitis and Diverticulitis presented 100% adherence in her strains. Invasion was only observed in two strains of the patient with Chronic Colitis, suggesting the presence of *E. coli* with AIEC phenotype only in the latter patient and not in patients with Ulcerative Colitis.

CONCLUSIONS

- 100% of *E. coli* strains obtained from the patients' biopsies were multiresistant, strains presented 100% resistance to Tetracycline and Ciprofloxacin and 93.75% were resistant to Trimethoprim with Sulfamethoxazole, Gentamicin and Ampicillin.
- All strains of *E. coli* from patient with chronic colitis and diverticulitis were resistant to Rifaximin using MIC method.
- Only 2 strains from patient with Chronic Colitis and Diverticulitis presented AIEC phenotype, strains of patients with Ulcerative Colitis showed a DAEC phenotype.

Evaluation of the Effect of Silver Nanoparticles (AgNP's) Against Pathogenic Bacteria Causing Foodborne Diseases

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Introduction: Foodborne diseases (FBD) are a public health problem that affects almost 1 in 10 people worldwide each year. An indispensable process for the prevention of FBD is disinfection, however, cases of bacterial resistance to common disinfectants have been reported, so it is necessary to develop new antimicrobial agents for use on surfaces in contact with food. Nanotechnology is a useful tool for obtaining new materials such as AgNP's whose antimicrobial properties are being widely studied. **Objective**: To evaluate the antibacterial activity of AgNP´sAc and AgNP´sEt obtained from A. potatorum against *Listeria monocytogenes* and EHEC O157: H7 by quantifying bacterial growth (CFU / mL) for possible use as a food surface disinfectant. **Material and methodology**: To evaluate the possible bacteriostatic or bactericidal effect of AgNP´s on *L. monocytogenes* and EHEC O157: H7, the antibacterial effect of AgNP´s and its precursors (AgNO3 and extract of A. potatorum) was compared by macrodilution and pour plate method. Subsequently, the minimum inhibitory concentration (MIC), the minimum bactericidal concentration (CBM) and the minimum contact time (TMC) of AgNP's solutions were determined. The MIC was obtained by macrodilution, while the CBM and the TMC were determined by pour plate method, using an initial inoculum of $5X10^5$ CFU / mL of *L. monocytogenes* and EHEC O157: H7. **Results**: The results obtained showed that AgNP´sAc had a bacteriostatic effect against *L. monocytogenes* and a bactericidal effect in EHEC O157: H7, while AgNP´sEt had a bactericidal effect in both bacteria, contributing to its potential development as an agent disinfectant on surfaces in contact with food. **Discussion**: From the CMI tests, it follows that the AgNP´sAc and AgNP´sEt of A. potatorum have a bacteriostatic effect regardless of their nature (aqueous solution and ethanolic solution) and the bacterial species used. From the results of CBM, it is inferred that the bactericidal effect of AgNP´s is dependent on the concentration of AgNP´s used, the nature of AgNP´s (aqueous and ethanolic solution) and the bacterial species evaluated. In addition, a variation of the TMC of AgNP's between Gram negative and Gram positive bacteria is demonstrated. **Conclusion**: AgNP´s have antimicrobial effect against *L. monocytogenes* and EHEC O157: H7.

Comparative stress response among *Salmonella enterica* strains of ST19 and ST213 genotypes to host digestive tract environment

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Salmonella enterica is one of the most important etiological agents of foodborne diseases, being able of survive prolonged stress periods outside the host. During its transit through the host digestive tract, *S. enterica* faces the acid conditions in the stomach, the exposure to reactive oxygen species and bile salts, the low oxygen tension in the gut, and antimicrobial peptides, inter alia. Nevertheless, this bacterium presents several strategies to confront such challenges, including the formation of filamentous cells, changes in cell membrane lipid composition, biofilm conformation and the coordinate expression of different stress resistance genes. The Sequence-Type 19 (ST19), as determined by Multi Locus Sequence Typing, is the founder genotype of serotype Typhimurium variants, and the most abundant around the world. It has been documented that the ST213 genotype is displacing the ST19 at Michoacán state. ST213 is an emergent genotype first described at Mexico, suggesting that new genetic variants of *S*. Typhimurium are being generated within the state and the country. We hypothesized that ST213 is more resistant to stress within the host and more virulent than ST19, thus being relevant for public health. This work compares the *in vitro* resistance to stress of host conditions between ST213 and ST19 strains isolated of foodstuff from Michoacán; the reference strain ATCC14028 (ST19) was also included. Cells from cultures at stationary phase were exposed to pH 1.5, simulating the lowest stomach pH. This stress was lethal to the reference strain after 20 min, however, the strains ST19 (11 CFU/mL) and ST213 (10 CFU/mL) survive after 60 min. All strains showed low percentages (0.43%-4.0%) of filamentous cells before and during stress; but this morphotype was transient, because the normal morphology is resumed once the stress condition has been removed. The resistance to oxidative stress at different concentrations of H_2O_2 was determined by optical density for six hours. Under oxidizing conditions strain ST19 grew in concentrations of 3-20 mM H_2O_2, however ST213 strain could not grow in concentrations higher than 3mM, and 14028 (ST19) strain was inhibited in 3 mM-30 mM concentrations. Obtained results show a differential response to stress among studied strains, but no better response to stress conditions of ST213 over ST19 have been observed. Further work is necessary in order to evaluate public health relevance of emergent ST213 genotype.

Bacterial richness analysis of "Bola de Ocosingo" cheese assessed by PCR-DGGE

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"Bola de Ocosingo" is an artisanal cheese manufactured with raw cow's milk, exclusively from the Ocosingo region in Chiapas. It is an unusual dairy product compared with other artisanal Mexican cheeses because it is formed from two kinds of cheese, one bundled inside another: the core, consisting of a fermented high-fat content pasta which is ripened several days at tropical room temperature. The other part is the rind, a "pasta filata" cheese that dehydrates in several hours, becoming into a hard-protective material which serves as a rustic packaging. There are scarce studies about the bacterial community composition of this kind of cheese.

Denaturing gradient gel electrophoresis (DGGE) has become one of the most widely exploited molecular methods for the investigation of the microbial diversity of food ecosystems, including cheeses; but no comparison has been made of the DGGE profiles obtained when different hypervariable (V) regions are amplified from the same community DNA samples. DGGE of 16S rDNA amplicons is a suitable tool in analyzing bacterial communities although the target gene is not limited to rDNA and the choice of the hypervariable region is still controversial. In this study, several previously published PCR primer-sets for DGGE were used to study the bacterial community of "Bola de Ocosingo" cheese.

The primer sets used to amplify different hypervariable regions of 16S rDNA and a fragment of rpoB gene, were: 338F/B518R (V3), Pedio2:644/FirR:1244 (V4-V7), WBAC1/WBAC2 (V7-V8) and rpoB1698F/rpoB2041R (rpoB). Amplicons were evaluated by DGGE obtaining completely different banding patterns. Specific richness values in "Bola de Ocosingo" cheese indicate that profiles using 338F/B518R (V3) showed more bacterial species (forty-nine) and profiles using Pedio2:644/FirR:1244 (V4-V7) showed the lowest number of bacterial species (ten); rpoB1698F/rpoB2041R (rpoB) and WBAC1/WBAC2 (V7-V8) showed eleven and fifteen bands, respectively. The relative dominance values were obtained from the banding patterns, depending on the intensity of the bands. DGGE allows the sequencing of amplicons so that the identity of the dominant bacteria in each banding profile could be known to have a more complete view of the bacterial community composition.

In conclusion, our results show that the bacterial community in "Bola de Ocosingo" cheese, through DGGE analysis, is best accomplished by the amplification of the V3 region of 16S rDNA. If a longer amplification product is desired, then the V7-V8 region should be targeted.

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Antimicrobial and antibiofilm effect of *Lophocereus schottii* against *Escherichia coli* and *Salmonella typhimurium*

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Antimicrobial resistance has become a serious public health concern worldwide, due to the emergence of multiresistant bacterial strains associated with a wide number of diseases and deaths every year especially for biofilm-formation bacteria. In addition, an increase of multiresistant bacteria cases has been observed in the last year. Therefore, it is necessary to find new effective alternatives for the treatment of bacterial diseases. In this sense, plants may represent a viable alternative due to their diversity of secondary metabolites, related with several benefits effects. The objective of this study was to investigate the antimicrobial potential of methanolic extracts of *Lophocereus schottii* (MELS) against planktonic and biofilms cells of *E. coli* and *S. typhimurium.* Antimicrobial effect against planktonic cells was determined thought the microdilution broth method. Antibiofilm effect was analyzed by the crystal violet assay, evaluating the effect of MELS to prevent and remove the biofilm mass production. In addition, remaining viable biofilm cells were determined by colony forming units. Minimal inhibitory concentration (MIC) values of MELS against *E. coli* and *S. typhimurium* were 900 and 1000 µg/mL, respectively. The preventive effect of MELS in the biofilm formation of *E. coli* showed a 72 % and 54 % biofilm formation reduction using a concentration of ½ MIC and ¼ MIC, while a 65% and 32% biofilm reduction was achieved for S. typhimurium at the same tested concentrations. Otherwise, it was observed that MELS concentration of 2.5 MIC and 5 MIC removed 52 % and 59 %, respectively of *E. coli* performed biofilms (24 h), while a 30 % (2.5 MIC) and 36 % (5 MIC) of biofilm removal was observed for S. *typhimurium*. On the other hand, the evaluation on the *E.coli* viable biofilms cells demonstrated that MELS produced a log CFU reduction of 2.8 and 2.9 at 2.5 MIC and 5 MIC, respectively. For *S. typhimurium* biofilms a log CFU reduction of 3 and 2.8 at 2.5 MIC and 5 MIC was observed, respectively. The obtained results demonstrated that MELS may represented a promissory natural alternative for the control of planktonic and biofilms cells of *E. coli* and *S. typhimurium.*

Molecular epidemiology of *Acinetobacter calcoaceticus-baumannii* complex using classical molecular typing and genome sequencing.

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Acinetobacter baumannii is an emerging nosocomial pathogen and with epidemiological relevance due to health care associated infections. *A. baumannii* has been related with ventilator associated pneumonia, bacteriemia, urinary tract infections, meningitis, and wound infection. The aim of this study was to employ molecular typing methods for *A. calcoaceticus-baumannii* complex (Acb) isolated from children of "Children´s Hospital of Mexico Federico Gomez" by plasmid profile, pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and CRISPR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeats). Additionally, the genome sequencing of five strains associated to Leukemia was performed. A total of 66 strains of Acb isolated in the period of 2015 to 2017 were included in this study. The *A. baumannii* strains were identified with the MALDI-TOF-MS-Biotyper, 11% (n=7/66) were *A. pittii*, 4% (n=3/66) *A. nosocomialis*, and 85% (n=56/66) *A. baumannii*. The plasmid profiles obtained by the Eckardt technique showed 1 to 7 plasmids with sizes between 2.4 and 121 Kb. The macrorestriction patterns obtained by PFGE showed generated eight groups (A-H) and 47 pulsotypes, as well as, 15 subgroups with an identical restriction pattern and a correlation coefficient of 0.99. MLST analysis showed a total of 16 sequence type (ST), the ST 156 (n=18/66) was the most prevalent, followed by ST 132 (n=7/66). The AYECas1 and CRISPR-Cas IFb systems were identified in the 38% (n=25/66) of the *A. baumannii* strains and them, the 80% (n=20/25) were identified the CRISPR-AYECas1 system as the most prevalent; while the CRISPR-Cas IFb system was identified in 8% (n=5/25). The five strains selected to sequence their genome, showed an MDR profile, belonging to ST 758, and grouped into two different groups by PFGE; however, the SNV determination showed that these strains contained a variation up to 46 SNV, which confirmed that the five strains were genetically different. The strains of the Acb complex showed a high degree of diversity. The molecular typing (PFGE and MLST) and epidemiology of the strains, provided the best information about of their genetic diversity. The genome sequencing also is important tool that provides information to understand the epidemiology, resistance, and virulence factors of these strains.

Prevalence of *Helicobacter pylori* in dental plaque and stool samples of asymptomatic children of the state of Guerrero

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H. pylori is a Gram-negative bacterium that colonizes the gastric epithelium of over 50% of the population worldwide. The infection by *H. pylori* can be acquired since childhood and, if it remains untreated, it can cause chronic gastritis, peptic ulcer and gastric cancer. However, in children *H. pylori* can cause iron deficiency anemia and growth faltering. It has been proposed that *H. pylori* can enter to the gastrointestinal tract trough consumption of contaminated water but can remain in the mouth by adhering to the dental plaque. The aim of this work is to determine the prevalence of *H. pylori* in dental plaque and stool samples of asymptomatic children in the state of Guerrero, Mexico, as well as to determine the frequency of the *vacA* and *cagA* genotypes in dental plaque samples. Dental plaque and stool samples were obtained from 136 children between 6 and 12 years old. *H. pylori* detection in dental plaque was performed by PCR using specific primers for the 16S rRNA gene, while the detection in stool samples was performed by immunochromatography using the CerTest kit. Identification of the *vacA* and *cagA* genotypes was performed by PCR using specific primers. We found an overall *H. pylori* prevalence of 71.3% (97/136). Of the *H. pylori* positive children, 30.9% (42/136) were positive in dental plaque samples, 53.6% (73/136) in stool samples, and 13.2% (18/136) in both samples. In dental plaque, the highest proportion of *H. pylori*-positive children were 6-7 and 8-9 years old (46.7% and 31.5%, respectively). Most of the strains in dental plaque samples were *cagA* positive (64.3%), and the most frequent *vacA* genotypes were s2m1 and s2m2 (4.8%). 50% of *H. pylori*-positive children in stool samples were 8-9 years old, and 67.6% were 10-12 years old. In conclusion, we found a high prevalence of *H. pylori* in asymptomatic children and the highest proportion of infected children was detected by the antigen test in stool samples.

sabA diversity of *Helicobacter pylori* strains isolated from patients with chronic gastritis

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H. pylori is a type I carcinogen for gastric cancer. In Mexico, the infection prevalence varies between 40% and 80% among patients with gastric pathology. The infection with this bacterium can cause chronic gastritis, peptic ulcer and gastric cancer. In order to stablish the infection, *H. pylori* uses several adhesins, like SabA, a 70 kDa outer membrane protein encoded by the *sabA* gene. *sabA* is one of the most diverse genes of *H. pylori*. Its expression is regulated at the transcriptional level by a poly-T (homopolymeric thymine) region located upstream of the -35 element of the promoter, and a region of CT (cytosine-thymine) dinucleotide repeats located in the 5' region of the gene. The length of the poly-T region controls the transcription initiation of *sabA* by affecting the binding of the RNA polymerase, a length of T_{13} allows a higher expression. On the other hand, the length of the CT region can change the reading frame, a functional protein can be produced with CT repeats of 4, 7 or 10. The aim of this work was to analyze the diversity of the poly-T and CT regions of the *sabA* gene in *H. pylori* strains isolated from patients with chronic gastritis. Fifty *H. pylori* strains were isolated from biopsies of patients with chronic gastritis. A 613 bp fragment of the *sabA* gene, containing the poly-T and CT regions, was amplified by PCR using specific oligonucleotides. The fragment was sequenced, and the sequences were analyzed using the SnapGene Viewer program. Our results showed that of the 50 strains analyzed, 41 (82%) were *sabA* positive. Of the 41 *sabA*-positive strains, 83% (34) had the *vacA* s1m1 genotype, 76% (31) were *cagA*+, and 39% (16) were also *babA*2+. The sequence of the poly-T and CT regions of *sabA* showed that the poly-T region varied in length from T_{11} to T_{23}, while the CT region contained repeats that varied from CT_6 to CT_{13}. Considering the length of the poly-T region (12-20) and the number of CT repeats (7, 10 and 13), 63% (26/41) of the *sabA*+ strains can express a functional protein. In conclusion, *sabA* has a high frequency in strains isolated from patients with chronic gastritis. According to the number of poly-T and CT repeats, 26 strains would produce SabA, and of these, 18 would show a high expression.

Acid-fast Stain vs Polymerase Chain Reaction (PCR) in Tuberculosis diagnosis

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Tuberculosis (TB) is one of the principal causes of death worldwide, its etiologic agent is a group of bacterias known as *Mycobacterium tuberculosis* complex (MTBc). According to national official norms, Pulmonar TB is diagnosed by acid-fast stain (AFS), a fast and economic method that lacks specificity. Polymerase Chain Reaction (PCR) with a specificity of 99%, molecular technique which has been used in the identification of the MTBc upon the amplification of molecular markers such as *gyr*B gene.

The aim of this work was to analyze acid-fast stain and PCR specificity in TB diagnosis employing *gyr*B gene as a molecular marker in TB diagnosis, through the evaluation of 56 AFS positive samples from public health institutions of Oaxaca, Mexico. All samples were analyzed by PCR in order to amplify a 1020 bp *gyr*B fragment, and detected only in 26 of the samples, wich belongs to the MTBc. In this case we shown that AFS specificity with respect to the molecular test is 53% calculated by the Bayesian method, concluding that this method is not reliable in TB diagnosis. It is worth mentioning that AFS method shows the presence of acid-fast bacilli whether belonging to the MTBc or not. The high specificity of detecting MTBc by PCR *gyr*B is very relevant for the treatment of the patients. And the introduction of other markers for the specific identification by PCR of mycobacteria other than tuberculosis will be helpful for the specific treatment of patients with diseases that are clinically similar with a different causative agent.

Obtention of an IgG monoclonal antibody against the *Brucella abortus* lipopolysaccharide

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Abstract: The specificity and affinity of monoclonal antibodies (MAb) are completely stable over the time. MAb have been useful in the diagnosis of infectious diseases, such as brucellosis, a chronic bacterial disease that affects several species of domestic and wild animals. The World Health Organization estimates an average of 500,000 new cases of human brucellosis worldwide, making it one of the zoonoses with the highest incidence in Latin America. The gold standard method for the diagnosis is the isolation and identification of *Brucella abortus* by culture, which can take up to four weeks, due to the slow growth of the bacteria and the rigorous nutritional requirements in primary isolation. Since the culture is technically difficult, and dangerous in many laboratories because of the risk of infection through aerosols, it is necessary to implement new diagnostic methods (1). In this work, the *B. abortus* lipopolysaccharide (LPS) was used as an antigen for the production of Mab. The *B. abortus* LPS was coupled by chemical conjugation with bovine serum albumin. In this way, the LPS became a T cell-dependent antigen, which induced the IgG antibody response in mice immunized with the conjugate (2). The application of 25 µg/mL dose of conjugate subcutaneously with Titermax® as adjuvant, generated a greater antibody response, compared to the response induced with Freund's complete adjuvant. The spleen cells of the mouse with the highest Ab response were fused with myeloma cells Ag8, and the resulting hybrids were developed in the selective medium HAT. The IgG-producing cells against LPS were detected by an indirect enzyme immunoassay, using LPS as capture antigen. The cultures of positive cells were cloned and subcloned to ensure that they were clones derived from a single cell. To verify LPS recognition over the bacteria, indirect immunofluorescence assays were performed using *B. abortus* 2308 and *B. abortus* RB51. Eight IgG producing clones were obtained against *B. abortus* LPS. However, these antibodies did not recognize the native antigen on the *B. abortus* strains.

Keywords: *Brucella abortus*; monoclonal antibody; Immunology.
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Isolation of antimicrobial substances produced by sliding bacteria.

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Since the beginning of the "era of antibiotics," great interest has been raised in exploring the biosynthetic abilities of different plant, animal, and microbial species to obtain from them natural substances with therapeutic or nutritional properties. They could have different uses in pharmacology and in the maintenance and conservation of food in order to generate products with a longer shelf life and at the same time offer the consumer food with less industrial processing.

The aim of this work is to characterize the antimicrobial activity of substances produced by sliding bacteria by establishing the production and purification conditions of said compounds obtained from different strains and checking their antimicrobial activity on bacteria present in rough milk.

The production of antimicrobial substances was evaluated in 6 different cultures of sliding bacteria grown in CY medium at pH 7 incubated at 32 °C for 24, 36, 48 and 72 hrs. The supernatants were collected by centrifugation, passed through a 0.22 micron Millipore filter to ensure removal of residual bacteria and precipitated with 5 volumes of cold acetone. Precipitates were solubilized in 50 mM sodium phosphate buffer, pH 7. The inhibitory activity of the supernatant fractions was evaluated by the agar diffusion test on type bacteria and antibiotic multiresistant bacterium.

Once the inhibition effect was verified, samples were electrophoresed in polyacrylamide gels (SDS-PAGE) to check for the presence and mass determination of the proteins present in the extracts. Subsequently, pepsin was used to confirm its protein nature, determining that the inhibition effect was sensible to enzymatic hydrolysis.

Using the plate dilution method we checked if the supernatants could also decrease the microbial load of a sample of rough milk.

Antimicrobial substances from 6 strains of sliding bacteria were obtained, achieving the inhibition of type strains (*E. coli, Salmonella and S. aureus*) and a multidrug-resistant antibiotic bacterium (*Klebsiella oxytoca*). In addition, it was possible to delay the dyes reduction effect in a sample of rough milk, significantly increasing its quality.

Molecular characterization of *Staphylococcus aureus* isolates from cheese

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A number of molecular typing methods have been developed for characterization of *Staphylococcus aureus* isolates and DNA sequencing of repeated region of protein A (*spa*) gene is a well established discriminatory method for outbreak investigations. The aim of this study was to carry out a characterization molecular of *S. aureus* strains isolated from cheese samples. Two ATTCC control strains (27543 and 43300*mecA*+) and a total of 22 *S. aureus* isolates from fresh cheeses were analyzed. The isolates were tested for antimicrobial susceptibility by disk diffusion and the PCR method to detect the *mecA* and *spa* genes. Of the twenty-four isolates, 8.33% (n=2) had reduced sensitivity to oxacillin and 12.5% (n=3) had reduced sensitivity to gentamicin. None of the isolates were resistant to vancomycin or levofloxacin. The presence of the methicillin-resistance *mecA* gene was not confirmed for any of the isolates. *Spa*-typing of the isolates revealed 7 distinct *spa*-types grouped into 5 clonal groups determined by a cladogram of genetic distance, also found four new sequences of repeated that could comprises new *spa*-types. The most predominant *spa*-types among the isolates were t127 (30.4%, n=7), t224 (26%, n=6), t189 (8.6%, n=2). The five clonal groups were associated with multilocus (MLTS), ST-1, ST-97, ST-188, ST-8 and ST-6 type sequences frequently isolated from bovine clinical mastitis infections and milk contamination used in the cheese making. The results suggest that the contamination of fresh cheeses might have zoonotic origin by contaminated milk and suggest that an effective surveillance in the food production chain should be implemented in order to monitor *S. aureus* contamination, which could be a possible route of transmission of *S. aureus* to humans.

Key words: *Staphylococcus aureus*, Antimicrobial, *mecA*, *spa*-Typing.

Search for enterotoxin A and antimicrobial susceptibility of strains of *Staphylococcus aureus* isolated from cheese

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Foodborne diseases are widespread and constitute a priority public health problem, the most frequent etiological agent of food poisoning is *Staphylococcus aureus*. This microorganism produces the so-called staphylococcal enterotoxins that have biological activity of pyrogenicity and superantigenicity. A wide variety of these toxins (SE) SEA to SEE, from SEG to J, SEK, SEL, SEP, SEM and SEO are known; being enterotoxins A and D the most frequently associated with food poisoning. The objective of this work was to search a collection of 50 strains of *S. aureus* (from cheese) for the presence of *nuc* gene that codes for thermonuclease and the seA gene that codes for enterotoxin A. Also, susceptibility was determined by plaque diffusion antimicrobial as described in CLSI 2015. In 100% of the strains analyzed, the *nuc* gene was detected and 62% (31/50) of the strains the *seA* gene was amplified for enterotoxin A. Regarding the antimicrobial susceptibility, it was found that 100% of the strains showed resistance for the antibiotics penicillin, dicloxacillin and ceftazidime, for ampicillin 98% resistance, 34% resistance for cefuroxime, 14% for tetracycline, erythromycin and gentamicin respectively and 8% resistance for trimethoprim-sulfamethoxasol. According to the results found, 98% of *S. aureus* strains isolated from cheeses show antimicrobial multiresistance and harbor the genetic information of the SEA enterotoxin.

Cronobacter sakazakii isolated in popular consumption plants in the Mexico's valley

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Cronobacter sakazakii measures approximately 3 x 1 µm, is mobile with perimeter flagella and belongs to the *Enterobacteriaceae* family. It has been strongly linked to the infant formula powder, in a wide variety of foods such as dairy products, dried meats and herbaceous material such as some plants and spices among others, which, in addition, it has been proposed that *C. sakazakii* is found naturally in the environment. Information as an etiological agent of conditions in vulnerable groups is scarce, however, most outbreaks in newborns due to the consumption of some infant formula powder are documented.

In Mexico, there are few studies on this pathogen, for which reason the search for *C. sakazakii* was done in 25 samples of chamomile and 25 samples of coriander acquired in the municipalities of Tultitlán, Tlalnepantla de Baz, Ecatepec de Morelos and the city hall Venustiano Carranza. Two methodologies were used; the manual of Analytical Bacteriology of the Food and Drug Administration (BAM-FDA) and the one of Guillaume-Gentil (2005) both adjusted to the working conditions. Of the total samples, 13 suspicious colonies were obtained by biochemical tests of which only 5 were confirmed by amplifying the *gyrB* gene.

The results obtained in this work showed that the appropriate methodology for herbaceous samples was the one proposed by Guillaume-Gentil (2005), that is, adjusting the concentration of bile salts, allowing to incubate at room temperature (25 ° C).

The importance of this study was to highlight the presence of *C. sakazakii*.

Evaluation of Disinfectants on Bacterial Strains Isolated in Sheep and Goats by the Rideal and Modified Walker Method

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Introduction. The compounds of phenol, commercially prepared benzal, 96% ethanol, sodium hypochlorite, and benzalkonium chloride are commonly used as disinfectants in human medicine, in livestock and in the food industry. It is therefore essential to know better the behavior of these agents in non-standardized strains of sheep and goats. Therefore, the **objective** of this work confronts the antimicrobial activity of 5% phenol, 1% commercial benzal, 96% ethanol, 1% sodium hypochlorite, and 10% benzalkonium chloride. By the method of Rideal and Walker modified against Staphylococcus aureus, Bacillus subtilis, and pseudomona aeruginosa of sheep and goats. **Methodology** They were physicochemically Gram-positive and negative. They were diluted (10-5 and 10-6), seeded in BD Brain Heart Infusion (BHI) plates in duplicate and incubated at 37 ° C for 48h. And they were pumped in UFC by digital counting. For the phenolic coefficient test, the microbiological activity of 5% phenol was compared against reference strains, the MIC was determined, samples were prepared in serial double dilutions of the four disinfectants (dilution range: 1: 2 to 1: 512). Suspensions of: Staphylococcus aureus, Bacillus subtilis, and pseudomona aeruginosa were prepared by adjusting them to tube 5 of the Mac Farland nephelometer: To the dilutions of the disinfectants (4.5 per tube) 0.5 ml of the suspension were added and they were seeded in soy agar Trypticasein poisorbate at intervals of 5, 10 and 15 minutes. Incubating at 37 ° C for 24 hours. Growth was observed at the maximum dilution of the disinfectant that kills the microorganism in 10 minutes, but not in 5 minutes, and was divided between maximum dilution of the phenol, result less than one was considered a bad disinfectant. The statistics were carried out in the SPSS and Minitab programs. **Results** In all cases, the correlation coefficients between the different methods used are close to the Phenolic coefficient at 1. The data are closely related to each other. This justifies the Rideal and Walker Modified Method for the disinfectant assessment test. The sensitivity test by diffusion in agar to the dilutions of the disinfectants (4.5 per tube) with 0.5 ml of the suspension in reseeding in trypticasein poisorbate soy agar at intervals of 5, 10 and 15 minutes showed sensitivity for routine use, with the compounds of 5% phenol, 1% benzal already commercially prepared, 96% ethanol, 1% sodium hypochlorite, and 10% benzalkonium chloride, Therefore, by virtue of the results obtained and the correlation of the various data provided by the CMB techniques, suspension and diffusion in agar, the validity of the Rideal and Walker Method modified as appropriate for the assessment of these disinfectants was proved. **Conclusion**. the use of the trypticasein polysorvate soy agar medium allows reliable results. Against different bacterial strains of non-standardized fields of sheep and goats, it does not intervene in the result.

Microbiological Study of Pasteurized Milk Using Three Methods.

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Presentación: cartel Área: Microbiología de alimentos y clínica

Introduction. Milk is an easily altered product, where coconut, Gram positive and Gram-negative bacilli can proliferate. Also, coliforms that ferment lactose with gas production within 48 hours of incubation at 35 ° C. The Petrifilm method is a modification of the count of bacterial colony forming units (CFU) in plaques. SimPlate has a high-count range (> 1600 more likely number per single dilution) and requires fewer dilutions per sample than conventional plaque technique (TPC), is an alternative to plate aerobic bacteria (PCA) counts and estimate populations of aerobic mesophilic microorganisms in samples of pasteurized milk. Therefore, it is **Objective** aimed at this work; compare the Petrifilm and SimPlate method with the conventional plate technique (TPC), for the count of aerobic mesophilic and total coliforms in pasteurized milk. **Methodology.** 50 samples of one liter of pasteurized milk from different dairy industries in Mexico City. they were divided into 10 subsamples, and a 25 ml aliquot was used for each subsample and analyzed separately. Thus performing 10 analyzes for each sample. 10^{-1}, 10^{-2} and 10^{-3} milk dilutions were made for total coliforms and the 10^{-1} dilution direct sample was used for aerobic mesophiles, the dilutions were 10^{-1} and 10^{-3}. In the conventional technique, the agar medium-red-violet-bile-lactose (RVBA) for total coliforms and Standard account agar (ACE) for aerobic mesophiles was added to the sample. In the Petrifilm technique, the upper film was lifted by depositing the sample in the center of the plate; In both techniques, total coliforms were incubated at 35 ° C ± 2 ° C for 24 hours and aerobic mesophiles at 35 ° C ± 2 ° C for 48 hours. In the SimPlate technique, the sample and the culture medium were inoculated in the dish by homogenizing and distributing in the wells, it was incubated at 35 ° C ± 2 ° C for 24 hours both coliform and for aerobic mesophiles. Colony counts first became Log10 account to match the underlying assumption of normal distribution and relevant statistical analysis was performed. **Results.** The correlation coefficients between the 3 methods were: total coliforms: 0.94 between the cultivation technique and SimPlate, and 0.93 with Petrifilm; for aerobic mesophiles only between the culture technique and Petrifilm the coefficient is acceptable and not when using SimPlate, being 0.64. This result is explained because: in the SimPlate method the dish has 84 wells, if the microbial load is high, all the wells come out positive and an uncountable result is obtained and not a real number of CFU / ml as in the case of Petrifilm. **Conclusion.** Both the Petrifilm method and SimPlate is the best choice for total coliform count in pasteurized milk; but for aerobic mesophiles in this trial the Petrifilm technique was more effective.

Detection of bacteria potentially producing biogenic amines in *Octopus maya* (Voss y Solís, 1966)

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Octopus maya is one of the most important fishing resources in Mexico, with Yucatan being the state where this fishery generates the greatest economic production. Almost 80% of the catch is exported to countries of the European Union (EU). A poor handling of the product at the time of capture and lack of control in the cold chain before entering the processing plant causes the product to contain a high concentration of bacteria producing amino decarboxylase enzymes at the time of processing. These bacteria generate an accumulation of biogenic amines (ABs) at levels that can become toxic to the consumer. The aim of the present work was to detect the presence of genes responsible for the decarboxylation of histamine (*hdc*), tyrosine (*tdc*) ornithine (*odc*) and lysine (*ldc*) in bacteria isolated from *O. maya*. The detection of these genes is of crucial importance in the monitoring of the food safety of cephalopods, being the molecular techniques powerful tools for this purpose. The study was conducted with eight strains isolated from the octopus's muscle. DNA extraction was performed with trimethyl ammonium cetyl bromide (CTAB), the presence of the genes was detected by qualitative Polymerase Chain Reaction (PCR). Recombinant DNA technology was used to obtain enough genetic material for sequencing. The eight strains amplified a 1440 bp fragment corresponding to the *odc* gene. Only *Enterobacter* sp. 73 was identified as the carrier of the four genes analyzed. The *ldc* and *odc* genes of strain 73 showed 100% identity with the sequence of *Enterobacter cloacae* and *Enterobacter* sp., respectively. The *ldc* gene of strain 6A showed 97.7% identity with the *ldc* gene of *Citrobacter freundii.* The presence of bacteria species capable of forming ABs in *O. maya* was determined with certainty. Therefore, it is suggested to improve the management, capture and storage measures of the organisms to avoid that the concentration of ABs represents a problem for the commercialization and health of the consumers.

Keywords: *Octopus maya*; decarboxylase genes, recombinant DNA, sequencing.

Strains Characterization of *Saccharomyces cerevisae* Isolated from Natural Fermentations of Sotol.

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Background and Objective: The selection of *S. cerevisiae* strains has been done in other alcoholic beverages, such as the Tequila, Beer, Wines, etc. The fermentation carried out with selected and inoculated strains of *S. cerevisiae* is quicker and of a better quality, to select the strains studies had been carried out to identify and typify from spontaneous fermentations. There have been physiologic tests to evaluate, characterize, select and domesticate these strains depending the industrial interest, therefor, the objective of this work is to characterize three different strains of *S. cerevisiae* from an 18 isolated strain group of natural fermentations of Sotol and select those that present the best ethanol performance. Methods: A PCR-RFLP in the region ITS1-5.8-ITS2 was carried out. The results were compared with the yeast.id data base. Next, a typification was carried out with MS-PCR with GTG_5 and M13 and a PCR with Interdelta fragments with the 12δ and 21δ primers. These tests differentiated three strains, those who were named as SMF1, SMF4 and SMIC3. These strains were exposed to different stress conditions such as: thermic and osmotic shock, low pH and high concentrations of ethanol, all of these to evaluate their tolerance by the presence of yeast growth by the Miles and Misra method. The three strains were inoculated separately in the natural mosto of Sotol to compare the performance of ethanol and the consumption of the fructose and glucose carbohydrates, as well as the ethanol, glycerol and acetic acid production.Results: The PCR-RFLP showed that the 18 isolated strains of the natural fermentations of Sotol are *S. cerevisae* and with the typification it was possible the differentiate three strains; SMF1, SMF4 and SMIC3. These did not showed any difference after being exposed to the different stress conditions. The fermentation process with natural mosto of Sotol, carried out by the SMF1 and SMF4 was similar, nevertheless, the SMIC3 strain showed flocculation and a low performance of ethanol. Conclusions: The MS-PCR and Interdelta analysis methods can distinguish differences between close strains. Despite of the molecular differences, SMF1, SMF4 and SMIC3 showed a similar tolerance. The SMF1 and SMF4 strains can be proposed to obtain a good efficiency of ethanol in Sotol fermentations.

Key words: *Saccharomyces cerevisiae,* Sotol

Characterization of *Enterococcus* sp. isolated from miscarriage products during the first trimester of pregnancy.

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Bacterial etiology in abortion, besides of traditional microorganisms is statistical unknow and the mechanisms involved are uncertain, therefore, the characterization of isolates of this nature are quite relevant.

Enterococcus sp. is an intrahospital pathogen of high importance because of the great resistance to antimicrobials, disinfectants and their incredible genetic plasticity.

The aim of this study was to characterize the isolates of *Enterococcus* sp. from miscarriage products and compare them with isolates of the same microbial gender obtained from environmental sources

The isolation of clinical strains was performed in Casman with blood 5%. The environmental strains were first enriched in Rothe Broth and isolated in bile esculin agar.

Antimicrobial resistance was determined by disc diffusion method and microdilution in Muller Hinton Broth, the presence of extended spectrum B-lactamase was evaluated by synergy with two discs. And biofilm production by qualitative evaluation in Congo red gelosa and quantification in microplate with violet crystal.

The data analysis was performed using a binomial matrix and Jaccard coefficient, in order to evaluate the similarity of the isolates.

In the clinical group there is a evident resistance to ciprofloxacin while in the environment group there is an important resistance to nitrofurantoin. There is also 37% BLEE in the clinical group and 62% in the environmental group. In the case of the biofilm production assessment, the clinical group has 65% of high production strains against 38% in the environmental group.

The data analysis shows groups with high similarity between both clinical and environmental strains, but also, small groups with low similarity.

There is no correlation between the origin of the strains isolation and the phenotypic characteristics evaluated.

Biological nitrogen fixation in pozol fermentation

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Natural cereal fermentation represents an important technology for food conservation that allows to obtain a wide variety of fermented foods with improved organoleptic characteristics, functional qualities and nutritional value, with a final positive effect on human health (1). In these fermented foods, the nutritional value is strongly influenced by the protein content and protein composition of the cereal (2).
In pozol, a Mexican fermented beverage, made of nixtamalized maize, the fermentation process produces an increase in soluble protein, some amino acids (lysine and tryptophan), vitamins (niacin and riboflavin) and nitrogen content, that has been speculated may be due to biological nitrogen fixation during fermentation (3,4). Since, nitrogen fixation can be an economic, simple and low-cost method to improve the nutritional value of fermented cereal-based foods, the objective of the work was explore the nitrogen fixation during pozol fermentation.
Pozol dough were purchased in Villahermosa, Tabasco. The fermentation was followed for 48 h at 37°C. Nitrogen and protein content was determined according the official methods AOAC (1990). Nitrogenase activity was evaluated through acetyl reduction assay (ARA) and nitrogen fixing bacteria were isolated and identified.
Proximate analysis composition of pozol samples showed that fermentation process produced an increase on the nitrogen and protein content. Positive nitrogenase activity was found in all pozol samples, from which a total of twenty-six nitrogen-fixing bacteria were isolated. Three genera were identified by sequencing the 16S rRNA and nifH genes: *Kosakonia*, *Klebsiella* and *Enterobacter*. Finally, the inoculation of one isolated nitrogen-fixing bacteria in the dough demonstrated *in situ* increase in acetylene reduction. Our results confirmed biological nitrogen fixation pozol, the first demonstration of this phenomenon in a fermented food.

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Probiotic potential of *Lactobacillus* strains isolated from an artisanal raw-milk cheese

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Probiotics have been described as viable microorganisms that exhibit a beneficial effect on the health of the consumer (including humans and animals). Various species of lactic acid bacteria (LAB) have been used as probiotic. Several strains of *Lactobacillus* (the first genus that has been explored as probiotic), *Streptococcus, Lactococcus, Enterococcus Pediococcus* and *Leuconostoc* have been successfully tested as probiotic. The main tests that are required in order to stablish a probiotic potential of bacteria are related to demonstrate their strees tolerance (temperature, different pH levels, bile salts, gastric juice), adhesion abilities (autoaggregation, hydrophobicity, adhesion to mammalian cells) and antipathogenic activity (antimicrobial metabolites production, co-aggregation with pathogens). In this context, it is known that an important source of probiotic microorganisms are artisanal dairy products (including raw-milk cheeses). This study is aimed in stablish the probiotic potential of *Lactobacillus plantarum* and *L. paracasei* strains isolated from artisanal raw-milk cheese. Nine strains (5 *L. plantarum* and 4 *Lactobacillus paracasei*), previously isolated and genetically identified by 16S rRNA and specie-specific PCR, were included. Preliminary, probiotic profile assessment included ability to grow at different pH levels (2, 5, 7), NaCl concentrations (2, 5, 7), temperatures (20, 35, 45 °C) and bile salts (0.3 and 1% of sodium taurocholate). Results show that, in general, *L. plantarum* strains were able to grow at pH 5 and 7, but only some strains at pH 2; tolerant to NaCl (up to 7%), capable to grow at 35 °C but only some strains at 20 and 45 °C, and tolerant to bile salts (0.3 and 1 %). Meanwhile, *L. paracasei* strains showed good adaptability and growth at pH 7, but only some strains at pH 2 and 5; tolerant to 2 and 5 % of NaCl concentration but not as well at 7%; tolerant to grow at 35 and 45 °C, but slower at 20 °C. *L. paracasei* didn't show tolerance to bile salt (only one strain showed good growth in presence of 0.3 and 1% of sodium taurocholate). We conclude that, at least four of the selected *Lactobacillus* strains, could be good candidates to be used as probiotics in human and/or animal supplementation.

Isolation and identification of filamentous fungi in vegetables in the central area of the state of Puebla

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The term mold is used to designate certain multicellular filamentous fungi, which can be recognized by their macroscopic and microscopic aspects. These characteristics are usually sufficient to determine the genus to which the fungus belongs. In this work, fungi present in vegetable crops in the central zone of the state of Puebla, cultivated in San Pedro Cholula and San Gregorio Zacapechpan were identified. Most of the species identified are phytopathogens. Phytopathogenic fungi are a challenge for agriculture and vegetable production, since they cause biological and economic losses. Samples of five kinds of vegetables were taken: parsley, spinach, coriander and two types of lettuce. To carry out the isolation, 10 g of each vegetable were taken and added to 90 ml of isotonic saline, the samples were shaken for 10 minutes and inoculated in PDA and SDA medium. After the recovery, isolation and microculture processes, the following results were obtained: in parsley the genus Alternaria was frequently isolated, followed by Fusarium and Geotrichum; in the spinach were found genres such as Fusarium, Cladosporium and Acremonium, as well as the genus Ulocladium identified repeatedly; in the cos and apricot lettuce the genus Penicillium predominated, in addition the genera Alternaria and Botrytis were found; finally, in the coriander, the genera Ulocladium and Alternaria were found.

Some of these fungi are important causes of alterations in fruits and vegetables, since they change their physical aspects, nutritional values, organoleptic characteristics and make their conservation difficult (Trigos, Ramírez, & Salinas, 2008). This is because, by invading the vegetable tissue, they can secrete substances as a result of their secondary metabolism; it can also be inferred that the same genus of fungus can affect more than one type of vegetable, as is the case of Alternaria, which affects parsley, lettuce and coriander.

It is necessary to know the presence and distribution of potentially pathogenic fungi for the production of vegetables, since, from that, suitable fungicides can be selected. One of these alternatives may be the use of bacteria of the genus Bacillus, as the high potential of Bacillus strains has been demonstrated as antagonists against phytopathogenic fungi that affect vegetables. That is why it is proposed the deep investigation of biological controls to improve the production of vegetables and influence the decrease of phytopathogenic fungi in them.

RISK OF ACQUIRING TOXOPLASMOSIS INFECTION BY TRANSFUSION OF HEMODERIVATES

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Abstract- *Toxoplasma gond*ii is a parasite of the protozoan group, it causes serious disease in pregnant women, and immunocompromised people causing eye damage, within its physiological characteristics it has an improved tropism towards the central nervous system of the host, where on arrival it can cause serious damage.
In immuno-competent people it can be found in its latent form in different organs of the human body and the host remain asymptomatic for a long time

Objective- To detect the presence of *Toxoplasma gondii* measure antibodies in sera from voluntary donors and to know risk factors.
Method- participated 457 blood donors, were processed by chemiluminescent the IgG and IgM antibodies, concentrations of 6.1-449 IU / mL for IgG. **Results-** 85 sera positive for IgG (16.87%) and IgM (1.04%). 96% of the participants reported consuming washed food, 37% living concrete houses with boiled water, 41% overcrowding at home, 74% have cats on the periphery of their home, 95% have not been transfused with hemoderivates products, age between 18- 58 years **Conclusions-** a positive serology was found in people who donated blood in 17.91%, due to the expression of antibody levels it can be said that they present with infection to the parasite, however the lack of symptoms demonstrates that they do not suffer from the disease, although the detection is not carried out Toxoplasma gondii there is a risk of transmitting it to the sick receivers patient.

SEROPREVALENCE OF INFECTIOUS CIRCULATING ANTIBODIES IN RELATION TO THE BLOOD GROUP IN BLOOD DONORS.

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Blood group antigens have some receptors for certain pathogens such as bacteria, viruses and parasites, it is important to know if there is specific affinity between the blood group and each of the pathogens because they can be transmitted by blood transfusion

To determine the seroprevalence of infectious circulating antibodies relative to the blood group in blood donors.
Material and method: Retrospective descriptive cross-sectional study, studies were carried out for detections of antibodies for syphilis, HIV HBV, HCV, Chagas) for donors of Blood Bank of H.G. Tijuana, from January to December 2016, chemiluminescence tests with reagents of specificity greater than 96% and with a 95% confidence interval, the titles for brucellosis were processed with the Rose Bengal method. Demographic data such as gender and age were selected for statistical purposes.
Results: Total donors 7356, age range 19-52 years, 34.49% women and 62.51% men. The population reactive to infectious circulating antibodies was 11.13%. By blood group 63.84% were O, 26.53% group A, 7.69% for B and 1.87% for AB. The percentage of Viral seroprevalence: HIV; group A 2.81, group B 2.12, group O 2.44, Hepatitis B; group A 1.69, group B 2.65, group O 1.14, Hepatitis C; group A 1.53, group B 1.76, group O 3.04. Bacterial: Syphilis; group A 3.53, group B 6.0, group O 3.95, Brucellosis; group A 0.15, group B 0.0, group O 1.31. Parasitic: Chagas; group A 0.30, group B 1.06, group AB; 0.30, group O 0.78, Toxoplasmosis O 59.8 A 26.8 B 7.0, AB 5.6.
Conclusions: The largest number of participants correspond to group O. The prevalence of HIV remains homogeneous in patients regardless of blood type. The prevalence of hepatitis B is higher in donors of group B, however for hepatitis C it is higher in donors of group O.
Bacterial seroprevalence for syphilis is related to donors of group B, quite the opposite for brucellosis. In Chagas the tendency is for group B extending to AB, however for Toxoplasmosis the predominance is in group O followed by A.
It is considered important to carry out studies plus epidemiological cohort and molecular studies to validate the molecular affinity of infectious agents and ABO groups.

Phenotypic and genotypic characterization of bacteria in meat for human consumption in a population of the Sierra Sur of Oaxaca, 2019-2020

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Abstract

Antimicrobial resistance is a global public health problem that has increased alarmingly in recent decades. Due to the misuse of antibiotics in the human population, surveillance and restriction measures have been implemented. Although WHO has also proposed surveillance in the veterinary field, there are few studies of the use of antimicrobials and the impact this might have.

Objective: To analyze and characterize the presence of bacteria in meat for human consumption in a population of the Sierra Sur of Oaxaca, 2019-2020. **Methodology:** Samples of meat for human consumption were collected from butchers mapped through the ArcGis 10.5 program; sample processing was carried out at the University of the Sierra Sur through food safety testing by sowing in selective and differential media. Bacterial identification was carried out by biochemical tests. Susceptibility tests were performed by the Kirbi-Bauer method. **Results:** The study currently has few isolates since it began at the beginning of the month, however we consider participation important, since to date enterobacteria have been identified with resistance to antimicrobials of first choice in human consumption and a *Kleibsiella sp.* resistant to imipenem. Sample processing will continue so as to obtain more information to present on the day of the congress.

Keywords: Carbapenem resistance, antimicrobial surveillance in food.

Structure-function relationship of *Streptococcus infantarius* subsp. *infantarius* amylopullulanase.

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Pozol is a traditional refreshing beverage prepared by resuspending a nixtamalized and fermented maize dough. A complex microbiota, formed by bacteria, yeasts and filamentous fungi develops in the fermentation of pozol; being lactic acid bacteria the most abundant group. During the nixtamalization of maize, soluble sugars such as sucrose and glucose are lost, leaving starch as the main carbohydrate available in the dough, so in order to grow, many of the microorganisms present must have an amylolytic system that allows the use of starch as carbon source.

Streptococcus infantarius subsp infantarius 25124 (Sii- 25124) is the bacterium that has been reported as predominant throughout the pozol fermentation. Previous studies showed that Sii-25124 produces two different amylases, a cytoplasmic α-amylase (~ 35 kDa) and a membrane-associated amylopululanase (AmyPul) (~ 250 kDa).

Amypul is an atypical amylase because of their multimodular structure and big molecular weight. The *in-silico* analysis showed two catalytic domains and 4 carbohydrates binding domains (CBM). The first catalytic domain (AmyA) is associated to the hydrolysis of α(1-4) linked glucoses and the second catalytic domain (PulA) is associated to the hydrolysis of α(1-6) linked glucoses, at the same time the four CBMs allow the interaction with the insoluble substrate (starch granules). Together, these characteristics make this enzyme very interesting not only for its basic study but for their industrial applications, since the saccharification industry has to use at least two enzymes with α(1-4) and α(1-6) specificity.
To evaluate the function of these domains, the cloning of different recombinant proteins is proposed. First, the two isolated catalytic domains in order to evaluate their specificity and then, the catalytic domains with 2 CBMs in order to evaluate the activity in insoluble substrates. Genes were cloned and expressed in *E. coli* for protein production. Biochemical characterization is in process.

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Correlation between Polymerase Chain Reaction and Cervical Cytology Results in Patients from Oaxaca, Mexico

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Cervix cancer is a public health problem being the fourth cause of death between women globally. Human Papilloma Virus (HPV) is one of the risk factors related to this pathology. There are effective diagnosis methods which allow appropriate detection and guide to suitable treatment administration. Nevertheless, new cervix cancer cases are registered daily because of the lack of efficient protocols for an acute diagnosis.

A transversal descriptive study was conducted in order to analyze the correlation between Polymerase Chain Reaction (PCR) and conventional cervix cytology with Papanicolaou (PAP) results. Some 112 patient files with PCR for HPV detection and PAP were analyzed, in 45 of them HPV presence was demonstrated by PCR while in 67 the virus was absent. PAP showed that 13 out of 112 patients had intraepithelial cervical lesion, from which 7 also had HPV infection and 6 didn't; 6 corresponded to low degree lesions and 7 to Atypical Squamous Cells of Undetermined Significance (ASCUS). Of those 6 with low degree lesion, 3 were HPV positive and 3 were HPV negative; between the 7 patients with ASCUS, 4 were HPV positive and 3 HPV negative.

Statistical analysis results showed 1.8 odds for HPV infected women to develop an intraepithelial cervical lesion in comparison to no HPV infected women.

Effect of a Hand-washing Workshop on the decrease of total aerobes on the hands of social service students of the Pharmacy Degree

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INTRODUCTION: The World Health Organization (WHO) estimates the annual incidence of nosocomial infections worldwide in 1.4 million cases as a result of inadequate hand washing. In our country, universities which use clinical fields were urged to develop strategies that guarantee the correct implementation of this technique to avoid risking the health of patients and professionals in training. The Bachelor of Pharmacy, having an area of specialization in Hospital Pharmacy sends social service students to hospitals to be trained in their areas of *expertise*, therefore, it established a pilot program of hand-washing workshop and the objective of this work is to determine the effect of said workshop by counting total aerobes on the hands of the students of the Pharmacy Degree who will do their social service in different hospitals in the State of Morelos. **METHODOLOGY:** Students were taken samples of each hand with a sterile swab at the following times: once they arrived at the facilities where the workshop would be held, once they had washed their hands without having started the workshop, once they received the workshop where they were instructed in the technique of hygienic hand washing issued by the WHO. Each of the samples were placed individually in 3mL of sterile saline solution, from which they were seeded using the spatula technique in Petri dishes containing 20 mL of Tryticasein Soy agar, the boxes were incubated at 37°C during 48 hours; later, the colonies of each box were counted to calculate the bacterial titers and the data were analyzed using the Excel program. **RESULTS:** All students enrolled in the eighth semester of the Pharmacy Degree with area of specialization in Hospital Pharmacy 11 (100%) participated in this study, starting with total aerobic microbial loads from 0 to 1000 CFU; after washing their hands before receiving information from the hand-washing workshop, 5 of them (45.45%) decreased their microbial load, 5 of them (45.45%) increased it and one of them maintained it. After the hand-washing workshop, 8 students (72.72%) decreased the number of bacteria in their hands and 3 (27.247%) increased it. **CONCLUSION:** The number of students who decreased their microbial load of aerobic total increase after receiving the workshop. However, as perspectives of the present work, it is proposed to start with the workshop in earlier semesters and implement a follow-up every six months so that 100% of students decrease the number of CFUs in their clean hands.

Comparison of the microbiological and chemical characteristics of pulque from Claveles, Guanajuato in two seasons.

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The "pulque" is a traditional drink obtained from the sap agave fermentation produced by hand., whose consumption has been reduced in recent years. The fermentation occurs by the naturally present microbiome, so it is important to know the microorganisms involved to improve the production process and create a standard for it is industrialization. The aim of this project was to determine the chemical characteristics of sap agave and pulque in the summer and winter seasons and their relation to the microorganisms presents in both.

The sap agave and pulque were sampling in "Los Claveles", Guanajuato in the month of December 2018 and July 2019. For the preparation of dilutions and microorganisms counting were carried out according to NOM-110-SSA1. The surface extension technique was performed on specific agars to identify microorganisms according to their metabolism. Reducing sugars and pH were determinate by standard methods as a part of chemical characterization.

Interest strains were selected in each medium and isolated. Sixteen Gram positive (G+) strains were isolated from sap agave on MRS agar, on, GYC agar was isolated twelve G+ strains, on standard methods were isolated six G+ and seventeen gram negative (G-). From MacConkey agar were isolated twentytwo G+ and four G-. Fourteen yeasts were isolated on extract malt agar and 8 yeast on agar Dextrose Sabouraud. From pulque was isolated twenty four strains G+ from MRS agar, and twelve G+ strains GYC agar. Eigth G+ and three G- strains were isolated from Standard Methods, Twelve yeasts were isolated from Dextrose Saubourad agar and six from Malt Extract agar. It was observed that after the fermentation there is an increase in the lactic bacteria, acetic and yeast, a decrease in aerobic mesophilic, and no presence of coliform bacteria or gram negative was found. In chemical determinations a decrease in the pH value was observed, having a general average in sap agave of 5.59±0.02 and 4.17±0.02 in pulque, while reducing sugars have an average of 2.58±0.33g/L and 2.85±0.28g/L in pulque, these similar values that could be due to a change in metabolism after a possible decrease in total sugars. Until now 103 bacteria and 40 yeasts were isolated, however, further analysis is necessaries in order to describe the relationship between the chemical sap agave and pulque chemical characteristics and the microorganisms isolated.

AREA: Microbiología de alimentos y clínica

Determination of virulence profile of Shiga toxin-producing *Escherichia coli* from Chicken Carcasses from Retail Markets in Culiacan, Sinaloa, Mexico

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Introduction. Shiga toxin-producing *Escherichia coli* encompasses a group of emerging food pathogens that can be divided into O157 and non-O157. Both groups are zoonotic enteric pathogens responsible for serious gastrointestinal infections and diseases such as hemolytic uremic syndrome and hemorrhagic colitis. STEC strains can be isolated from raw poultry representing a high risk to public health. The aim of the present study was to determine the virulence profile of STEC strains isolated from chicken carcasses. **Materials and methods.** Thirty whole chicken carcasses were purchased from various local retailers. The liquid from each package was collected and enriched in a two-step enrichment in tryptic soy broth with an incubation for 2 hours at 25ºC and then 8 hours at 42ºC. The enrichments were then subjected to an immunomagnetic separation with anti-O157 beads, were plated directly on CHROMagar O157 (C-O157) selective medium and were further incubated for 18-24 hours at 37°C. For the confirmation of STEC strains and identification of the main virulence factors, a multiplex PCR was performed. **Results.** According to the results out of 30 chicken samples, 12 (40%) were confirmed to be STEC positive. Among the STEC-positive samples, 16.6% of them had the *stx1* virulence gene, 83% the *stx2* gene, whereas only 1 sample where positive for both and for the *ehxA* virulence gene. **Conclusions.** Considering the virulence profiles, our results indicate that raw poultry can be a vehicle for potentially pathogenic STEC strains increasing human disease outbreaks.

Rapid detection of influenza viruses A and B using the system influenza A + B Veritor ™ BD compared with the RT-qPCR in Mexican patients during the winter period of 2018-2019

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Influenza causes significant morbidity and mortality during outbreaks or epidemics. Timely diagnosis of influenza is important for optimal patient management and infection control. Several diagnostic methods are used for the detection of influenza. Viral culture has been the gold standard for influenza detection, but it has a long response time (3 to 10 days). RT-PCR is used most frequently in clinical settings and is considered the most sensitive test for influenza detection. Rapid diagnostic tests for influenza (RIDT) are faster, take 10 minutes and are a practical method for detection, but the obstacle in clinical use is poor sensitivity. To improve sensitivity, several systems of influenza point of care test readers have been developed. BD Veritor ™ System Flu A + B is a digital immunoassay for direct and qualitative detection and differentiation of influenza A and B viruses in different respiratory samples. These digital immunoassays (DIA) allow lower detection limits, reduce operator variability and allow negative control that reduces the likelihood of a non-specific binding.

OBJECTIVE: The objective of this study was to evaluate the sensitivity and specificity of the BD Veritor ™ System Flu A + B (BD) trial in patients with influenza and influenza-like syndrome treated in the INER.

MATERIAL AND METHODS: We use respiratory samples from patients with patients with influenza-like influenza syndrome treated during October 2018 and February 2019 at INER. The collected samples were kept in viral transport medium and stored in a freezer at 80 ° C. The samples were analyzed using the BD Veritor ™ System Flu A + B (BD) assay. The sensitivity and specificity of the RIDTs were calculated using the results of RT-PCR as standards.

RESULTS. Among 187 respyratory samples, 184 was nasopharyngeal specimens (98%); were positive by RT-PCR 45% (84/187). Usigng the BD Veritor ™ System Flu A + B (BD) 35 samples were positivie for influenza A and 6 for influenza B virus. The sensitivity was the 60% an and specificity was the 100% wthit the BD Veritor ™ System Flu A + B (BD). The average RT-PCR threshold cycles (Ct) for specimens that were positive for influenza A or B was of 26. The mean Ct values were lower for samples that were positive by BD Veritor ™ System Flu A + B, when compared to the mean Ct values for samples that were negative by RIDT.

CONCLUSION. We found a lower sensitivity to that reported by other authors. The negative tests by the veritor system had higher values of Ct.

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CONCLUSION. We found a lower sensitivity to that reported by other authors. The negative tests by the veritor system had higher values of Ct.

"Phenotypic and genotypic detection of *Clostridium difficile* isolated from asymptomatic carriers"

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Clostridium difficile is a Gram-positive, obligate anaerobic, spore-forming bacterium, found in the environment and in the intestinal tract of animals and humans. It is the most common cause of infectious diarrhea associated with the use of antibiotics, and has emerged as a leading nosocomial pathogen in developed countries. The infection caused by *Clostridium difficile* (CDI) is a toxin-mediated disease, characterized by infectious diarrhea. Symptoms range from mild to severe diarrhea, pseudomembranous colitis (PMC), toxic megacolon, or even death. The increase in the incidence and severity of the CDI has resulted in a significant economic burden on health systems due to the costs associated with treatment, and prolonged hospital stays. Asymptomatically colonized patients may shed spores to their environment and, consequently, to other patients Prevalence estimates that asymptomatic *C. difficile* colonization varies considerably between different patient groups. Among healthy adults with no prior risk factors for CDI, *C. difficile* colonization prevalence varies between 0 and 15%. Epidemiological evidence suggests that an increasing number of cases of CDI is related to populations that were generally considered of low risk. In Mexico, there are few reports about the presence of *C. difficile* in carriers, therefore in the present study, we collected samples from the comunity to isolate *C. difficile* and to determine by PCR the toxin genes (*tcdA*, *tcdB*, *cdtA* and *cdtB*). Samples were inoculated in specific media plates for *Clostridium difficile* called CCFA (Cycloserine-cefoxitin-fructose Agar) and BHI agar was also used as maintenance medium. The *tpi* gene has high specificity for *C. difficile* identification. It encodes the triosa phosphate isomerase, and has been used in other studies to identify *C. difficile* strains. In the present study we collected 25 samples from adults and 5 from children. We detected *tpi* gene in clones from 5 different people, 1 child and 4 adults. Currently we are detecting the presence of *C. difficile* toxins in this *tpi* positive strains. It seems likely that infections caused by C. difficile become an important problem in Latin America. In Mexico, the prevalence of *Clostridium difficile* is poorly reported so it is important to know the prevalence of this bacteria in asymptomatic people that represent an epidemiological risk and to avoid serious public health problems.

Study of antibiotic resistance mechanisms in *Acinetobacter spp.* isolated from hospitalized patients.

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Introduction: Currently in Mexico there has been an increase in infections associated to health attention caused by *Acinetobacter* spp., and there are few studies of the presence of antibiotic resistance mechanisms. **Objective:** To determine the mechanisms of antibiotic resistance in *Acinetobacter spp*. causing infections in the Hospital Regional I.S.S.S.T.E., Puebla. **Materials and methods:** 42 isolates (2015-2018) were identified by Vitek2, and *rpoB gene* sequencing, the resistance profile was determined (Kirby-Baüer and MIC), participation of efflux pumps (MIC with and without Phenylalanine-Arginine-β-Naphthylamide (PAβN) at 25mg / l), resistance genes (PCR and sequencing) and clonal relationship by pulsed-fields gel electrophoresis (PFGE) were identified. **Results:** 33 strains of *A. calcoaceticus-baumannii* complex, 4 *A. haemolyticus*, 1 *A. schindleri*, 2 *A. ursingii*, and 2 *A. lwoffii* were identified. 23 *A. calcoaceticus-baumannii* strains were multidrug-resistance (MDR) and presented resistance to ciprofloxacin (CIP), amikacin (AN), and carbapenems. The 23 strains carried $bla_{\text{OXA-51}}$ (100%), $bla_{\text{OXA-24}}$ (100%), $bla_{\text{OXA-23}}$ (13%), bla_{NDM} (56%), bla_{IMP} (4%) and *aac (6´)-Ib* (4%) and did not carry $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{GES}, bla_{KPC}, bla_{SHV}, bla_{PER}, $bla_{\text{VEB-}}$, bla_{VIM}, bla_{GIM}, bla_{SPM}. IS*Aba1* and IS*Aba125* were not found flanking bla_{OXA} and bla_{NDM} genes. The participation of efflux pumps in the resistance to meropenem was observed in 10 strains (MIC decreased 2-fold), for amikacin in 6 strains (MIC decreases 2 to 8-fold), and for ciprofloxacin in 5 strains (MIC decreased in 2-fold). Two circulating clones were detected by PFGE. **Discussion:** In this hospital, the species of the *A. calcoaceticus-baumannii* complex predominated, and presented high levels of antimicrobial resistance due to the presence of bla_{NDM}, and the participation of efflux pumps mainly. We found two clones circulating with a similar resistance genotype. **Conclusions:** This study shows that antimicrobial resistance is mediated by multiple mechanisms, which must be taken into account in decision-making for the treatment and containment of resistant strains in this hospital.

Characterization of UPEC clinical isolates in pregnant women.

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Urinary tract infection (UTI) is nowadays a public health problem in Mexico. It is characterized by the presence of bacteria in the urine (bacteriuria). UTI can be symptomatic or asymptomatic, complicating its diagnosis and treatment. *E. coli* is the main causal agent. When *E. coli* colonizes the urinary tract, it is called uropathogenic *E. coli* (UPEC). This bacterium has been associated with the development of neonatal sepsis, nosocomial outbreaks and with recurrent infections in pregnant women. In addition, this bacterium has high rate of antibiotic resistance. Therefore, the identification of the genetic profile of *E. coli* strains isolated from pregnant women, might lead us to establish their clonality and to relate them to clinical presentation and complications.

246 *E. coli* clinical strains were obtained from urine culture samples of women with urinary tract infection collected in the INPer. The UPEC strains were selected by the detection of *fim*H, *iut*A, *fyu*A, *hly*A, *traT*, *rpa*I, *pap*A and *ibe*A genes by multiplex PCR. For the classification of the UPEC in phylogroups, the methodology of Clermont and collaborators was used. The clonal characterization of the clinical isolates was carried out by PFGE, using the XbaI enzyme in the CHEF Mapper® XA system. The bioinformatic analysis was carried out in the Bionumerics 7.6 software. In 99% of the clinical isolates of UPEC, at least one of the 8 virulence factors were identified; the most frequently amplified genes were: *fim*H (88%), *fyu*A (83%), *iut*A (66%) *traT* (55%), The genes that were in less than 50% of the isolates were: *rpa*I, *ibeA*, *hly*A and *pap*A. Regarding the phylogroups, it was found that 45% of the isolates belonged to phylogroup B2, 37% to phylogroup A, 12% to phylogroup D and 6% to phylogroup B1. PFGE analysis of 42 clinical isolates did not show a predominant group; but, two clones presented the same restriction profile. The frequency of antibiotic resistance was as follows: Trimetroprim / Sulfamethoxazole (41%), ampicillin (53%), levofloxacin (50%), piperacillin (50%) tetracycline (100%) and trimethoprim (100%).

The *fim*H, *fyu*A, *iut*A and *tra*T genes were the most frequently amplified genes and are useful as molecular markers to identify UPEC isolates. The *ibeA* gene was only identified in the phylogroup B2, suggesting that this phylogroup is associated with meningitis development. The *E. coli* strains belonging to phylogroups B2 and A are the most frequently isolated in pregnant women. The highest frequency of virulence genes was found in *E.coli* antibiotic sensitive strains of phylogroups B2 and A, indicating that the presence of virulence factors is independent of antibiotic resistance. No clonal relation was found among the clinical isolates of UPEC suggesting that they are strains acquired in the community.

Drug susceptibility testing of *Mycobacterium mucogenicum* isolates from different sources.

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Background: *Mycobacterium mucogenicum* belongs to the group of fast-growing nontuberculous mycobacteria (NTM). It causes localized or systemic mycobacteriosis, and so it compromises patients' lives. This species is one of the most frequent NTM isolated in Mexico City from potable water and some food, being those the carries of this opportunistic pathogen to people. In Mexico, there are no reports of drug susceptibility testing (DST) of *M. mucogenicum* to guide the scheme of treatment of infected patients. Also, it is not known if the drug susceptibility profile is the same between clinical and environmental isolates. Hence, this study aimed to assess the drug susceptibility of four strains isolated from different sources. Methods: three strains isolated from different environmental sources from Mexico City were evaluated (1. From potable water, 2. From sprouts ready to eat, 3. From Mexican chili sauce), and the strain *M. mucogenicum* ATCC 49649 was used as a reference of a clinical isolate. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration of 12 antibiotics were determined (FOX, IMP, KAN, AMK, TOB, STR, DOX, MINO, CLR, CIP, MX, SXT) through the resazurin microtiter assay (REMA) and the colony-forming units (CFU) counting of survived bacteria, respectively. Results: Only two antibiotics showed the same MIC for all isolates. Most of the results of MIC and MBC were similar between environmental isolates, while results were more different and even higher concentrations were needed to inhibit the growth or to eliminate bacteria from the clinical isolate (ATCC 49649). The MIC was the same than the MBC in about 33.3% of all assays, which means that the inhibitory concentration found in those assays also had a bactericidal effect. In conclusion, the environmental isolates had a similar susceptibility profile between them, while the clinical isolate did not. The most sensible isolate was that from sprouts ready to eat, followed by the isolate from chili sauce and then the one from potable water and finally, the least sensible was the clinical isolate. The drug susceptibility profile of every strain must be influenced by the environmental stress that faced before they were isolated.

MICROBIOTA AND MICROBIOME

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VI Meeting of Biochemistry and Molecular Biology of Bacteria (BBMB)

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Metagenomic analysis of a bacterial biofilm grown on an asphalt rock in the Gulf of Mexico

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Oil degradation by bacteria has been widely studied. At present, ~79 bacterial genera that can use hydrocarbons as the sole source of carbon and energy has been reported. Most of these genera are able to degrade lighter hydrocarbons fractions but few of them use the heaviest fractions of petroleum (resins and asphaltenes). In this work, we studied a bacterial community capable of growing on the surface of an asphalt rock obtained from the Gulf of Mexico. The rock surface biofilm (BF) and surrounding supernatant (SN) bacterial diversity was analyzed using V3-V4 16S rRNA amplicon sequencing with the Illumina MiSeq platform. Results showed that BF samples (142 genera) and SN samples (149 genera) were very similar. However, a lower bacterial diversity in BF samples was observed according to alpha diversity indexes (Chao1 BF = 702.78, Chao1 SN = 1159; Shannon BF = 2.47, Shannon SN = 2.53). Enrichment of both samples using asphalt as a carbon source for 4 months at 4°C, resulted in a bacterial diversity decrease. Nevertheless, there was an increase in relative abundance of hydrocarbon degrading bacteria genera (such as and their probable mutualists. Four isolated bacterial strains present in the sample (*Idiomarina*, *Pseudomonas*, *Rhodococcus* and *Pseudoalteromonas*) were used to test their degradation activity of the heaviest fractions of oil . The metabolic potential of *Idiomarina* sp. for heavy oil fraction consumption, was explored by Whole Genome Sequencing at chromosome resolution using the Oxford Nanopore Minion technology. The ~2.9Mb genome revealed high similarity to *I. loihiensis* L2TR and *I. loihiensis* GSL 199 strains, isolated from a hydrothermal vent at 1,300-m depth on the Loihi submarine volcano, Hawaii and Great Salt Lake, Utah, respectively. Interesting features related to hydrocarbon metabolism were found in the genome, but further genome improvement and error correction are needed. The bacterial diversity at the rock bacterial biofilm and the isolated bacteria genomic information can contribute to the understanding of recalcitrant hydrocarbon degrading metabolism, which can be potentially used for bioremediation and other biotechnological applications.

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Bacterial co-occurrence networks from traditional agroecosystems from contrasting climates

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Traditional milpas are diverse agroecosystems based around maize, squash and beans, with dozens of species of cultivated and useful wild plants commonly present. Milpas have been cultivated for millennia in Mesoamerica, and their soils microorganisms might have been subject to selection pressures during the process of plant domestication. Many milpas are still managed traditionally, with low tillage and minimal use of agrochemicals. Therefore, their microbial communities might preserve important functions for plant health and productivity in the absence of modern agricultural inputs. Thus, the study of milpa soils could help us understand the biotic interactions that sustain plant productivity, which might be exploited in the future for sustainable agriculture, including the development of biofertilizers. We are only starting to understand the dynamics of milpa-soil microorganisms and their interactions with plants. In a recently published study, we found enrichment of members of the phyla Verrucomicrobia and Actinobacteria, as well as well as order Burkholderiales, in the vicinity of maize-roots, as compared to bulk soil. Since Actinobacteria and Burkholderiales have known plant-beneficial representatives, Verrucomicrobia could also be enriched for beneficial interactions with the plants. In this work, we compare the soil microbial communities of two distant milpas with contrasting altitude and climate. Although similarities in community structure were found, the milpas showed clearly different microbial communities, as assessed by beta-diversity analyses. In both milpas, we found modules of co-occurring bacteria with negative correlations between them, suggesting competition between modules. Strikingly, the most significant positive and negative correlations were conserved in the co-occurrence networks of both milpas. These results suggest that similar mechanisms drive the community ecology of these contrasting milpas, either by similar niche-preferences or by biotic interactions between bacteria.

Pre and probiotics effects on the growth and intestinal microbiota of an endemic fish from Mexico with aquaculture potential.

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Pike silverside fish of Pátzcuaro´s lake *Chirostoma estor* is endemic to the lacustrine basin of the Mexican highlands and has ecological, nutritional, economic and cultural importance, and it is currently in danger. Although the complete life cycle of this species has been achieved in captivity, a diet that optimizes its growth to reach a commercial size has not yet been developed. An option to solve this problem may be the addition of prebiotics and probiotics in the fish diet. Therefore, in this work, prebiotics (inulin 2%, cell wall of *Saccharomyces cerevisiae* 0.025%), probiotics (*Lactobacillus acidophilus* 1X10^6 CFU/g), and synbiotics (*L. acidophilus* + inulin and *L. acidophilus* + cell wall of *S. cerevisiae*) were added as part of the diet of juvenile pike silverside fish *C. estor.* One of the aims of the present study was to determine the influence of these supplements on the growth of the fish in captivity and also, determine the influence over the gut microbiota determined by Illumina sequencing and bioinformatic analysis. The results of the experiment concerning growth indicate that synbiotics (combinations of prebiotics and probiotics) have a greater influence ($P < 0.05$) on growth, compared to prebiotic and probiotic treatments added to the diet individually (including control treatment without supplements). We are analyzing gut microbiota metagenomics data at the moment, with the aim of observing the influence of prebiotics, probiotics and synbiotics over the microbiota richness, abundance and community structure. As a preliminary conclusion, the application of synbiotics has the best positive influence over the growth of juvenile pike silverside fish *C. estor* and possibly the best stimulating effects of a good functional microbiota profile.

Fecal bacterial profile of the Mexican wolf (*Canis lupus baileyi*) in reserve and zoo environments

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The Mexican wolf (*Canis lupus baileyi*) is an important top predator within Mexican ecosystems, but is currently in danger of extinction. Intestinal bacterial communities of animals are a general indicator of their well-being and health status. To date there are no records of the fecal bacterial profile in Mexican wolves. Our objective was to determine and compare the taxonomic composition of the fecal bacterial microbiota of two communities of Mexican wolves, one *in situ* (Michilia Reserve) and the other *ex situ* (Ocotal Zoo) in Mexico, using next generation massive sequencing. Twelve fecal samples of Mexican wolf were collected; DNA was extracted, V3-V4 regions of the 16S rRNA gene were amplified and sequenced in MiSeq Illumina; the results were analyzed with QIIME using EzBioCloud as a reference. We identified 29,259 high-quality bacterial sequences in Michilia and 37,057 in Ocotal. In Michilia five phyla were determined (Firmicutes 61.48%, Proteobacteria 20.57%), 25 families (Lachnospiraceae 34.16%, Succinivibrionaceae 16.33%, Peptostreptococcaceae 14.64%) and 87 genera (*Anaerobiospirillum* 16.33%, *Clostridium*_g21 14.87%, *Blautia* 12.54%). In Ocotal seven phyla were found (Fusobacteria 73.10%, Firmicutes 25.22%), 26 families (Fusobacteriaceae 73.10%, Lachnospiraceae 8.72%, Ruminococcaceae 8.22%) and 88 genera (*Fusobacterium* 73.10%, *Sporobacter* 4.97%, *Blautia* 4.72%). The composition of the bacterial communities in Mexican wolf feces resembles other studies on canids, especially in the population of Michilia. There was a highly significant difference in fecal bacterial diversity between the two communities analyzed (PERMANOVA pseudo-F = 6.96, P = 0.001). The food available to the wolves is different at the two locations; in Michilia it is comprised of dry food and horse meat, while in Ocotal the wolves are fed white meat (chicken, rabbit, domestic guinea pig). Likewise, in Michilia the wolves are separated in small packs or all alone in large areas of natural forest, while in Ocotal they all coexist within single zoo habitat. The type of management given (type of food, living conditions, and stress) could be influencing their fecal microbiotas. We recommend additional research to clarify the reasons for these differences that can contribute to the conservation of this endangered species.

Microbiota of diarrheagenic *Escherichia coli* is characterized by the presence of Phylum Proteobacteria, Family Enterobacteriaceae and genera *Escherichia-Shigella*

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Diarrheagenic *Escherichia coli* (DEC) pathotypes are the main etiological group isolated from children with acute and persistent diarrhoea in less developed regions of Latin America and developing countries from Asia and sub-Sahara Africa. DEC pathotypes are *E. coli* strains that have acquired diverse virulence factors by horizontal gene transfer, based on these factors and their pathogenic mechanisms DEC are classified in six pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli* (EAEC), diffusely-adherent *E. coli* (DAEC), shiga toxin producing *E. coli* (STEC). After the introduction of rotavirus vaccine, DEC pathotypes have become the main pathogens associated with diarrhoea among children under five years old requiring hospitalization in Merida, Yucatan, and DAEC, EAEC, and EPEC, were the most prevalent pathotypes. Nowadays, the host microbiota, which is a diverse community of microorganisms on or in the host, is considered a major host component for disease susceptibility. The aims of the present study were to identify and compare the microbiota of children with DEC-diarrhoea and without. DNA was extracted from faeces of children with diarrhoea, seeking medical care at the paediatric emergency room of “Hospital General O’Horan, Secretaria de Salud”, from whom the only enteropathogen identified was DAEC, EAEC and EPEC, and from children without diarrhoea, visiting the hospital for vaccination or simple fractures, negative to any of the 15 enteropathogens sought out. The 16S rRNA gene was amplified and sequenced using Ilumina-MiSeq. Sequences were processed using QIIME2 software and clustered into operational taxonomic units (OTUs). Differential OTUs abundance analysis (>1%) revealed that at phyla level: Proteobacteria was significantly more abundant in the microbiota of DEC-diarrhoea children vs. control children, while the abundance of Firmicutes and Verrucomicrobia were significantly less in patients. Of the Proteobacteria phylum the Enterobacteriaceae and *Escherichia-Shigella* were the most abundant family and genera in the DEC-diarrhoea children, respectively. In contrast, among the control children the families that were significantly more abundant were Erysipelotrichaceae (*Erysipetaloclostridium*), Rikenellaceae (*Alistipes*), Bacteroidaceae (*Bacteteroides*), Tannerellaceae (*Parabacteroides*) and Akkermansiaceae (*Akkermansia*). The later genus has been found to be more abundant in healthy children worldwide, while genus *Prevotella*, which contains a set of genes for cellulose hydrolysis, had similar abundance in both children’s groups, as in children from other less developed regions of the world. In control children, microbiota genus diversity was higher than in DEC-diarrhoea children. The microbiota parameters identified among children with DEC-diarrhoea are in line with other studies of microbiota and disease. The Proteobacteria phylum was also more abundant in the microbiota of Chilean children with DEC- and viral-diarrhoea.

Bacterial communities associated with heterocystous cyanobacteria in the cycad coralloid root may have a role in symbiosis

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Heterocystous cyanobacteria, prokaryotic heterotrophs that fix nitrogen in specialized cells called heterocysts, can form symbiotic relationships with various hosts. Among these symbioses we find that formed with cycads, one of the oldest gymnosperms. The cyanobacteria at the coralloid root, the cycad symbiotic organ, has been found to be associated with a diverse bacterial community. Metagenomic explorations of these cycads' bacterial communities from different geographical locations revealed a taxonomic core comprised of *Caulobacterales*, *Rhodospirillales* and *Rhizobiales*. These bacterial orders are also found in bacterial communities associated with cyanobacteria in symbiosis with other hosts. With these results in hand we aim at reconstructing nearly natural sub-communities to explore specific interactions with mechanistic implications, under biologically meaningful ad hoc conditions of this system. For instance, our results indicate that the nitrifying activity of *Rhizobium* found in symbiosis between the aquatic fern *Azolla* and cyanobacteria is also part of the cycad-cyanobacteria symbiosis, suggesting that the cycad symbiosis is also dependent upon bacteria-bacteria interactions. This is a hypothesis that can be experimentally tested, bringing about a higher order of resolution for the study of complex microbiomes.

Taxonomic and functional changes in the microbiota of the white shrimp (*Litopenaeus vannamei*) associated to postlarvae ontogenetic development

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Although *Litopenaeus vannamei* is one of the most important species used in aquaculture, little is known about the functions provided by the intestinal microbiota for host development. This study aims to know the taxonomic and functional changes in *L. vannamei* gut microbiota during its postlarval development in a recirculation system with controlled conditions for 80 days. For this, DNA extraction from the intestinal microbiota and subsequent massive sequencing of the V4 variable region of 16S rRNA gene and functional prediction was performed. The results show that bacteria of the Vibrionaceae family predominated at Farm, 0 and 20 culture days, while the Rhodobacteraceae family increased from 20 culture days, unlike Vibrionaceae, which showed a constant decrease as the shrimp reached the youth and adult stages; though Intrasporangiaceae family remained constant in organisms 40 to 80 days old. Microbial-mediated functions predicted by PICRUSt showed that some level 2 KEGG pathways such as biosynthesis and biodegradation of secondary metabolites as opposed to the requirement in carbohydrate metabolism. The Level 3 KEEG pathways, display glycine, serine and threonine metabolism, functions related to lipid metabolism such as fatty acid biosynthesis and linoleic acid metabolism and digestive system like digest and absorb carbohydrates and bile secretion. The principal component analysis (PCA) shows that taxonomic profile and functional prediction have a similar pattern of distances, with differences according to the development time. where two main groups were observed, the 0D and 20D, and another at 40D, 60D and 80D, presenting a trend of microbial stabilization of the host according to its growth suggesting that the microbiota tends to be variable during the first postlarvae phase, but it becomes more constant towards the adult phase. These results suggest that intestinal microbiota is selectively found according to *L. vannamei* requirements during its development, working jointly host-host.

Interactions between bacterial genera from a hydrocarbon degrading marine microbial consortium

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In the Gulf of Mexico, hydrocarbon-degrading bacteria are ubiquitous and can utilize these compounds as carbon and energy sources. During the hydrocarbon degradation processes, a change in microbial population diversity can be observed, both *in situ* and *in vitro* conditions. The hydrocarbon presence can shape bacterial communities selecting species capable of degrading them, but also other organisms that can benefit from the metabolites released by the degradation process. Additionally, the presence of species that can withstand the oil presence are found but usually not thriving in those conditions. However, bacterial interactions in these communities, like mutualism, competition, parasitism and commensalism are not completely understood yet. In this work, we analyzed these interactions in a microbial community, isolated from marine sediment samples (from southwest Gulf of Mexico), where high concentrations of aromatic hydrocarbons were detected. The consortium was grown for 85 days using marine mineral media with kerosene as sole carbon source. Population changes were monitored using V3-V4 16S rRNA amplicon sequencing with the Illumina® MiSeq platform. MetaMIS software (v1.02) was used to identify interactions between bacterial genera present using a network approach. We identified a diversity of 767 genera which was reduced to 66 after 85 days. *Alcanivorax, Halomonas* and *Idiomarina* genera represented > 10 % of total relative abundance each and were enriched together up to 95 % after 85 days. These genera have been reported as hydrocarbon degraders. In particular, the *Alcanivorax* genus benefited from growing in aliphatic kerosene hydrocarbons and had a positive relationship with most other genera. In contrast, the opposite relation from other bacteria towards *Alcanivorax,* was mostly parasitism. Genera showing mutualism relationships in more than 50 % of their total relations, were also identified, suggesting an important maintenance role in the consortium. Between those are *Halomonas* and other rare genera (relative abundance < 1 %) such as *Bacillus, Roseovarius, Methylophaga, Pseudidiomarina* and *Geobacillus*, capable of different hydrocarbon degradation processes, were also detected. Our results shed some light on bacterial population interactions and their response to ubiquitous and enriched hydrocarbon environments.

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Characterization of lactic acid bacteria, isolated from musts during the production of artisan mezcal in Oaxaca.

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Introduction. The fermentation of artisan mezcal in Oaxaca is one of the stages most important of the process, since this is where sugars are transformed into ethanol, CO 2 and other chemical compounds that make up that complex mixture called mezcal. Said transformation is carried out by a large number of yeasts and bacteria; The latter have been little studied despite their importance (Pérez *et al.,* 2016).
Methodology. Three samples of the must of a wooden tub in three fermentation were collected different depths, in sterile bottles, and they were measured both *in situ* and in the laboratory the following physicochemical parameters: pH, temperature, and Brix degrees with a refractometer brixometer. In the laboratory, the bacteria were isolated in MRS (Man, Rogosa and Sharpe) which is a selective medium for lactic acid bacteria, added with nystatin (200 mg / L) to inhibit the growth of fungi and yeasts; serial dilutions were made in 1% peptoned broth and 50 µl of the 1 X 10^{-6} dilution by extension in plate. Subsequently, strains were purified by stria depletion in MRS and AN (Nutritive Agar) with nystatin (200 mg / L) until Pure strains were obtained. Once purified, they were stained with Gram, to identify its characteristics in a compound optical microscope. They were also characterized morphologically the colonies present in the plates, and biochemical tests were performed such as SIM, LIA, TSI, Simmons, catalase and nitrate reduction based on MacFadin 2008, (Fig 1). With all the above, genera of lactic acid bacteria were identified as: *Lactobacillus*, *Weisella* and *Leuconostoc*.

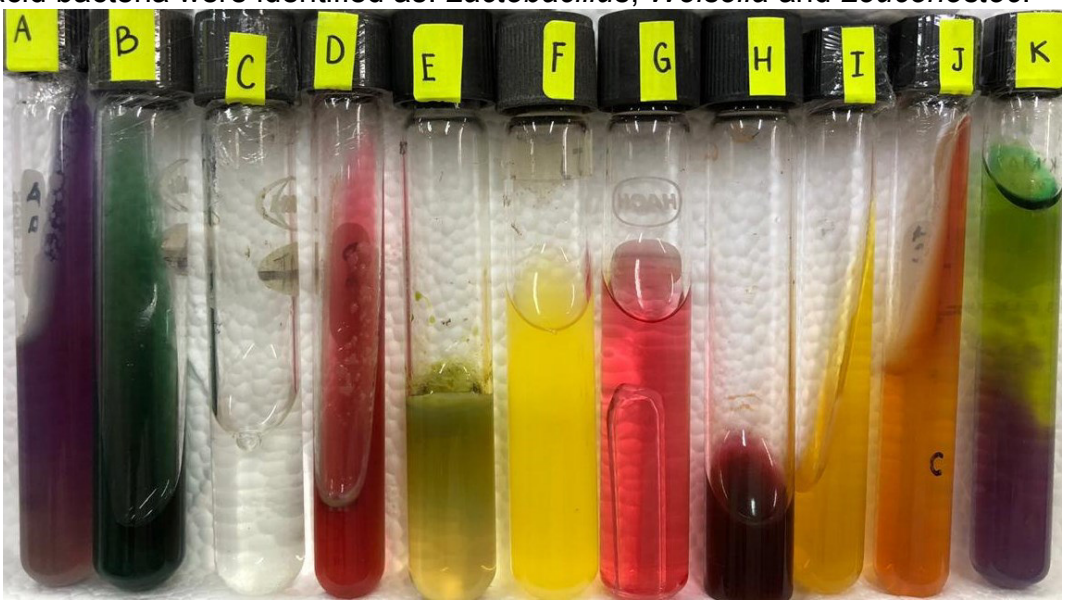

Figure 1. Biochemical tests for the identification of lactic acid bacteria.

Metatranscriptional characterization of gut intestinal microbiome in obese and obese with Metabolic Syndrome Mexican children

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Obesity has reached epidemic proportions and is considered the epidemy of the century. Mexico is on the top countries with significant incidence and prevalence in both adult and child populations. Additionally, Obesity is the first factor to develop Metabolic Syndrome (MS), which is diagnosed if at least three of the following characteristics are present: large waist circumference, hypertriglyceridemia, low levels of HDL, hyperglycemia, and hypertension. Furthermore, SM is also associated with chronic diseases such as type II *diabetes mellitus*, nonalcoholic fatty liver, gastrointestinal cancer, and cardiovascular diseases. Therefore, studies to understand the cause of SM in obese infants is critical.

Previous research has determined that an imbalance of the intestinal microbial composition has a relationship with obesity and MS. However, little is known about the functional activity (metatranscriptome) of the microbiome in both pathologies. In this work, the metranscriptional profiles of the intestinal microbiome of Mexican infants (7 to 10 years old) were determined. To this end, we selected two groups of obese children: one group, including obese without metabolic complications (O) and another group, including obese with MS (OMS). We first established a protocol for RNA extraction and preparation of simultaneous *RNA-seq* libraries. After the massive sequencing using Illumina®, we preprocessed the sequences and applied several bioinformatics tools to get the taxonomy assignment and functional annotation of the transcripts. The metatranscriptional analysis allows us to know the functional activity of the intestinal microbiome and its relationship with obesity and MS. The study of RNA has methodological complications due to the sensitivity of the sample to be degraded. However, we developed a method to obtain RNA of high quality and prepare simultaneous libraries of RNA-Seq, decreasing the time and cost of its preparation. We found significant differences between the metatranscriptional profiles of O and OMS groups.

Additionally, we observed differences between groups in richness and diversity of the expressed species and functions. Our results show that OMS overexpressed functions related with production and conversion of energy, biosynthesis, and transport of lipopolysaccharides, biosynthesis of secondary metabolites and metabolism of polysaccharides; these functions are directly related with a higher extraction and accumulation of energy. We also found specific species related to MS, like *Enterococcus faecium, Clostridium perfringens, Prevotella* spp. y *Paeniclostridium sordelli*.

Microbial profiling of gestational diabetes pathophysiology: First steps towards dysbiosis characterization using amniotic fluid, placenta, meconium and colostrum samples

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Obesity affects 600 million people worldwide and is a precursor to a vast array of comorbidities, such as diabetes. In Mexico, we expect 30% of pregnancies to develop gestational diabetes mellitus (GDM) due, in part, to obesity by the year 2020. Newborns of mothers with GDM are prone to congenital anomalies, hypoglycemia, macrosomia, insulin resistance and birth mechanical trauma. Recently, causal relationships have been established between the microbial composition of human breast milk and some aspects of newborn health. However, the impact of the microbial composition of breastmilk from a mother who experienced GDM is less clear. As obesity and subsequent GDM are frequent occurrences in Mexico, it is critical to understand how these morbidities may impact amniotic fluid, placenta, meconium and colostrum microbiome composition, and subsequently the health of future generations. Here, we present microbial population dynamics from different sources of individuals with GDM, and obesity in contrast to healthy subjects. This data will help to design hypotheses to understand the causes of the observed dysbiosis in the microbiome of newborns born from obese mothers with GDM and will provide insights to establish strategies to explore the possibility of restoring healthy phenotypes by means of a culturomics approach.

Gamma aminobutyric acid-mediated neuroprotection conferred by dietary *Escherichia coli* strain HT115 in *Caenorhabditis elegans*.

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The bacterivore worm *Caenorhabditis elegans* and its cognate bacterial diet comprise a simple, genetically tractable model to investigate the role of bacterial metabolites in host physiology. Recent research described that in mood disorders and neurodegenerative diseases the microbiome composition and abundance are altered and this has provided a glimpse at the neuroactive potential of specific bacterial metabolites. Nonetheless, whether bacterial metabolites directly influence neuronal degeneration is largely unknown. This study used a *C. elegans* strain expressing a neurotoxic allele of the MEC-4(d) DEG/ENaC channel which causes the progressive degeneration of the touch receptor neurons (TRN), to evaluate the effect of various laboratory and environmental dietary bacteria on neurodegeneration dynamics. While degeneration of TRNs was steadily carried and completed at adulthood when feeding on *E. coli* OP50, the strain routinely used for *C. elegans* maintenance, it was significantly reduced in other bacterial strains. Strikingly, neuroprotection reached more than 40% in the *E. coli* HT115 strain. HT115 protection lasted well into old age and encompassed other neuron types. Small amounts of HT115 on OP50 bacteria as well as UV-killed HT115 were still sufficient to produce neuroprotection. Early growth of worms in HT115 protected neurons from degeneration during later growth in OP50. HT115 promoted the nuclear translocation of the DAF-16/FOXO transcription factor, a phenomenon previously reported to underlie neuroprotection caused by downregulation of the insulin receptor in this system. Moreover, a *daf-16* loss of function mutation abolishes HT115-driven neuroprotection. Comparative genomics, transcriptomics and metabolomics approaches pinpointed the neurotransmitter γ-aminobutyric acid (GABA) as a metabolite differentially produced between *E. coli* HT115 and OP50. An HT115 mutant lacking glutamate decarboxylase enzyme genes (GAD), which catalyzes the conversion of glutamate into GABA, lost the ability to stop neurodegeneration. Moreover, *in situ* GABA supplementation or heterologous expression of glutamate decarboxylase in *E. coli* OP50 conferred neuroprotective activity to this strain. Together, results demonstrate that bacterially produced GABA exerts an effect of neuroprotection in the host, highlighting the role of neuroactive compounds of the diet in nervous system homeostasis.

Prevalence of *Porphyromona gingivalis* and *Tannerella forsythia* bacterial species in *E. gingivalis*-ST1 and/or ST2-kamaktli subtypes carriers

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The human oral microbiome analysis involves a complex interaction among bacteria, parasites, virus and fungi. *Entamoeba gingivalis* is one of the two major parasites that have been associated with periodontal disease in human oral cavity. Recently, we differentiated one *Entamoeba gingivalis* subtype that showed high nucleotide variation in 18S-ITS2 ribosomal region that we named ST2-kamaktli subtype (Garcia et al, 2018). Our interest has been focused on identity the microorganisms from the human oral cavity associated with different health states: healthy periodontium, periodontal disease and orthodontic treatment. So, in this study we determined the association between *Entamoeba gingivalis* and the ST2-kamaktli subtype with respect to two bacterial species strongly associated with periodontal diseases (*P. gingivalis* and *T.forsythia*) in the oral cavity of the three previously mentioned patient groups. In general, we observed a high bacterial prevalence in all three groups. At least one of the two bacterial species was present in 41% of the persons from the healthy periodontium group, in 72% of the periodontal diseases group and in 82% of the orthodontic treatment group. We show and discuss the association of *P. gingivalis* and/or *T. forsythia* with *Entamoeba gingivalis* and/or kamaktli subtype.

Linking up the metabolically active versus total *Vibrio* spp. population in the digestive tract of *Litopenaeus vannamei* during their post-larval development

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Shrimp aquaculture is continuously affected by bacterial diseases capable to produce mortality of post-larvae and juveniles' shrimps resulting in irreparable economic losses for aquaculture industry. In this sense, *Vibrio* spp. has been determined as responsible for important losses in shrimp aquaculture. Microbial communities could handle and determine the guest protection versus pathogens bacteria. Specifically, gut microbiota, has taken huge relevance for its role as symbiont for host´s lifecycle. Therefore, microbial ecology interactions related to their life cycle development and their interactions with and within biotic factors of white shrimp, require to be explain in order to improve shrimp production. The abundance of total and metabolically active populations of *Vibrio* sp. in digestive tract of shrimp, *Litopenaeus vannamei*, during their post-larval development. were analyzed using qPCR and retrotranscribed qPCR (RT-qPCR) targeting the 16S rRNA gene sequence. A lab-scale shrimp bioassay was performed for 80 days in a recirculating system aquarium with strict controlling conditions, including sterilized marine water. The results indicate that *Vibrio* population from white shrimp´s gut is associated with it development process. Multivariate analyses revealed that DNA has an important participation in shrimp development respect RNA. Therefore, culture days contributed significantly to explain the changes in *Vibrio* population, with two main clusters observed, one group with farm and 20 culture days and a second group at 40, 60 and 80 culture days. Also, the samples were taken from water and intestine, to verify the influence of microbial community in the organ for shrimp development.

Contribution of hospitals in the microbiome inside Mexico City Subway System.

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Hospitals act as hotspots to the acquisition and spread of antibiotic resistance and currently, this is an important problem of public health. Therefore, we hypothesize that stations near to hospital environments should have different abundance and diversity compared with other stations.

Moreover, Subway System is essential for most of the Mexico City population, and every day thousands of people contributed and are exposed to a wide variety of microorganisms that are present in this environment. There are various sources from microorganisms, and each one has a different contribution to the microbial composition of indoors, although human occupants are a major source, other main sources are the outdoors, because of the air and dust that can introduce into them.

To achieve our objective, we collected samples of the air and surface turnstiles. Subsequently, half of the samples were direct-plated onto LB media (with and without antibiotics) and the other half was used to genomic DNA extraction. Later, the 16S rRNA gene was amplified and the sequencing was performed. About the culture experiment, we counted the number of UFCs.

The results showed that the microbial composition presented on-air and surface turnstiles, seems not to be different between stations near to hospitals and the other both. Meanwhile, the most abundant phyla on both samples were Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. The most abundant genera on turnstiles were *Acinetobacter, Corynebacterium, Propionibacterium, Staphylococcus*, and *Stenotrophomonas* and in the air samples were *Deinococcus, Kocuria, Paracoccus*, and *Rubellimicrobium.* Moreover, we found, resistant bacteria to ampicillin, kanamycin, chloramphenicol, meropenem, cefotaxime, erythromycin, tetracycline, ciprofloxacin, and neomycin. Nevertheless, we didn't observe a higher proportion of resistant bacteria near the hospitals.

Our preliminary conclusion is that the contribution of hospitals to resistant bacteria inside the subway is not quantitatively or qualitatively relevant.

Distribution of rhizospheric bacterial diversity of corn from a production area of Jalisco

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Corn (*Zea mays* L.) is one of the main crops in Mexico and the world because it is the first cereal in grain yield per hectare. Los Altos, Jalisco is considered one of the most important agricultural production areas in Mexico, where corn cultivation is an important economic activity that sustains livestock activity and human food. The study of the microbiota present in this area will found the development of a sustainable production in the area. Considering that the use of chemical products is increasingly restricted due to harmful effects, the need to use biological products to promote plant growth and biological control of pests and diseases is evident; as it is too the implementation of sustainable agriculture through the generation of tools that evaluate the changes made in the soil microbiota by biotic and abiotic factors. The objective of this study was to carry out a descriptive analysis of the geographical microbial diversity distribution associated with the corn rhizosphere of a productive zone in the state of Jalisco. Rizospheric soil samples were collected from corn crops of three plots per municipality of a producing area of Los Altos de Jalisco using a combined method, the samples were processed by massive sequencing of hypervariable regions V2, V3, V4, V6 -7, V8 and V9 of the 16S rRNA gene. With the massive sequencing data, an analysis of alpha and beta diversity was performed, obtaining the abundance percentages of the main microbial groups present in the corn rhizosphere. The geographic distribution maps of the bacterial groups with the higher relative abundance (Actimonycetes, Proteobacteria and Firmicutes) in the rhizosphere of corn crops were generated. The results show the geographical distribution of the sampling points associated with the relative abundance of the main bacteria phyla in each site. The dominant group in most of the rhizospheric samples was actinomycetes, with a central geographic distribution including municipalities like Jalostotitlan and Arandas, contrasting with the bilateral abundance distribution of the proteobacteria who were the second most abundant group, while the least abundant of the bacterial groups was the firmicutes with a unilateral distribution with an increase in abundance from west to east. The generation of these maps of microbial diversity present in this region will under an analysis of distribution of communities associated with the rhizosphere of this crop, that allows visualizing and associating the diversity with the nutritional conditions of the soil to form a reference of the state in wich they find and thus contribute to development of sustainable agriculture in this producing region.

Impact of bacteriophages associated with childhood obesity in the intestinal microbiome in a model murine.

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Over the last decade, obesity has faced one of the most serious public health challenges, due to its fast prevalence increase and co-morbidities with several diseases, as the Metabolic Syndrome (MS), diabetes mellitus II and cardiovascular diseases. In addition, growing evidence has identified the gut microbiota as a potential factor in the pathophysiology of both obesity the related metabolic disorders, given its influence on metabolic and immune functions of the host. However, most of these investigations have focused only on the role of bacteria, leaving aside other components that can regulate the composition and function of the bacterial communities of the gut microbiota, within which are bacteriophages playing an important role in their ability to infect bacteria.

In this study, we investigated the bacterial taxonomy and diversity of the gut microbiota of a murine model (group A) with normal weight, transplanted with human intestinal microbiota and infected with bacteriophages isolated from fecal samples of healthy and obese with Metabolic Syndrome (OMS) children and compared with the native gut microbiota of a normal-weight murine model (group B) infected with bacteriophages extracted from fecal samples from healthy children and OCM. This study was conducted in three stages; the first stage was to establish a protocol for the depletion and transplantation of human intestinal microbiota to a murine model; the second stage was characterized by the establishment of a protocol for the isolation of bacteriophages from fecal samples and subsequent inoculation to the murine model; finally, in the third stage and for three months, metagenome changes as a consequence of bacteriophage infection were analyzed using sing next-generation sequencing from the V3-V4 region of the bacterial 16S rRNA gene. Group A transplanted with human gut microbiota showed greater variation in bacterial richness and diversity during the process of gut microbiota depletion and transplantation. The results showed that group A infected with bacteriophages isolated from fecal samples of OCM children had differentially higher levels of bacteria of the genus *Clostridium*, *Turicibacter*, *Enterococcus*, while the group B infected with bacteriophages isolated from fecal samples of healthy children showed higher differential levels of bacteria of the genus *Clostridium*. compared to the B group infected with bacteriophages of OCM children that showed differential levels of the *Faecalibacterium* genus. Changes in the relative abundance of different taxa of the bacterial community were mostly observed in taxa of lesser abundance. However, these changes occurred for brief periods of time. Our analysis revealed that bacteriophages caused an imbalance of the intestinal microbiota (dysbiosis) in the murine model.

Mycobiota associated with dieback in Mexican Lime (*Citrus aurantifolia)* affected by Huanglonbing (HLB).

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Mexican lime (*Citrus aurantifolia*) production has been deeply impacted by the Huanglonbing (HLB) disease, caused by the floem limited bacteria *Candidatus* Liberibacter asiaticus in the state of Colima. Additionally, it has been observed that infected trees also show symptoms of citrus dieback, mainly affecting their canopy. The aim of this work was to morphologically and molecularly characterize the mycobiota associated with mexican lime trees affected by both HLB and citrus dieback. In order to achieve this, dead twig samples were collected at INIFAP-Campo Experimental Tecomán and fragmented into 5 cm pieces, then superficially disinfected with 1% sodium hypoclorite, and incubated at 28 °C for 72 h on Dichloran Rose Bengal Chloramphenicol Agar (DRBC). Afterwards, the fungal colonies observed were transferred to Potato Dextrose Agar (PDA) and monosporic cultures were obtained by serial dilutions of a 0.01% Triton X-100 spore suspension. The morphological characterization of the monosporic isolates was performed on PDA at 5 and 14 days after inoculation; parameters such as texture, colony diameter, pigmentation, exudate production and reproduction structures were analyzed. Genomic DNA of these isolates was extracted using the DNeasy Plant Mini Kit (Qiagen) and amplification of their ITS sequences was performed with the ITS1/ITS4 primers using the Taq PCR Master Mix Kit (Qiagen) and a Veriti 96 well thermal cycler (Applied Biosystems) under the following conditions: initial denaturacion at 95 °C for five minutes, followed by 30 cycles of denaturation at 95 °C for 1 minute, annealing at 55 °C for 1 minute, and extension at 72 °C for 90 seconds; a final extension phase was performed at 72 °C for 10 minutes. So far, 21 unique isolates have been obtained, nine of which belong to the genus *Lasiodiplodia*, seven to *Aspergillus*, one to *Trichoderma*, one to *Fusarium* and three still not identified due to the lack of reproduction structures. Sequencing results are still pending.

Dynamic and Asymmetric Changes of the Microbial Communities after Cohousing in Laboratory Mice.

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Horizontal transmission of the microbiota between different individuals is widely used to normalize the microbiota in laboratory mice. However, little is known about the dynamics of microbial communities and the level of microbiota transmission after cohousing. We extensively analyzed the fecal microbiota in Jackson and Taconic C57BL/6 mice to study horizontal transmission after weaning. Changes in the microbiota were clearly detected on day 3, almost plateaued on day 7, and resulted in near-comparable composition by day 28 after cohousing. Notably, the transmission of bacterial species was asymmetric in kinetics and abundance, resulting in a microbiota that is more similar to that of Jackson mice than Taconic. Several operational taxonomic units (OTUs) increased their abundance rapidly after cohousing in Taconic mice whereas several OTUs including two mucus-associated bacteria increased their abundance with delayed kinetics in Jackson mice. These studies provide insight into the dynamics and normalization of the microbiota during horizontal transmission.

Characterization of bacterial diversity of healthy individuals and patients with ocular surface infection

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Introduction: The ocular surface system comprises the conjunctiva, cornea and its glandular epithelia in addition to the tear film; these tissues function as a protective barrier in the anterior region of the eye. This area can contain and maintain a great diversity of microorganisms such as viruses, parasites, fungi and in a greater amount of bacteria. There are some factors that could modify the conditions of the ocular surface, creating a suitable microenvironment to facilitate the growth of these microorganisms by initiating an infection process. Conventional microbiology techniques only show us dependent culture microorganisms, however the new sequencing techniques have helped us to understand the total composition of the diversity of microorganisms that could be present on the ocular surface, this information can now be obtained by sequencing of the 16S ribosomal gene. Objective: To characterize the bacterial diversity on the ocular surface in patients with infection and healthy individuals. Methodology: Samples from patients with ocular infection and healthy individuals were selected, DNA extraction was performed and quality was evaluated. It was amplified with specific primers for the V3-V4 region of the 16S ribosomal gene and subsequently genomic libraries were prepared, sequencing was performed on the Illumina Miseq platform; and the bioinformatic analysis was performed. Results: A total of 42 samples, 21 cases and 21 controls were selected, of which 28 samples are diagnostic of corneal ulcers, 8 of keratitis and 6 of conjunctivitis. The quality control of the sequencing data of the samples was performed through the FastQC program, the adapters were subsequently cut with the Trimmomatic program and once the sequences passed this process, the identification of OTUs with the QIIME program was performed, to obtain the taxonomic classification of the microbial diversities present in the ocular surface. Conclusion: Differences were observed in the abundances of bacterial diversity at the genus level in the samples compared to controls.

Keywords: Eye infections, Eye surface, 16S rRNA, Bacterial diversity

Microbiota response to stress situations.

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The aim of this work is to understand the influence of the intestinal microbiota in the brain chemistry and behavior and how dysbiosis of this microbiota is specifically related to anxiety, which is a common symptom in several mental illnesses associated with stress, such as depression.

The microbiota is necessary for normal response to stress, anxiety-like behaviors, sociability and cognition. Furthermore, the microbiota maintains homeostasis of the central nervous system regulating immune function and integrity of the blood brain barrier.

Experimental models and conditions have been used that have revealed the microbiota's function in stressful situations, including prebiotic and probiotic intervention, administration of antibiotics, fecal transplantation and the use of specific pathogen-free animals.

In addition, it has been shown that behavior can be affected by the manipulation of the bacterial composition related to mood, pain and cognition. Psychological stress can alter the intestinal microbiota.

Resilience and stress-related disorders may depend on the diversity and complexity of the gastrointestinal microbiota, since the gastrointestinal microbiota is considered as a facilitator of adaptation to stress and the immune response in the body, as it can affect the responses of anxiety and fear, and can reduce behavioral despair or anhedonia.

Anxiety and depression are health problems that are increasing worldwide, so effective and affordable treatments would benefit millions of people, therefore; and since probiotics have potential in the treatment and prevention of anxiety and depression, the study clinically regulation of intestinal microbiota as a potential treatment option and prevention in various psychiatric disorders is necessary.

Lactobacillus gasseri and *Sneathia sanguinegens* in women non-squamous intraepithelial lesion and cervical cancer with HR-HPV infection

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ABSTRACT

Introduction. Vaginal microbiota is of great importance in maintaining vaginal health. In the vaginal microbiota an increased of anaerobic bacteria as *Sneathia spp*. combined with reduced relative abundance of *Lactobacillus spp*. is involved in acquisition and persistence of HPV infection and the development of cervical precancer and cancer. The aim of this study was to determine the frequency of *L. gasseri* and *S. sanguinegens* in women non-squamous intraepithelial lesion (non-SIL) and cervical cancer (CC) with high-risk human papillomavirus (HR-HPV) infection. **Methodology.** Seventy eight DNA samples of scrapings and cervical biopsies of women from the state of Guerrero were analyzed; 16 non-SIL without HPV infection, 20 non-SIL and 42 with diagnosis of CC all with HR-HPV infection. The detection of *L. gasseri* and *S. sanguinegens* was performed by Polymerase Chain Reaction (PCR) endpoint. DNA from a strain of *L. gasseri* and of a positive sample of *S. sanguinegens* verified by sequencing was used as a positive control. As a negative control, the DNA was replaced by sterile deionized water. **Results.** In this study *L. gasseri* was found in 50% (8/16) of women non-SIL without HPV infection, the 65% (13/20) of women non-SIL/ HR-HPV infection and 21.4% (9/42) of women with CC/ HR-HPV (p=0.002) while the 31.2% (5/16), 40% (8/20) and 76.2% (32/42) were positive for *S. sanguinegens*, respectively (*p*=0.001). **Conclusion.** *L. gasseri* is frequently found in vaginal microbiota of women non-SIL while in women with CC and HR-HPV positive it is common to find *S. sanguinegens*.

Microbial diversity and structure of the parasitic plants *Phoradendron velutinum* and *Arceuthobium gilli.*

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Parasitic plants represent a serious problem for an ecosystem's health due to their impact on hosts such as theft of nutrients causing weakening of it. Molecular tools like amplicon metagenomic sequencing provide us with a large amount of information that allows us to identify an organism. The aim of this work was to determine bacterial diversity and structure of the microbial communities in 2 species of parasitic plants (*Phoradendron velutinum* and *Arceuthobium gilli*) using microbiologic and metagenomic approaches. A collection of 12 samples (3 for each species with its duplicate) was taken in Veracruz from May 1st to 3rd, 2018. Two specimens were taken for the species determination for personal from UNAM herbarium, the other one was used in our analysis. For the determination of bacteria present in the samples, crops were carried out on LB agar in order to extract DNA of the isolates with the Wizard® Genomic DNA Purification kit, 16s rRNA gen were sequenced from 6 bacteria in Macrogen in Korea and sequenced (Sanger) in both directions (5'-3' y 3'-5'), the sequences were analyzed in BioEdit, SeaView, Clustal X2.1, JmodelTest 2.1.7, MEGA6. In the other hand, metagenomic DNA was extracted from each sample with the DNeasy® PowerSoil® Kit and amplicon sequencing using Illumina MiSeq Platform of the 16s rRNA Bacteria V6-V8 region was carried out in Integrated Microbiome Resource (IMR) at Dalhousie University. Sequences were analyzed using Mothur v.1.41.0. The results obtained from the microbiologic approach showed that *Enterobacter spp*. was present in 6 isolates. Analysis of the metagenomic sequences at family level pointed as dominant *Enterobacteriaceae* (79.14%), followed by *Moraxellaceae (10.03%)*, at genus level the predominant one was *Enterobacter (30.14%).* It was obtained that the sequences of *Phoradendron velutinum* have more equitable abundances and greater diversity in microbial communities than *Arceuthobium vaginatum* but are still considered as relatively low diversity (>2). It has been reported that plants have managed to develop a symbiotic relationship with bacteria (mainly endophytic bacteria) for the survival of both organisms, and these are mainly found in the roots. Enterobacteria is a heterogeneous and wide family of gram-negative bacilli that are often the cause of a considerable number of infections. It has been reported in various studies that Enterobacteria are the main plant growth promoting bacteria, since they produce indoleacetic acid, fix nitrogen, etc. We found in the study that all the families reported here belong to the phylum gamma proteobacteria, which indicates that the parasitic plants contain microbial communities help their survival and in turn are pathogenic for the host.

Metagenomic Analysis of Gut Microbiota Associated with Obesity

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Obesity is an excessive accumulation of body fat, this condition leads to different metabolic alterations putting at risk human health. Although obesity is the expression of an imbalance between energy intake and expenditure, it is known that gut microbiota plays an important role in affecting energy balance. That is why the purpose of this study was to analyze the gut microbiota composition in the different taxonomic groups and its association with the serum levels of lipopolysaccharides in young with normal weight and obesity. We analyze the regions V3 and V4 regions of 16S rRNA using the Illumina Miseq system and bioinformatics methods, the Pierce™ LAL Chromogenic Endotoxin Quantitation Kit was used to determine serum levels of lipopolysaccharide. The obtained results showed that the levels of LPS are significantly higher in the obesity group (p=0.02) compared to the normal-weight group. Groups with normal weight and obesity showed a similar gut microbiota composition at family and gender level, with 62 families of microorganisms identified in both groups. In the group with obesity, the families with the highest relative abundance were *Ruminococcaceae, Prevotellaceae, Lachnospiraceae*, and *Bacteroidaceae*, of which *Ruminococcacea* and *Lachnospiraceae* belong to the *Firmicutes* phylum and the remaining families are found within the *Bacteroidetes* phylum, observing similar results in the normal weight group. At the genus level, 145 different genera were identified, being the genera *Prevotella* (*p*= 0.11), *Bacteroides* (*p*= 0.23), *Ruminococcacea_UCGAC0\002* (*p*= 0.15), *Alistipes* (*p*= 0.21) and *Sutterella* (*p*= 0.27) those who would show greater relative abundance, for the group with obesity the genera with greater abundance were *Prevotella, Bacteroides* and *Ruminococcacea _UCGAC0\0002*, being the same genera for the group with normal weight, where the relative abundances of the genera *Prevotella* and *Bacteroides* were the ones that showed the highest relationship with elevated levels of lipopolysaccharide (>1 EU/mL). Alterations in the intestinal microbiota may be associated with the development of obesity, metabolic alterations, as well as modifications in intestinal permeability.

Metagenomic analysis of Actinobacteria phylum in patients with Irritable Bowel Syndrome

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Irritable Bowel Syndrome (IBS) is a chronic functional bowel disorder with a prevalence of 35.5% in Mexico. Recently it has been described that gut microbiota have a key role in the development of IBS, several studies shown an alteration in gut microbiota in patients with IBS, as well as in some of the metabolites such as short chain fatty acids which could be associated with the IBS symptoms. In a previous study at the Faculty of Medicine (UNAM) the composition of gut microbiota in patients with IBS was described, they observed an increase in Actinobacteria *phylum*, which had not been previously described. Therefore, it is particular interest to study the relationship between Actinobacteria phylum increase in IBS patients and the physiology of the disease. In the present study, abundance of species from Actinobacteria phylum in IBS patients was determined. The taxonomic assignment was performed whit Kraken software(v1.0), for the genomic alignments the RefSeq database (NCBI, September 2018) was used. The statistical analysis was carried out with the DeSeq2 software, in R language, where the families and genus of Actinobacteria phylum with an increase/decrease in abundance were determined ($p = 0.05$). The analysis of the metagenomes allowed to identify 34 genus of Actinobacteria phylum with differences in their abundance between IBS and control subjects (*Corynebacterium sp., Streptomyces sp., Xylanimonas sp., Rhodococcus sp., Sinomonas sp., Micrococcus sp., Rothia sp.*, etc). Whereas, at species level, 57 shown differences in abundance among the two study groups. Interestingly, we found 10 and 5 species of *Corynebacterium* and Streptomyces genus, respectively, in a low proportion in IBS subjects. Additionally, an analysis of metabolic pathways in the study samples was performed. The assembly was done in the SPADES software(v3.11.1), and the annotation with the Prokka (1.12) and KASS (v 1.12) software, which used the KEGG (Kyoto Encyclopedia of Genes and Genomes) database. The results obtained showed, in general, a lower abundance of genes associated with metabolic pathways in subjects with IBS compared to control subjects. Among these metabolic pathways are two-component systems, ABC transporters, purine metabolism, glycolysis, oxidative phosphorylation, pyruvate metabolism, fructose and mannose metabolism, etc. Two-component systems, transporters and signaling pathways could be involved in the symptoms of IBS. It is still necessary to perform a deeper analysis of this data. Together, these results show that there are significant differences at several taxonomic levels of Actinobacteria phylum in the metagenomes of IBS subjects which may be involved in their pathophysiology.

Fecal bacterial profile of the Merriam´s kangaroo rat, *Dipodomys merriami*, in the Chihuahuan Desert

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The range of the Merriam´s kangaroo rat, *Dipodomys merriami*, (Rodentia; Heteromyidae) includes the Chihuahua Desert; it occupies environments characterized by extreme temperatures and variable food availability. Kangaroo rats feed on seeds and parts of plants and so have important effects on the spatial and temporal heterogeneity of vegetation. The objective of study was to determine the taxonomic composition of the fecal bacterial microbiota of the Merriam´s kangaroo rat within the Mapimí Biosphere Reserve, using massive next-generation sequencing. Seven individuals were captured using Sherman live traps to obtain fresh fecal samples; after fecal samples were obtained all individuals were released. From each sample, DNA was extracted, V3-V4 regions of the 16S rRNA gene were amplified and sequenced in MiSeq Illumina; the results were analyzed with QIIME using EzBioCloud as a reference. From the seven samples, 1,131,536 high-quality sequences were obtained (mean = 161,648). The results comprise 21 phyla (Firmicutes 52.53%, Bacteroidetes 40.8%), 46 classes (Clostridia 47.7%, Bacteroidia 40.8%), 74 orders (Clostridiales 47.7%, Bacteroidales 40.8%), 127 families (S24-7_f Bacteroidales 35.1%, Ruminococacceae 23%) and 427 genera (unknown genus in Bacteroidales 9.8%, Ef602759_g 8.5%, *Eisenbergiella* 6.8% and *Ruminococcus* 5%). A total of 548 taxonomic species were classified, but only 2% were known species (*Alistipes inops, Bacillus subtilis, Bifidobacterium animalis, B. pseudolongum, B. thermacidophilum, Christensenella minuta, Kallotenue papyrolyticum, Lactobacillus delbrueckii, Propionibacterium acnes, Sanguibacteroides justesenii, Saphylococcus petrasii, S. gallolyticus* and *Treponema porcinum*). According to different authors, the phyla Firmicutes and Bacteroidetes are associated with healthy intestinal communities and high-energy efficiency in several vertebrate species. This information is the first study identifying the taxonomic composition of fecal bacteria of *D. merriami*; we expect that this information will be useful to design future management and conservation strategies for this species in this Reserve.

Fecal bacterial microbiota of the pallid bat, *Antrozous pallidus*, in the Chihuahuan Desert

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The range of the pallid bat (*Antrozous pallidus)* includes the Chihuahuan Desert; while primarily an insectivore, it occasionally feeds on vertebrate prey such as lizards, smaller bats and pocket mice. Our objective was to determine the taxonomic composition of the fecal bacterial microbiota of the pallid bats within the Mapimi Biosphere Reserve, using next-generation massive sequencing. Six fecal samples of *A. pallidus* were collected; DNA was extracted, V3-V4 regions of the 16S rRNA gene were amplified and sequenced in MiSeq Illumina; the results were analyzed with QIIME using EzBioCloud as a reference. From the six samples, 884,426 high-quality sequences were obtained (mean = 147,404). Our results comprised 19 phyla, (Firmicutes 46.2%, Proteobacteria 24.7%), 40 classes (Clostridia 28.2%, Synergistia 17.6%), 74 orders (Clostridiales 28.2%, Synergistetes 17.6%), 141 families (Synergistaceae 17.66%, Ruminococcaceae 15.8%) and 379 genera (*Tamella* 16.83% and *Enterococcus* 15.30%). We identified 448 species, of which just 5% had been previously named and described. Those included *Beijerinckia indica, Campylobacter jejuni, Cronobacter dublinensis, Desulfovibrio africanus, Enterobacter asburiae, Enterococcus faecalis, E. saccharolyticus, Erwinia iniecta, Klebsiella pneumoniae, K. quasipneumoniae, Lactobaccillus sakei, Lactococcus lactis, Listeria grayi, Morganella morganii, Photorhabdus temperata, Pseudomonas chlororaphis, Salmonella entérica, Serratia marcescens, Sporomusa rhizae, Staphylococcus hominis, S. succinus* and *Tamella caduceiae*. Our results were consistent with the findings of other authors: typically, the phylum Firmicutes and Proteobacteria are the most abundant in fecal samples of several species of bats and other vertebrates, although their abundance may vary substantially. This information is the first analysis of the taxonomic composition of fecal bacteria of *A. pallidus*; we expect that this information will be useful to design future management and conservation strategies for this species in this Reserve.

Oral sodium butyrate induces substantial intestinal changes in IL-17 / IFNγ and stabilizes tight junction proteins in an experimental model of cholestatis

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Introduction. Short chain fatty acids play a crucial role in intestinal homeostasis. Dysregulation of the intestinal epithelial barrier is part of the complications of cirrhosis and it is intimately related to bacterial translocation and death by bacterial infections. Butyrate regulates anti-inflammatory processes, oxidative stress pathways, cellular proliferation and differentiation and importantly intestinal barrier function. However, butyrate's effect in experimental liver fibrosis hasn't been assessed yet and is therefore unknown. **Aim.** Evaluate butyrate's effect in the intestinal inflammatory response as well as in tight junction proteins in experimental cholestasis. **Material and Methods.** Male wistar rats were ligated in the common bile duct (BDL) for 8 or 15 days. Butyrate was intraperitoneal (500 mg/kg) or orally (1%) administered, respectively. Control groups with BDL received saline solution or water, respectively. IL-17, IL-10 and IFNγ gene expression in ileum was analyzed by qRT-PCR. Tight junction proteins: occludin, ZO-1 and Claudin-1 were analyzed by western blotting from ileum homogenates. Liver TGFβ, IL-6 and collagen type I were examined by qRT-PCR. Histopathological analysis from liver and different ileum sections were also assessed. **Results.** Butyrate IP administration decreased significantly IL-17 while promoting IL-10 and IFNγ expression in comparison with the control group; however, we did not found any substantial changes in tight junction protein expression with the exception of claudin-1 ($p=0.03$). At the hepatic level, we did not find changes in profibrogenic genes nor IL-6. On the other hand, orally administered butyrate drastically decreased IL-17 ($p=0.17$) and IFNγ ($p=0.004$). IL-10 increased as well as occludin ($p=0.05$) and claudin-1 ($p=0.02$) regard the control group. TGFβ, IL-6 and collagen type I decreased but without statistical significance. **Conclusions.** Chronic oral administration of sodium butyrate is efficient in attenuating pro inflammatory cytokines that directly impact in the stability of the small bowel's tight junction proteins.

Evaluation of domestic canaries (*Serinus canaria*) gut microbiota. Potential zoonotic pathogens and antibiotic resistance patterns.

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Background: Gastrointestinal microbiota play a vital role in maintaining organismal health, through facilitating nutrient uptake, detoxification and interactions with the immune system, and others physiological systems. However, the microbiota of birds has been poorly studied in Mexico. Domestic canaries *Serinus canaria* are available at pet shops and through bird traders. Some studies have demonstrated zoonotic transmission of bacteria from birds to humans. In addition, the emergence of bacterial resistance to antibiotic use in veterinary practice is considered a source of multidrug-resistant bacterial infections for humans. **Aim**: This study was aimed at evaluating the gastrointestinal microbiota and the prevalence of potential multidrug-resistant in breeder canaries. **Methods and results**: We included 24 birds in the study. Samples were immediately placed in sterile vials, kept in a coolbox in the aviary ("La Güera", León, Guanajuato) and later stored at -20 °C. Each sample were processed, and serial dilutions were prepared with sterile saline solution. Then, 0.1 mL of dilutions were spread on nutrient agar and plates were incubated at 37 °C for 48 h. Gram stain of isolated was determined initially. Identification of the microorganisms was performed using the Microscan 4 walkaway system. Plates PC33 (cocci and gram-positive bacilli) and NC68 (cocci and gram-negative bacilli) were used. We isolated 96 bacterial strains, among which the dominant species were *Enterococcus faecalis*, *Staphylococcus sciuri*, *Enterobacter cloacae* and *Rhodococcus equi*. A total of 75 bacterial strains were identified and some of which are a potential threat to public health (e.g., *Pseudomonas aeruginosa*, *Yersinia pseudotuberculosis*, *Klebsiella sp., Vibrio* sp., *Acinetobacter* spp. and *Staphyloccocus aureus*. Our results also show diverse strains with highest antibiotic resistance, which were mainly species of *Staphylococcus*. **Conclusion**: The study showed that gastroinstestinal microbiota was characterized by numerous species of bacteria and *S. canaria* should be monitored in order to preserve human health.

Keywords: Domestic canaries, gut microbiota, multidrug resistance.

Prevalence of bacterial communities and potential pathogens in surface waters of the Rio Grande/Bravo in Reynosa Tamaulipas.

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Introduction. The Rio Bravo/Grande is a body of water that has both an economic and public health impact because it is used for vegetable irrigation and recreational activities, which have an impact on bacterial communities. Therefore, the objective of this work was to detect prevalence the bacterial communities and potentially pathogenic bacteria for humans in surface waters of the Rio Grande/Bravo in Northern Tamaulipas by sequencing the 16S rRNA. **Materials and methods**. 17 water samples were collected along the Rio Bravo/Grande in the Reynosa city. DNA extraction was performed using the ZymoBIOMICS® DNA Miniprep kit (Zymo Research, Irvine, CA) following the manufacturer's instructions Sequencing was directed to the 16s rRNA gene using the Quick-16S ™ NGS Library Prep kit. The platform used was Illumina® MiSeq ™. For the Bioinformatic analysis DADA, DADA2, LEfSe, Qiime v.1.9.1 and the Zymo Research database were carried out. **Results.** Reynosa city presented a low abundance of taxa compared to the rest of the sites. At the filum level, the most abundant were *Proteobacteria* 40%, *Firmicutes* 28%, *Cyanobacteria* 9%, *Bacteroidetes* 4%, *Verrucomicrobia* 10%, *Plantomycetes* 1.55% and *Actinobacteria* 10.23%. **Conclusion.** The contamination by anthropogenic activities impacts in the bacterial communities and these are more prevalent in Reynosa city, in which microorganisms such as *Bacteroidetes* are associated with fecal contamination and, within these filum human pathogens such as *Flavobacteria, Sphingobacteria* and *Cytophagia* are commonly found.

Gut microbiome in small ruminants

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The aim of this work was to describe the microbiome in the digestive tract of goats and sheep from different regions of the Mexico using massive sequencing, to identify microbial agents of interest in production. Oral and rectal swabs were taken from apparently healthy adult goats and sheep from the States of Mexico, Sinaloa, Oaxaca, Tlaxcala, Puebla and Chiapas. The DNA was obtained with a commercial kit and sequenced on the platform Miseq Nextera XT 2x150. The results obtained from the sequencing were filtered by quality, obtaining the fastq files that were used to perform the bioinformatic analyzes necessary to obtain sequences and subsequent bacterial taxonomic characterization by gender and in some cases species. From the processing of the samples, more than 7 million filtered readings were obtained by quality and more than 25,000 assembled sequences were obtained. As a result of the comparison of the sequences in Blast the presence of the following bacterial genera was demonstrated: *Escherichia, Pseudomonas, Salmonella, Campylobacter, Serratia, Klebsiella, Pseudomonas, Vibrio, Bordetella, Enterobius, Aeromonas* and *Pasteurella*. Being the most frequent *Escherichia, Pseudomonas* and *Salmonella*. All these genera presented more than 98% homology with sequences reported in the gene bank, after analyzing the sequences obtained using blastn. In most cases, especially Escherichia, Pseudomonas and Salmonella, assembled cotings of more than 1,000 bp were obtained. Additionally, the presence of bacterial sequences was corroborated by the detection of phage sequences of the same bacteria. Of all these bacterial genera there are reports of their presence in ruminants from different regions of the world, but not necessarily in Mexico. In addition, some other findings were found with fewer sequences, or shorter sequences (less than 1000 nt) and therefore these require further analysis. In this study, the presence of several bacterial genera with high abundance and with sufficiently representative sequences that allow concluding that they are infecting herd populations in regions that are important in the production of sheep and goats.

Identification of actinobacterial strains isolated from rhizosphere of experimental wheat varieties from CIMMYT by MALDI-TOF mass spectrometry and 16s gene sequencing

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Actinomycetes or actinobacteria perform important biological functions in processes such as degradation of organic cellulose and chitin compounds in soil, which, in turn, can be exploited by other organisms. In addition to its capacity for the production of secondary metabolites, greatly appreciated in the pharmaceutical industry. Because of their transcendent role in the decomposition of organic matter and their participation in the carbon cycle, along with their antibiotics production, actinobacteria are considered an important component of healthy soils and their populations are widely studied to assess the correlation between the community composition and the presence of soil pathogens and the incidence of soil borne diseases.

In this research we aim to characterize the morphological traits of the actinobacteria population isolated from the rhizosphere of new varieties of wheat donated by Centro Internacional de Mejoramiento de Maíz y Trigo (CIMMYT) collected from experimental fields in Cd. Obregón, Sonora, México [1], and, identify the isolated strains through MALDI-TOF mass spectrometry and sequencing of the 16s gene.

The identified strains will give an insight into the composition of the cultivable actinobacteria populations present in healthy wheat rhizosphere and serve as a reference point for comparison against the cultivable populations of actinobacteria populations present in states of disease or in the presence of soil borne pathogens.

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Flora bacteriana de heces fecales de una cría de manatí en rehabilitación

Bacterial flora in stool of a captive manatee calf

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Abstract

In this study 90 bacterial families were identified from the rectal samples of a captive manatee calf, at the Division of Biological Sciences UJAT. Eight samplings were made, with 15 days intervals. Samples were taken with and inoculated into Agar Salmonella and Shigella and Agar Eosine and Methylene Blue plates using the quadrant streaking technique. Once isolated and purified, samples were identified with the miniaturized system API strips 20 E and API strips 20 NE. Three families were identified; the most abundant was *Enterobacteriaceae*, followed by *Aeromonadaceae* and *Pseudomonadaceae*; distinguishing six different species. The most frequent species were *Escherichia coli*, *Aeromonas hydrophila* and *Proteus mirabilis*, considered as commensal bacteria living in the intestine of different marine mammals: dugongs, dolphins, whales. A low percentage of *Pseudomonas aeruginosa*, *Pseudomonas luteola* and *Morganella morganii* was identified, they, are considered as part of the intestinal microbiota in several mammals. Knowing the bacterial flora of a colony in captivity is important for health monitoring and manipulation, as well as to prevent potential risks of zoonosis.

Bioprospection of actinobacteria and study of bacterial diversity from Calakmul *aguadas* (wetlands-like) using comparative genomics and metaprofiling.

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The Calakmul Biosphere Reserve is an area with calcareous soils and one of the most extensive preserved rain forest in the world where no studies on the microbiota have been reported to date. The *aguadas* are accumulations of water from the Reserve that can be temporary or permanent, which are an interesting ecosystem to compare microbial populations in different situations; those that have remained flooded for a long time and those that behave in a cyclical way depending on the rainy and dry season, being even an opportunity to study the impact of climate change on microbial populations. Bioprospection of microorganisms such as Actinobacteria or the natural product chemistry associated with their secondary metabolites are the main goal of this project. We take samples of sediments of three cyclic *aguadas* (flooded and dry) and performed convetional isolation in different semi-selective media for Actinobateria, since they are known as prolific producers of natural products. We are interested in bioprospection to address mainly agricultural problems, to achieve this, antagonism assays were carried out against five phytopathogenic fungi using the isolated actinobacteria. Some of these actinobacteria were found to inhibit the growth of three phytopatogenic fungi. To explore the genetic determinants and the potential as antagonist of actinobacteria from the *aguadas* of Calakmul, as well as their genomic potential, the complete genomes were sequenced. Using genome mining and comparing with other previously reported actinobacteria genomes, we found biosynthetic gene clusters (BGC) for siderophores which have been associated with fungi inhibition as well as other new BGC that should be studied.
Metaprofiling has been widely used due to its convenience to perform taxonomic and phylogenetic classification in large and complex microorganisms samples sequencing a molecular marker. In this work we select 16s rRNA gene as marker and compare the sequencing results of samples in rain and dry seasons to unmasks the differential composition of bacterial communities associated in part to climate changes and also to reveal the bacterial genetic potential in undisturbed sites.

GENE REGULATION AND SIGNAL TRANSDUCTION

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Functional genetic study of the interaction of GacS and LadS kinases in *Azotobacter vinelandii*

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Azotobacter vinelandii is a soil bacterium that produces two polymers of industrial interest: poly-β-hydroxybutyrate and alginate. In *A. vinelandii* alginate production is under the control of the two-component system (TCS) GacS/GacA. This TCS in turn controls the Rsm post-transcriptional regulatory system thus establishing a cascade that regulates alginate biosynthesis by controlling the expression of the *algD* structural gene. GacA activates the transcription of eight sRNAs (RsmZ1-7 and RsmY) that counteract the repressive activity of the RsmA protein that binds to the *algD* mRNA, preventing its translation. Interestingly the activity of GacS/GacA system can be modulated by accessory regulators. In *Pseudomonas aeruginosa*, RetS and LadS kinases has a negative and positive influence, respectively, on the expression of the sRNA *rsmZ* dependent of GacA. In this bacterium RetS exerts its regulatory activity through its interaction with GacS, this interaction functionally blocks the kinase activity. On the other hand, LadS positively stimulates the activity of GacS. *A. vinelandii* has a homologue of the RetS and a putative homologue of the LadS protein. In previous work in our laboratory, a qualitative two hybrid assay was performed between RetS with GacS and LadS with GacS, observing that RetS interacts with GacS, without observing such interaction between LadS and GacS. Subsequently, *retS* and *ladS* mutants were constructed, in the *retS* mutant the alginate production decreased, while the *ladS* mutant increased the polymer production, suggesting that RetS functions as a positive regulator and LadS as a negative regulator, in contrast to what was reported in *P aeruginosa.*

In this work, a double mutant *gacS ladS* was generated. With respect to the *gacS* mutant in the double *gacS ladS* mutant a decrease in alginate synthesis was found. This result indicates GacS is dominant with respect to LadS in the regulatory system. The phenotype observed also suggests that LadS requires the presence of GacS to carry out its function, which would imply an interaction between both kinases. However, two hybrid qualitative assay indicated that no such interaction occurs, therefore, a two hybrid quantitative assay was performed. We determined the β-galactosidase activity of the reporter in the two hybrid system LexA. It was observed that there is no interaction between GacS and Lads since the measured activity is similar to that of the control. On the other hand, the interaction between GacS and RetS causes a decrease in β-galactosidase activity compared to the control. The results obtained confirming that this system does not detect interaction between GacS and LadS kinases.

Study of the regulation of polyhydroxybutyrate (PHB) depolymerization in *Azotobacter vinelandii*

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Polyhydroxyalkanoates (PHA) are polyesters produced by various archaea and bacteria as reserve of carbon, energy and reducing power. These polymers are intracellulary accumulated under conditions of carbon source excess and nitrogen, phosphorous or oxygen limitation and are mobilized (utilized) when the carbon source is scarce. *Azotobacter vinelandii* is one of the bacteria capable of producing PHA, it synthesizes mainly polyhydroxybutyrate (PHB) and the importance of these compounds in industry is that they can be used to manufacture biodegradable plastics to replace petroleum-based plastics. PHB synthesis in *A. vinelandii* starts from two molecules of acetyl Co-A, through three enzymatic steps catalyzed by β-ketothiolase, acetyl Co-A reductase and PHB synthase, encoded by the *phbA, phbB* and *phbC* genes, respectively; while PHB degradation (mobilization) is carried out by the enzymes PHB depolymerases, hydroxybutyrate dehydrogenase, succinyl Co-A transferase and, again, β-ketothiolase. Although these two processes occur simultaneously, giving rise to a constant synthesis/mobilization cycle, some mechanism is needed to control the balance of this cycle, thus favoring synthesis or degradation depending on the metabolic conditions. Some regulators of the PHB biosynthetic genes are known in *A. vinelandii* but nothing is known about the control of PHB degradation, it is possible that both processes would be controlled by the same regulation systems.

In this work, we show that PhbF is one of the regulators involved in biosynthesis process, that acts repressing the expression of *phbP1* gene that encodes a phasin, a granule-associated protein that promotes granule formation only in presence of PHB. In *A. vinelandii*, several genes encoding possible PHB depolymerases have been found, one of them, *phbZ1*, shares its regulatory region with *phbP2* gene (involved in biosynthesis) and a possible PhbF binding site is found in the regulatory region shared by these two genes. In this study, the participation of PhbF regulator in the control of mobilization process was demonstrated by the analysis of *phbZ1* gene expression in both UW136 and UW136*phbF* strains through qRT-PCR and *phbZ1-gusA* transcriptional fusions, in which an increase was observed, as well as the analysis of their PHB accumulation phenotypes in which was observed a decrease when *phbF* gene was inactivated.

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Expression of the Fla2 flagellin in *R. sphaeroides*: analysis of the control mechanisms

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The bacterial flagellar biogenesis is a tightly regulated process widely studied in Gamma-proteobacteria, such as *E. coli* and *S. enterica*. In these bacteria the expression of the flagellar genes is controlled by the master regulator, FlhDC.

In Alpha-proteobacteria the mechanisms that control flagellar gene expression are poorly understood. However, it is known that in *Caulobacter crescentus* the expression of the flagellar genes is controlled by CtrA which activates a four-tiered regulatory hierarchy. The last step of this cascade culminates with the expression of *flaA*, encoding the filament protein flagellin. The synthesis and assembly of FlaA in this structure is post-transcriptionally controlled by FlaF and FlbT proteins. Presumably, FlbT binds to the 5´UTR of *flaA (fljK*) inhibiting translation and negatively affecting the stability of this mRNA.

In *Rhodobacter sphaeroides* the expression of FlaA is also dependent on FlaF and FlbT, which localize upstream of the open reading frame RSWS8N_19879. We believe that these three genes together with *flaA* form an operon. Previously, we showed that deletion of either of these genes (*flaF-flbT-19879*), abolished FlaA synthesis and hence these strains are unable to swim.

To determine if the absence of FlaA was due to the lack of transcription, a plasmid carrying the transcriptional fusion *flaA-lacZ* was introduced into the parental cells (AM1), as well as into the *flaF*, *flbT*, and 19879 mutant strains. All these strains showed similar values of β-galactosidase activity, indicating that the products of these genes are required for the synthesis of FlaA but not for its expression.

The aim of this work is to elucidate the mechanisms involved in the expression of FlaA mediated by FlaF, FlbT and 19879. For this, a deletion analysis of the 5'-UTR region was carried out. Our results indicate that FlaA translation is still dependent on FlaF, FlbT and 19879 regardless if the 5'-UTR is present or not.

To clarify the functional relationships between FlaF, FlbT, and 19879, we overproduced and purified these proteins in order to carry out the analysis of the possible interactions, and these studies are underway.

In silico and functional analyses of GrlR, the LEE pathogenicity island repressor in enteropathogenic *Escherichia coli*

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The human pathogens Enteropathogenic *Escherichia coli* (EPEC) and Enterohemorrhagic *Escherichia coli* (EHEC), as well as the mouse pathogen *Citrobacter rodentium*, belong to the attaching and effacing (A/E) pathogens family. A/E lesion formation and the infection in general, depend on a type III secretion system (T3SS) encoded in the LEE (Locus of Enterocyte Effacement) pathogenicity island and a set of effector proteins. The T3SS acts as a molecular syringe that translocates effector proteins into the host cell. A complex network of global regulators controls LEE PAI gene expression in response to different environmental and metabolic cues; nevertheless, the specific control of their expression requires regulatory elements present only in A/E pathogens: Ler (LEE-encoded regulator) acts as the master regulator by counteracting the repression exerted by H-NS (Histone-like nucleoid structuring protein). GrlA (Global Regulator of LEE Activator) binds directly to the *LEE1* promoter activating *ler* expression, while GrlR acts as a repressor through its interaction with GrlA. The role of residues in the GrlR:GrlR homodimer and 2XGrlR:GrlA heterotrimer interaction interfaces has been studied; nevertheless, there is a lack of knowledge about the importance of conserved residues among GrlR and its orthologs in other bacterial species.

GrlR orthologous sequences were retrieved from the UniprotKB and NCBI databases using a 0.001 e-value threshold to perform a multiple sequence alignment using the Clustal Omega software and identify conserved regions among GrlR orthologs. Secondary structures and 3D models were predicted using the CFSSP server and the I-TASSER software to determine structural domains conservation. Site-directed mutagenesis of conserved residues was performed by overlapping PCR. The phenotype of GrlR mutants was assessed by complementing an EPEC Δ*grlR* mutant and comparing the protein secretion profiles of the complemented strains.

We identified 262 GrlR orthologs, all of which corresponded to Betaproteobacteria, except for one found in the Gammaproteobacteria *Chromobacterium haemolyticum*. Residues M3, K4, G6, Y8, F12, S14, G20, G22, G33, G34, D35, G42, L52, and V64, were highly conserved. The conserved structures predicted among GlrR-like proteins were beta strands containing the Y8, F12, S14, G20, L52, and V64 conserved residues. Other conserved residues, G6, Y8, G22, G33, G34, and D35, are located in the homodimerization interface, while residues F12, S14, and G20 are in the heterotrimerization domain. The complementation assay revealed that the G42A mutant was unable to restore the repressor function in the EPEC Δ*grlR* mutant. These preliminary results suggest that some highly conserved residues, but not all, are essential for the GrlR repressor function, even when, in some cases, they are not located in a protein-protein interaction interface such as G42.

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Differences in HBsAg detection and oxidative stress gene expression between wild type and C107R mutant of Hepatitis B Virus genotype H

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Introduction: Hepatitis B Virus (HBV) infects hepatocytes, during its replicative cycle, the pregenomic RNA (pgRNA) is the main intermediary.10 genotypes of HBV had been identified (A-J) and in Mexico, genotype H is the prevalent. HBV is mainly diagnosed by the small-surface antigen (HBsAg) detection in serum. HBsAg "a" determinant (amino acids 121-147) is the target epitope for anti-HBsAg antibodies (anti-HBs). *In silico* studies demonstrate the importance of Cys107 and Cys138 to form a loop that is recognized by anti-HBsAg antibodies used by the standard diagnostic tests. In patients with chronic hepatitis B (CHB), the levels of oxidative stress are increased by the accumulation of viral antigens in the cytoplasm of infected cells.

Methodology: Huh7 cells were transiently transfected with replicons for HBV genotype H —H2 (wild type) and H6 (C107R mutant)—, using Lipofectamine 2000 (Invitrogen). HBsAg was detected in the cell culture supernatant from transfected cells [Monolisa™ HBsAg ULTRA (Bio-Rad)]. Immunofluorescence and western blot (of cytoplasmatic proteins) were performed using anti-HBsAg antibody clone A10F1 (Biolegend) as primary antibody, this antibody recognize the large-HBsAg protein. RNA was extracted from transfected cells. cDNA was obtained using the M-MLV. Relative gene expression was performed using real time PCR for pgRNA, *CAT* and *TGF-β1*. *GAPDH* was used to normalize all gene expression.

Results: HBsAg was detected by ELISA in the cell supernatant from HBV wild type replicon but not from the C107R mutant. Nevertheless, HBsAg production was confirmed using the monoclonal antibody A10F1 by immunofluorescence and western blot in both wild type HBV-H and the C107R mutant. There were no differences between wildtype genotype H and the C107R mutant on the expression of pgRNA. *TGF-β1* gene expression was slightly increased with HBV gt-H wild type and the C107R mutant (1.37 and 1.22 fold respectively) compared with the empty vector. The expression of *CAT* was significantly reduced with both HBV replicons, wild type and C107R mutant compared with the empty vector (3.1 and 2.7 folds lower respectively).

Conclusions: C017R mutant is able to replicate and to produce the HBsAg, but the serological diagnosis by standard methods is affected. Both replicons are able to modify *CAT* and *TGF-β1* expression; these molecular changes could play an important role in the development of liver disease.

Effect of *pdeL* phosphodiesterase and the pair phosfodiesterase diguanylate cyclase *pdeI-dgcI* on the expression of some virulence genes and biofilm formation in Enteropathogenic *Escherichia coli*.

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Enteropathogenic *Escherichia coli* (EPEC) produces an injury called A/E lesion, by using a type three secretion system (T3SS) to deliver bacterial proteins into the eukaryotic cell, and intimate adhere, to the human intestine, causing severe diarrhea in children under two years of age. This lesion is determined by the pathogenicity island named LEE and the *bfp* fimbria. On the other hand, the second intracellular messenger cyclic di-GMP has been associated with physiopathological roles such as the expression of virulence genes and biofilm formation in some bacteria. Our goal is to evaluate whether the enzymes phosphodiesterases and diguanylate cyclases that are involved in degradation and synthesis of this second messenger, respectively, affect biofilm formation or virulence in EPEC.

By using a methodology that allows us to delete genes with PCR products, two mutants of the genes *pdeL* and *pdeI-dgcI* were constructed. We carried out assays of biofilm formation in different media to determine the conditions under which these deletions affect biofilm formation. Furthermore, in order to evaluate if there is an effect on some virulence genes encoded inside and outside of LEE, assays of secreted proteins, Western Blot and a reporter *cat* fusion were performed.

Secreted protein profiles as well as Western Blot assays indicated that the deleted genes do not affect secretion and expression of proteins that are part of the LEE. By using a bfpA-*cat* fusion, it was determined that the double deletion pdeI-dgcI affects the transcription of *bfp*, which plays an important role in the first stage of the A/E lesion. Furthermore biofilm formation was favored in low nutrient concentrations media and at 30 C. We concluded that deletion of *pdeL* and *pdeI-dgcI* has no effect on LEE genes but on *bfp*. Also biofilm production was affected in mutants, but the precise role played by cyclic di-GMP on these phenotypes remains to be clarified.

Roles of quorum-sensing and Rsm systems on virulence factors production by *Pseudomonas aeruginosa* ID4365

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Pseudomonas aeruginosa PAO1 possess three Quorum Sensing Systems (QSS) named Las, Rhl and PQS that controls transcriptionally the synthesis of virulence factors (VF) such as elastase, rhamnolipids and pyocyanin. While, the Rsm system is involved in VF control at a post-transcriptional level. In *P. aeruginosa* PAO1 strain, the Rsm system is formed by four non-coding small RNAs called RsmV/W/Y/Z which antagonises the repression exerted by RsmA/RsmE proteins. The ID4365 strain is an environmental isolated that overproduced pyocyanin about 37 fold compared with PAO1 strain; however the control of the VF production by the QSS and Rsm system is unknown. Here, we investigated the role of the Las, Rhl and Rsm systems on the synthesis of the VF. Mutants strains in the QSS and Rsm were generated using homologous recombination technology; elastase, pyocyanin and rhamnolipids were measured as previously were described. *rsaL* transcriptional fusion was constructed by cloning *rsaL* promoter into the plasmid mini-ctx-*lux* and transferred into *P. aeruginosa*. An analysis of the *lasR* gene showed that a punctual mutation changed Glu^{24} by a stop codon, therefore ID4365 strain is a natural mutant in the Las system. Thus, we expressed LasR *in trans* and VF were measured, the results showed an increase in the VF compared with the wild-type strain; additionally LasR was able to activate an *rsaL'lux* transcriptional fusion, a canonical target for the Las system. Inactivation of *rhlR* abolished the synthesis of VF implying that in ID4365 strain these VF are controlled by the Rhl system. On the other side, overexpression of *rsmA*, but not *rsmE*, reduced pyocyanin production and overexpression of *rsmY* had a positive effect. Inactivation by deletion of *rsmA* showed an increase of pyocyanin production but a reduction in the synthesis of rhamnolipids and elastase. Furthermore, RhlR protein levels are diminished in the *rsmA* mutant strain. In conclusion, we showed that the Rhl system is responsible for elastase, rhamnolipids and pyocyanin production in *P. aeruginosa* ID4365 and that the Rsm system regulates negatively pyocyanin production but positively rhamnolipids and elasase synthesis.

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Inter- and intra-molecular interactions of the *Salmonella* Typhimurium regulator InvF.

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Salmonella is a pathogen that causes food-borne gastroenteritis in humans worldwide, and is also an important pathogen of food-producing animals including cattle, pigs and chickens. To invade host cells *Salmonella* encode several genes clustered in chromosomal regions called *Salmonella* pathogenicity islands (SPIs). One of these is SPI-1, a 40-kb region which includes 39 genes encoding a type III secretion system (T3SS-1), their chaperones and effector proteins, as well as some transcriptional regulators. The expression of the SPI-1 genes is controled by a regulatory cascade of several transcriptional regulators wich include HilD, HilC and RtsA. These regulators act in a complex feed-forward loop to activate each other and HilA. This regulatory cascade ends with InvF. InvF is an AraC/XylS-like transcriptional regulator that plays a central role in *Salmonella* virulence by controling the expression of genes within and outside SPI-1 that are required for *Salmonella* invasion of the host cells. As a member of the AraC family of transcriptional regulators it is possible that InvF forms multimers and associates with other proteins. Several studies have shown that the chaperone SicA is necessary for InvF activity, and some studies demonstrated protein-protein interactions, but the region in InvF where this interaction happens is not known, also it is not known whether InvF interacts with other proteins. The objetive of this study was to determine if InvF was able to form homodimers with itself and heterodimers with SicA, as well as to demostrate the region of InvF involved in such interaction with SicA. To do the latter a LexA-based two hybrid system for monitoring and analyzing protein homodimerization and heterodimerization was used, in addition to the use of purified versions of both proteins. On the other hand, we wanted to elucidate the rol of SicA in InvF stability in the absence and presence of the chaperone by using several protease mutant strains to determine whether SicA prevents or decreses InvF degradation. Results corroborated that SicA forms homodimers while they also showed that InvF is not able to form them. The stability assays shown that SicA decreses InvF degradation in *Salmonella*. Our results corroborated previous results and will allow us to define which InvF amino acid residues are involved in protein-protein interactions.

Effect of H-NS, Lrp and ppGpp on the P8 and P9 promoters of the *Salmonella* Typhi *leuO* gene

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Salmonella infections are one of the most frequent causes of foodborne illness. To establish a successful infection, *Salmonella* uses a large number of genes that encode a wide variety of virulence factors, some of which reside within the pathogenic islands, while others are located elsewhere in the genome, such as *leuO*.

LeuO belongs to the family of LysR type transcriptional regulators and is involved in virulence and bacterial response to stress, and can function both as an activator or repressor. It controls the expression of an eclectic set of genes in both *Salmonella enterica* and *Escherichia coli*, and is quiescent. It forms part of the H-NS regulon, a histone-like protein. In *S.* Typhimurium and *E. coli,* (p)ppGpp, an alarmone that responds to stress conditions, is an activator of *leuO* gene expression. It is not known whether this effect is direct on the *leuO* promoter, or is indirect through the activation of the synthesis of the Lrp regulator, a leucine response protein that in turn would activate the *ilvIH* promoter (*pilvlH*). Thus, it has been postulated that the expression of the *leuO* gene depends on (p)ppGpp during the stationary phase, due to nutrient limitation.

In this project, the effect of HNS, Lrp and (p)ppGpp on two recently found promoters within the *leuO* regulatory region, P8 and P9, two of the ten promoters reported by our working group, was evaluated *Salmonella* Typhi IMSS1. The activity of transcriptional fusions of two regulatory fragments, pRS9 (P9) and pRS8 (P8), was measured in several genetic backgrounds, wt, *hns*, *lrp*, and *hns lrp*, both in MN rich medium and in M9 minimal medium. Also, activity was determined in *relA*, and *relA spoT* strains to evaluate the effect of (p)ppGpp; in addition to simulating the strict response by synthesis of (p)ppGpp by induction of RelA' protein expression.

In conclusion, the transcriptional fusions pRS8 (P8) and pRS9 (P9) of the 5' regulatory region of *leuO*, show promoter activity in the wild type strain. The H-NS and Lrp regulatory proteins did not affect P8, and Lrp modestly regulated P9 in a negative manner. Also, (p)ppGpp positively regulates P9 and negatively P8 and these promoters do not respond to a pulse of (p) ppGpp.

The possibility of a different regulation of P8 and P9 in an individual context, in contrast to when all ten promoters are in the chromosomal arrangement will be discussed.

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Analysis of a putative operon in *A. brasilense* Sp245 involved in growth and motility.

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Azospirillum brasilense has been characterized as a growth promoting rhizobacterium (PGPB) that is capable to promote beneficial effects on plants and confer protection to phytopathogenic microorganisms through several mechanisms. However, to realize this objective it is essential that the bacterium establish a bacteria-plant association colonizing the host plant roots. Motility and chemotaxis are both the first factors playing an initial and critical role in colonization. These mechanisms confer to *Azospirillim* the ability to sense and respond to a gradient of chemical compounds and navigate to a most favorable environment, which is warranted through the signal transduction machinery that controls the regulation of motor organelle. The ability of bacterial cells to control motility during early steps in biofilm formation is also critical for the transition to a non-motile, biofilm lifestyle. Recent studies have clearly demonstrated the ability of c-di-GMP to control motility via a number of mechanisms, including transcription of motility-related genes and modulating motor function.

Herein, we described a putative operon containing five genes, the translation products predict that the genes encode for a diguanylate cyclase (CdgE), two histidine kinases (HkhB and HkhC), CheY-like response regulator (CheY-like), and LuxO-like transcriptional factor (LuxO-like). We constructed a polar mutant on *cdgE::gusA-Km*^{R} gene, and three deletion mutants *ΔdgcE*, *ΔhkhB* and *ΔluxO-like*. Phenotyping analysis indicated that *A. brasilense* 105A and *A. brasilense* 57 shown a significant delay of growth in a minimal medium supplemented with malate as carbon source, as compared to the wild type strain. In addition, motility assays were determined in minimal media supplemented with 10 mM malate, succinate, proline and lactate during 48 h of growth all mutant strains showed an important decrease in chemotactic rings, as compared with the wild type strain *A. brasilense* Sp245. Preliminary result of RTPCR indicates that genes *cdgE* and *hkhB* form a transcriptional unit. The data obtained suggest that the putative operon is implicated in motility and growth, and may be associated in a complex signaling pathway.

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Study of the activity of select promoters by the transcriptional regulator TyrR of *Azospirillum brasilense* Sp7

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Many species of beneficial soil bacteria, including Plant Growth Promoting Rhizobacteria, form microcolonies or biofilms when they colonize roots. During the colonization process of *Azospirillum brasilense* to the plant host, several genes have been shown contributed with different functions, including chemotaxis, aerotaxis, formation of biofilms, and the enzymes participating in metabolic pathways. The ability to detected and response to environmental signals is essential to survive in soil. Here we studied the transcriptional regulator named TyrR, which is involved in biofilm formation, exopolysaccharide (EPS) production, and D-alanine catabolism in *A. brasilense* Sp7 (Jijón Moreno *et. al* 2019). TyrR belongs to the superfamily of Enhancer Binding Proteins (EBP) exhibiting the characteristic domains found in these proteins. The TyrR protein binds to a consensus sequence (TyrR box) located in the promoter region of *tyrR* and *dadA* genes, this last gene encoding for D-amino acid dehydrogenase which is involved in catabolism of the D-alanine as a carbon source. The objective of this study was to understand how TyrR is involved in the transcriptional regulation of genes encompassing consensus TyrR box sequences; related genes encoded in chemotaxis, the EPS production, and formation of biofilms. A bioinformatic analysis of the *A. brasilense* Sp7 genome sequence, indicates that TyrR might regulate the expression of genes involved in signal transduction such as chemotaxis, histidine kinases, and Methyl accepting Chemotaxis Proteins (MCP). The functionality of the promoters was realized using the transcriptional fusion of presumptive promoters with the expression of the *gfp* and *gusA* for fluorescence and enzymatic activity of the β-glucoronidase assay, respectively, and tested in the *A. brasilense* Sp7 (wild-type) and is isogenic mutant *A. brasilense tyrR::Gm*^{R}, strains. This study will be providing novel information about the mechanism that regulate the colonization process of *A. brasilense* Sp7 to their host plants.

Reference

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Study of the motility of bacteria of the genus *Vibrio* under different salinity conditions

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The experiments were carried out with three strains of the genus *Vibrio* (*V. shilonii* AK-1, *V. harveyi* BB120 and *V. alginolyticus* VIO5). Motility tests were performed in a known medium TBSW, which is composed of 1% tryptone, 35 mM $MgSO_4$, 7 mM $CaCl_2$, 7 mM KCl. To determine the swim phenotype of each one of the bacteria, three 2 mL cultures were prepared in TBSW medium, which were incubated at 30 ° C in orbital shaking overnight. These cultures were used to inoculate 3 µl in solid TBSW medium with 0.25% soft agar in different concentrations of NaCl (120 mM, 175 mM, 200 mM, 300 mM, 400 mM, 500 mM and 600 mM). They were subsequently incubated in the dark conditions, for 8 hours and at a temperature of 30 ° C. In this experiment the diameters were measured by the "ImageJ" program and it was observed that there is phenotypic variation of the swim. Successively growth curves were performed, in TBSW medium. The growth was analyzed from the 600 nm wavelength at 1 hour intervals. In this experiment it was possible to observe the genetic, physiological and morphological behavior of the microorganisms studied in this work. Finally, swimming velocity values were obtained through a video analysis of 30 cells using the “CellTrack” program, for each growth condition (120 mM, 300 mM and 600 mM NaCl) at a length of 600 nm wave at a point of optical density 0.6, for each strain (*V. shilonii* AK-1, *V. harveyi* BB120 and *V. alginolyticus* VIO5). To obtain the calculation of swimming velocities, cells that showed a displacement greater than 11 µm / s were considered. The values presented show the average swimming speed ± standard deviation of each bacterial population. In this experiment, the significance was assessed by analyzing one-way variances (ANOVA) followed by a 95% confidence Tuckey test = α: 0.05. This analysis demonstrates the existence of significant differences between the salt conditions of these populations. Multiple sequence alignment of the PomA and PomB proteins was performed with the MUSCLE program, with this alignment it is shown that the bacteria studied are similar in their amino acids, this does not indicate that they are identical. Finally, it is concluded that the motility of the bacteria of the genus *Vibrio* differs genotypically, phenotypically and morphologically under different concentrations of NaCl. Multiple alignment was a tool that identifies the evolutionary patterns that these bacteria suffered in order to survive in different hosts.

Expression and function of *cdgD* gene encoding a hybrid DGC-EAL protein from *Azospirillum brasilense.*

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Azospirillum brasilense is a nitrogen fixing bacterium, which has been employed as inoculant in world-wide for its beneficial effect on host plants owed to multiples mechanisms, that is proposed are responsible of a better nutritional status of plants such as corn, wheat, sorghum, rice among others. These mechanisms include direct effects on plant metabolism, by providing nutrients (iron, phosphorus and N, for example) and synthesizing molecules with phytohormone activity (auxins, cytokinins, gibberellins, nitric oxide), or indirect effects such as an antagonism against phytopathogens and increased resistance to pathogens. To this end, the bacterium has to contain an array of genes participating in controlled of a successful colonization to plant, such as quimiotaxis and biofilm formation, which are the initial steps for the beneficial effects observed in plants of agronomic interest.
In *A. brasilense* has been described occurs several genes encoding for proteins involved in biosynthesis and degradation of the second messenger cyclic-di-GMP, which controls key functions such biofilm formation and motility in bacteria and actively could participate in colonization to host-tissues. In this study we analyzed the expression and function of the *cdgD* gene. The *cdgD* gene was expressed both in cell growing under planktonic and biofilm conditions as determined by the qRT-PCR assay. In addition, it was observed a differential expression when the nitrogen source (potassium nitrate or ammonium chloride) was added to minimal media. An insertional mutant in *cdgD*::Km^R gene was constructed, the results obtained shown a decrease in biofilm production as compare with the wild-type (WT) strain. Furthermore, when the mutant was complemented with the plasmid pVK*cdcD* the formation of biofilm was restored and motility was significantly higher as compared to the WT, when the amino acid proline was added to medium as N source. Then, the data indicated that *cdgD* encoded for a bifunctional diguanylate and phosphodiesterase protein that has a role in biofilm formation and motility. A transcriptional fusion using the promoter fused to a gene encoding for an autofluorescent protein (*pcdgD-mCherry*), and subsequently used to analyzed the gene expression both under abiotic conditions as well as in association with wheat roots, under hydroponic and soil conditions. Interesting, the gene is expressed under both conditions as analyzed by Confocal Laser Scanning Microscopy.
We suggested that, the CdgD hybrid protein may be able to respond to environmental signal cues recovered from the plant root exudates, and direct the bacterium towards the plant. *Azospirillum*-plant association begins with the adsorption and adherence process of this bacterium into root plant. Then, it will be significantly to study the genes which might contribute to this initial interaction of *A. brasilense* with its host-plants.

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CtrA regulation mediated by ClpXP in *Rhodobacter sphaeroides*.

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CtrA is a response regulator conserved in alpha-proteobacteria. CtrA regulates many cellular processes including motility. CtrA is activated by phosphorylation mediated by the hybrid histidine kinase, CckA, and the phosphotransfer protein ChpT. In *C. crescentus* it has been reported that degradation of CtrA is also controlled by CckA. The proteolysis mechanism involves the ClpXP proteasome and the adaptors proteins CdpR (phosphorylated by CckA), RcdA and PopA. These proteins are conserved in some alpha proteobacteria but not in *R. sphaeroides*.

R. sphaeroides has two different flagella: Fla1 commonly present in the wild type strain (WS8N), and Fla2 that is only expressed when *fla1* is not expressed and CckA has a gain of function mutation. We have found that CtrA is not detected in the wild-type strain even that its expression depends on a constitutive promoter. To determine if CtrA stability could also be controlled in *R. sphaeroides* in spite of the absence of CpdR, RcdA and PopA, we tested a version of CtrA lacking the last three residues which are similar to the signal recognized by ClpXP. We observed an improvement in the swimming capacity of the strains expressing CtrAΔ3 that correlates with an increased in the amount of protein detected by western blot. The role of the ClpXP proteasome was evaluated by determining CtrA half-time life in Δ*clpX*. We found that in the absence of ClpX, the half-time life of CtrA increased. However, this strain did not express the flagellar genes and the stady-state amount of CtrA is reduced as compared with the parental strain. These results suggest that ClpXP could also regulate a repressor of *ctrA*, besides of controlling CtrA degradation.

Identification of a gene encoding for a phosphodiesterase and likely diguanylate cyclase hybrid protein in *Azospirillum brasilense* Sp245.

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The role of bacterial surface components in combination with bacterial functional signals in the process of biofilm formation has been increasingly studied in recent years. Plants support a diverse array of bacteria on or in their roots, stems, and leaves. These plant-associated bacteria have important effects on plant health and productivity. Biofilm formation on plants is associated with mutualist responses, but how plants regulate such associations is unclear. Certain bacteria in biofilm matrices have been found to induce plant growth and to protect plants from phytopathogens. *Azospirillum brasilense* is a Gram-negative, free-living bacterium that associates with plant roots, excretes plant hormones and fixes nitrogen in the rhizosphere, which has a role in plant growth-promotion. The cyclic dimeric GMP (c-di-GMP) is one of the bacterial intracellular second messengers that regulate biofilm formation in many bacteria. C-di-GMP is generated by cyclization of two GTP molecules by enzymes called diguanylate cyclases (DGC). DGC enzymes contain the enzymatic active site amino acid motif GGDEF. The c-di-GMP is degraded by enzymes called phosphodiesterases (PDE). In addition, there are enzymes functioning as DGC and PDE named hybrid proteins. Here, we identified a gene encoding a hybrid protein. By using bioinformatics tools was determined that the protein contains a transmembrane region, two CACHE, and two PAS sensory domains, as well as both GGDEF and EAL highly conserved domains, suggesting that hybrid protein could be functional as DGC and PDE as well. Then, we purified the protein and the enzymatic activity for PDE activity was demonstrated. In spite that GGDEF domain was highly conserved the DGC activity was not detected. To be certain that DGC function is absent a mutant by insertion with a kanamycin cassette was inserted in CCC97222.1 gene and the complemented mutant were constructed. Further phenotyping studies will confirm if are present both activities, and how such functions are controlled.

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A new alternative against urinary tract infection: design and generation of uroplakin-silenced bladder cells line

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Introduction. Urinary tract infections (UTIs) associated to Uropathogenic *Escherichia coli* (UPEC) are a health problem due to their high morbidity rate, and an increase in multidrug-resistant as well as the lack of an effective vaccine. UPEC strains are the main etiologic agent of the UTIs, harboring type 1 fimbriae, the most prevalent virulence factor, which contribute to the adherence and invasion in the uroepithelium. The FimH adhesin is localized on the distal part of these fimbriae and is recognized by glycomannosylated proteins via uroplakin (UPK) on the uroepithelium. **Hypothesis**. The silencing of UPK may be a potential alternative for reduces the UTIs by UPEC. **Objective.** Design a small hairpin (sh)RNA against UPK1a that impact in their expression in human bladder cells line. **Material and methods.** shRNA against UPK1a (shUPK1a) was manually designed and analyzed with the software RNAstructure. Scramble shRNA-UPK1a (S-shUPK1a) was generated with scramble generation tool (GenScript) and used as transduction control. shRNAs-UPK1a were designed and cloned into pLVX-shRNA2 vector for perform a viral particles packing with the lentiviral system. Recovered viral particles were transduced to human bladder cell line HTB5 and the efficiency of transduction was measured by percent of fluorescent cells. shRNA-associated knock down cells lines were evaluated through of ELISA and Cytometry flux assays. **Results**. The designed shUPK1a and S-shUPK1a included three specific palindromic regions: *BamH*I (5´) or *EcoR*I (3´), seed, and *UPK*1a (21 nucleotides) sequences, which are linker with a loop of 10 nucleotides and a poly A terminator in 3´. The shRNAs showed a stable structure of stem and loop (≥99%) with a free energy of -51.9 for shUPK1a and -51.3 for S-shUPK1a. Forward and complementary reverse of single-strand shRNAs were chemically synthetized and aligned with a temperature among 95-25°C, and alignment buffer (pH 7.5, 10 mM Tris, 1mM EDTA, and 50 mM NaCl). Double strand shRNAs were cloned into pLVX-shRNA2 and packed into lentiviral particles. Transduction assays with these particles in HTB5 cells showed a GFP expression after 48 h post-infection. Silencing of UPK reduction of ~47% ($p<0.0001$) was observed with the ELISA and ~80% using flux cytometry assays; in both case when compared with the negative controls. **Conclusions**. The shUPK1a was able to significant silencing to UPK in the HTB5 cells.

Co-expressed gene modules share similar function and regulation

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The biological systems respond to environmental perturbations through many compounds that interact among them, making up complex networks. In particular, gene co-expression networks have been widely constructed in the last years thanks to the increasing of experimental information, such as microarrays and RNA-sequence data.
These networks allow the identification of groups of co-expressed genes that can function in the same process, which may be related to biological functions of industrial, medical and academic interest.
Many of these genes of interest are related to the transcription process, which is regulate by transcription factors, which are proteins with DNA-binding domain. Other genes of interest are related to metabolism, that is modulated by means of proteins called enzymes, within these we can define a compendium of enzymes that can intervene in different metabolic maps which we call promiscuous.
Due to the relevance of these two types of genes it is important to see how they are distributed in different genomes, even more so in different functional modules.
In this study, gene co-expression networks for 17 organisms from database COLOMBUS using (WGCNA) was performed, which were clustered into modules of genes with similar expression patterns in each species.
These modules were analyzed to determine relevant modules through a hypergeometric analysis using a set of TFs and a set of enzymes for each genome. The richest modules were characterized using Pfam families and KEGG metabolic maps.
Additionally, we performed an enrichment of biological functions with Gene Ontology. We identified that the most abundant families are related to systems of two components and type TetR_N, while the most abundant metabolic maps are related to central metabolism, although the samples come from different experiments both in number and variability. This study provides insight into how regulation and metabolic maps similar are expressed between organisms different.

Functional gene association networks analysis suggests that the ORF VCA0231 from *Vibrio cholerae* codes for a common iron uptake regulator in proteobacteria.

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Iron and riboflavin are the two main micronutrients required as cofactors for a myriad of enzymatic redox reactions in all life kingdoms. Notably, an important metabolic interrelation between these two molecules has been described in several organism. In bacteria, iron and riboflavin coordinate to reciprocally regulate the transcription of their provision pathways. Nonetheless, no mechanistic study of this effect has been performed in any species. Recently, we determined the transcriptomic responses to riboflavin in the pandemic pathogen *Vibrio cholerae*. Genes downregulated by riboflavin included several iron uptake-related genes as well as the ORF VCA0231, encoding a member of the AraC family of transcriptional regulators with unknown function. Here, we performed a genomics analysis of gene associations in order to determine putative physiological functions for the product of VCA0231. Initial assessment of association networks within the *V. cholerae* genome using the STRING database predicted ten functional partners. Seven of such putative partners are proteins involved in siderophore uptake of other forms of ferric iron uptake. Further analysis with the Enzyme Function Initiative Tools identified 248 homologs distributed in alpha, beta and gamma proteobacteria. Sequence similarity network analysis clustered these homologs in four groups. Next, Genome Neighborhood analysis were done to identify Pfam families statistically associated within the first three genes encoded upstream and downstream of the homologs in the bacterial genomes. Four Pfam families associated to cluster 1, seven to cluster 2, eleven to cluster 3 and six to cluster 4 were detected. The TonB-dependent receptor plug domain Pfam family, involved in siderophore uptake, was associated with all clusters. Other Pfam terms also involved in iron uptake were specifically associated to each cluster. Together, results suggest that VCA0231 encodes a member of a family of regulators of transcription of genes involved in iron uptake in proteobacteria. Given that this gene is under the regulation of riboflavin, it comprises a candidate to mediate the iron-riboflavin regulatory coordination described in *V. cholerae*.

Role of the histidine kinase LadS involved in alginate production in *Azotobacter vinelandii*

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Azotobacter vinelandii is a free-living and aerobic bacterium that inhabits in alkaline soils. The biotechnological concern of the bacteria is its ability to generate alginate in aerobic environments. The alginate is a heteropolymer used as a thickening and viscosifier agent. In *A. vinelandii*, the pathway for alginate synthesis is controlling by the activity of GDP-mannose-dehydrogenase encoded by the *alg*D gene. The gene *alg*D is regulated by a post-transcriptional regulation system constituted by sRNAs that belong to RsmZ, RsmY or RsmX families, and a repressor protein called RsmA. After the transcription of *alg*D, the presence of RsmA protein obstructs the translation of *alg*D, promotes the degradation of the mRNA and causes a negative impact in alginate production. In fact, the sRNAs appearance antagonizes the effects of RsmA by encouraging the RsmA capture and promoting the translation of the mRNA generating a positive impact in alginate production. The presence of eight sRNAs from the RsmZ family, and one sRNAs from the RsmY family has been confirmed in *A. vinelandii,* and its expression is controlled by the two-component system GacS/GacA. GacS is a histidine kinase that media the phosphorylation of GacA. Once GacA is phosphorylated, it activates the transcription of the sRNAs genes.

In *Pseudomonas aeruginosa*, a phylogenetically close bacterium of *A. vinelandii,* has been reported that another histidine kinase called LadS stimulates the GacS phosphorylation. A homologue of LadS was found in *A. vinelandii*, but contrary to expected, the mutation of *lad*S enhances the alginate production. This evidence suggests that LadS acts as a negative regulator in the sRNAs expression. The purpose of this research was to study how LadS in *A. vinelandii* participates in the expression of rsm-sRNAs that in turn controls alginate production. The *lad*S mutation was transferred to previously constructed recombinant strains that possess *gus*A transcriptional fusions for the *rsm*Z1, *rsm*Z2 and *rsm*Y genes (belonging to the rsm-sRNAs family) and a strain that has a chromosomal *alg*D-*gus*A translational fusion. The transcriptional fusions expression was determined to measure the activity of beta-glucuronidase encoded by the *gus*A reporter gene. Preliminary results show a relation between the expression of the *rsm*Y and LadS in contrast to the observed to *rsm*Z2. It appears that LadS have regulation in alginate production by the expression of *rsm*Y in *A. vinelandii*.

The LEE-encoded regulator GrlA, promotes the expression of the type III secretion effector gene *nleH1* in enteropathogenic *Escherichia coli*

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Enteropathogenic *Escherichia coli* is a human pathogen that causes childhood diarrhea in developing countries. Like enterohemorrhagic *E. coli* and *Citrobacter rodentium,* it causes a particular phenotype in the host cell called the attaching-and-effacing lesion (A/E), which is characterized for the intestine microvilli resorption and the bacteria intimate adherence at the colonization site where it induces the formation of an actin rich pedestal like structure. The genes required for these phenotype are encoded within the pathogenicity island LEE (locus of enterocyte effacement), which consists of 41 ORFs encoding a type III secretion system (T3SS), effectors, chaperones and regulatory proteins. In addition to the LEE genes, the EPEC genome contains, mainly within prophages, several additional genes encoding effectors generically known as Nle (non-LEE encoded). EPEC virulence genes are under the control of a regulatory cascade involving the LEE-encoded regulators Ler, GrlA and GrlR. GrlA directly binds to the *ler* regulatory region to activate its expression, while Ler antagonizes the negative regulation exerted by H-NS on LEE genes.

Little is known about the regulation of the *nle* genes. We have previously reported that a subset of these genes, coding for different non-LEE-encoded effectors such as NleH1, NleH2, NleG, and NleB2, share a conserved regulatory upstream sequence containing a 13-bp inverted repeat named NRIR (*nle* regulatory inverted repeat) that is required for their optimal transcriptional activation. Here, we determined that this subset of genes, also share the promoter features of the *ler* gene, including the putative GrlA binding sequence motif ATGT, located within the spacer promoter sequence. Taking the *nleH1* regulatory region as a study model, here we aimed to study the role of GrlA in the regulation of NRIR containing genes, by testing the activity of *nleH1-cat* transcriptional fusions in a *grlA* mutant derivative of EPEC E2348/69, as well as by complementation experiments with a plasmid expressing GrlA in a heterologous genetic background such as *E. coli* K-12 strain MC4100. The expression levels directed by the *nleH1* promoter (*nleH1p)* were reduced in the Δ*grlA* mutant in comparison with the wild type strain. Consistently, overexpression of GrlA in the K-12 background increased the activity of *nleH1p* even in the absence of the NRIR element; however, expression levels were significantly higher when both the NRIR and the ATGT motifs are intact. Overall, these results suggest that the NRIR synergizes with GrlA to activate this subset of *nle* genes. These results are a step forward to elucidate the complexity of EPEC virulence gene regulation, in particular on the connection between the LEE pathogenicity island and genes encoding virulence factors outside the LEE.

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Mutational analysis of genes encoding a putative non-ribosomal peptide synthetase involved in the synthesis of phaseolotoxin in *Pseudomonas syringae* pv. phaseolicola NPS3121

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Pseudomonas syringae pv. phaseolicola is the causal agent of halo blight disease in bean (*Phaseolus vulgaris* L.). The disease causes losses in the crop yield of this legume. *P. syringae* pv. phaseolicola produces a non-host-specific toxin known as phaseolotoxin, which causes a chlorotic halo. A chromosomal fragment called “Pht cluster” contains genes is involved in the synthesis of the phaseolotoxin and the expression of these genes was shown to be thermoregulated. On the other hand, the *PSPPH_4550* gene, coded outside the Pht cluster, also participates in the phaseolotoxin synthesis. This gene has been identified as part of a non-ribosomal peptide synthetase (NRPS). It has been proposed that the organic moiety of phaseolotoxin is synthetized by NRSP. Likewise, it has been described that the transcription of these genes occurs mostly at 18°C, the temperature at which phaseolotoxin is produced optimally. The present study was focused in the mutational analysis of *PSPPH_4550* neighboring genes and their participation in the phaseolotoxin synthesis.

Replacing the wild-type genes with an interrupted allele in *P. syringae* pv. phaseolicola chromosome, mutants were constructed by double recombination events. To this, PCR-derived amplicons containing selected open reading frames from the chromosomal fragment *PSPPH_4540* to *PSPPH_4559* were cloned into pCR4-TOPO vector. These genes were interrupted with an antibiotic resistance gene. The resulting plasmids were introduced by electroporation into *P. syringae* pv. phaseolicola, and antibiotic resistance was used to select double recombination events. The fidelity of the double recombination was confirmed by PCR. Phaseolotoxin production by wild-type strain and mutants were assayed by the *E. coli* JM103 growth inhibition assays.

Null mutants were obtained and were grown under conditions allowing phaseolotoxin production. Several mutants were unable to produce phaseolotoxin as shown by the growth inhibition assay, indicating that these genes are involved in their production.

In conclusion, our results suggest that *PSPPH_4550* neighboring genes belong to a chromosomal fragment involved the synthesis of the organic moiety of phaseolotoxin, in a similar way to Pht cluster.

Study of the gen Avin_15460 (*gacS2*) involved in the synthesis of alginates in *A. vinelandii*.

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Azotobacter vinelandii is a gram negative γ-proteobacterium that produces copious amounts of the exopolysaccharides known as alginates. In *A. vinelandii* the alginates biosynthesis is controlled by the two component regulatory system (TCS) GacS/A. The TCS are generally formed by a histidine kinase (HK) and its effector protein called response regulator. In *A. vinelandii* GacS/A specifically regulates the expression of the *algD* gene, this gene encoding a GDP-dehydrogenase a crucial enzyme in the alginates synthesis. However, GacS/A does not directly control *algD* expression; this is done through the control that GacS/A exerts on the Rsm regulatory system. Rsm is a post-transcriptional regulatory system that consists of a protein called RsmA and nine small regulatory RNAs (sRNAs). RsmA is a repressor protein that binds to *algD* mRNA and blocks its translation. In turn, Rsm-sRNAs counteracts the repressor activity of RsmA allowing messenger expression. In *Pseudomonas aeruginosa* besides GacS other HKs controls the expression of the sRNAs belonging to the family Rsm. Among these HKs are RetS, LadS, PA1611, PA1976, and PA2824. In *A. vinelandii* we found homologues for RetS, ladS and PA2824 (SagS). SagS has been related with the control of the sRNA rsmY through an alternative route of transcriptional regulation, which is independent of the GacS/A system.

In order to study the role of the SagS homologue (Avin_15460, which we call *gacS2*) in alginates biosynthesis, we generated a *gacS2* mutant and measured the polymer production. To rule out a polar effect of the mutation, the mutants were complemented. To establish whether *gacS2* controls the expression of the sRNAs belonging to Rsm family (*rsmZ1*, *rsmZ2* and *rsmY*), *gacS2* was mutated in strains containing transcriptional fusions of the *rsmZ1*, *rsmZ2* and *rsmY* sRNAs. The expression of the sRNAs was quantified through the measurement of beta glucuronidase activity in wild-type and mutant strains.

Characterization of GSU1771 regulator involved in electron transfer and energy generation in *Geobacter sulfurreducens*

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Geobacter sulfurreducens is bacteria capable to couple the anaerobic respiration to the reduction of metals. In addition, this bacterium can generate bioelectricity from the oxidation of organic compounds, transferring the resulting electrons to electrodes. In *G. sulfurreducens*, the extracellular transfer of electrons is directed by more than 100 *c*-type cytochromes and a conductive structure type Pili. Recently it was reported that a strain that accumulated several mutations, reduces more efficiently insoluble Fe(III) oxides. Among the mutations that this strain present is the insertion of an IS element into the coding region of the *gsu1771* gene. The *gsu1771* gene codes for a protein member of the transcriptional regulators SARP-type described in *Streptomyces* species. In this work, the participation of the GSU1771 regulator in the expression of the important genes in the extracellular transfer of electrons, energy generation and biofilm production was evaluated. The mutant strain Δgsu1771 exhibits a growth delay in NBAF basal medium. However, it can reduce Fe(III) soluble to Fe(II) faster than the wild-type strain (WT). Interestingly, mutant strain Δ*gsu1771* produces more *c*-type cytochromes than the WT strain, as was observed through the fractional extraction of internal membrane, periplasm and outer membrane proteins, and heme-staining. We also determined that the strain Δ*gsu1771* produces more PilA protein (structural protein of the Pili) than the WT strain as was observed by immunodetection. By RT-qPCR in mutant strain Δ*gsu1771*, we observed that *omcB*, *omcC*, *pilA*, *omcE*, *omcS*, *omcZ*, *dcuB* and *frdCAB* genes were upregulated. DNA-protein binding assay reveals that GSU1771 protein binds to promoter regions of *pilA*, *omcE*, *omcS*, *omcZ*, *dcuB* and *frdCAB* genes. By Cyclic voltammetry (an electrochemistry assay to evaluate the electroactive properties of biofilms) and by fluorescence microscopy, we determinate that Δ*gsu1771* strain produced a biofilm which electric charge transfers more efficiently and is thicker than biofilm of the WT strain. These results point out that GSU1771 represses the transcription of the *pilA* gene and some c-type cytochrome involved in metals reduction and bioelectricity production, positioning this strain as potential to be used in bioremediation and bioelectricity generation processes.

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Study of pyocyanin synthesis by the reiterated operons *phzA1-G1* and *phzA2-G2* in *Pseudomonas aeruginosa* ID4365.

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Pseudomonas aeruginosa is a Gram negative bacterium which is an opportunistic pathogen to humans. These bacteria are able to produce a broad range of virulence factors such as rhamnolipids, elastase and pyocyanin. Pyocyanin is a redox-active compound that in eukaryotic cells is able to increase the production of reactive oxygen species increasing oxidative stress and thus causing damage in the host cells. In the reference strain PAO1 (a clinical isolated), pyocyanin is produced by two reiterated operons named *phzA1B1C1D1E1F1G1 (phz1)* and *phzA2B2C2D2E2F2G2 (phz2)*. The strain ID4365 is an environmental isolated that overproduce pyocyanin about 37 fold more than PAO1 and this strain contains the two reiterated operons as well; however, contribution of each operon for pyocyanin synthesis remains unknown. In this work we determine the role of *phz1* and *phz2* for pyocyanin production. To achieve this, we constructed two plasmids: *pEXTcZ1R* (to replace *phz1* operon) and *pEXSmZ2R* (to replace *phz2* operon) which were used to transform ID4365 strain. Then, candidates were verified by PCR in order to determine the absence of the operon and the substitution by the resistance cassette, tetracycline for *phz1* and streptomycin for *phz2*.

Mutant strains and the wild type strain were evaluated in its capacity to produce pyocyanin in two medium, LB and PPGAS (a low phosphates medium) by 24 h and 37 °C. Results showed that in LB medium both operons contribute at the same level for pyocyanin production since its inactivation of each diminished 70% compared with wild type strain. On the other side, in the low phosphate medium PPGAS, inactivation of *phz2* reduce 60% compared with wild type strain; however, inactivation of *phz1* almost abolished the pyocyanin production compared with wild type strain indicating that in this medium the role of *phz1* is more important than *phz2*. In conclusion, the contribution of each operon for pyocyanin synthesis is dependent of the culture conditions.

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Molecular characterization of SehB, a type II antitoxin of *Salmonella enterica* serotype Typhimurium

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Salmonella enterica serotype Typhimurium is a bacterium that causes gastroenteritis and localized diarrhea in humans. The genome of *S.* Typhimurium codes for diverse virulence factors, among which are the toxin-antitoxin (TA) systems. One of the most recently studied is the TA system type II SehAB, where SehA is the toxin and SehB is the antitoxin. It was previously reported that the absence of the SehB antitoxin affected the growth of *S.* Typhimurium during growth in LB medium. In addition, the SehB antitoxin can interact directly with the SehA toxin neutralizing its toxic effect as well as repressing its own expression. We analyzed the effect of eight single-amino acid mutants in both N- and C-terminus of SehB antitoxin on its transcription repressor function, as well as its ability to form homodimers and to interact with the SehA toxin. *S.* Typhimurium Δ*sehB* was transformed with different constructions (pMPM-K6, pK6-SehBWT, pK6-SehBY32A, pK6-SehBL42A, pK6-SehBL52A, pK6-SehBI60A, pK6-SehBS107A, pK6-SehBL121A, pK6-SehBL129A and pK6-SehBF138A), and the expression of *sehA* toxin gene was determined by RT-qPCR. Compared to the wild-type protein (pK6-SehBWT), all changes in amino acid residues at both the N- and C-terminus affected the repressor function of SehB antitoxin. Moreover, electrophoretic mobility shift assays showed that these amino acids residues were required for the DNA-binding activity. By using LexA-based two-hybrid system, mutations in the amino acid residues at N-terminus (Y32, L42, L52 and I60) showed a lower capacity for homodimer formation of the SehB protein. In agreement with our results, the eight amino acid residues of SehB antitoxin are required for the repressor activity: Y32, L42, L52, I60 are required for its homodimerization, while S107, L121, L129 and F138 are only involved in the DNA-binding activity.

Transcriptional analysis of putative genes involved in the synthesis of c-di-GMP under biofilm conditions in *Geobacter sulfurreducens*

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In *G. sulfurreducens,* metal reduction and bioelectricity production require the participation of several elements, including multiple *c*-type cytochromes, electrically conductive pili and the formation of electroactive biofilms. The hallmark of the electroactive biofilms is electronic heterogeneity, mediated by coordinated interactions between the conductive pili and cytochromes associated with the matrix, constituted by the xap exopolysaccharide. Experimental evidence demonstrates that *G. sulfurreducens* mutant strains that do not produce any of these factors have a negative phenotype in biofilm formation and electricity production. In different bacterial species, the second messenger c-di-GMP is involved in controls the biofilm production, however, the role of this signaling molecule in *Geobacter* genus has not yet been investigated. *G. sulfurreducens* genome has 29 genes that code for possible diguanylate cyclases (DGC), enzymes involved in the c-di-GMP synthesis. In this work we study the role of the genes encoding proteins with predicted DGC activity under biofilm condition in *G. sulfurreducens*. First, to determine which DGC genes are expressed under conditions of biofilm against planktonic cells, we performed an expression analysis for the 29 genes by RT-qPCR. RT-qPCR shows that 12 DGC genes are upregulated under biofilm conditions versus planktonic cells, of which *gsu1037*, *gsu2016*, *gsu3376*, *gsu0895*, *gsu1870* and *gsu1937* present the highest expression levels (3-22 more times). It is predicted the all proteins encoded by these genes show a domain GGDEF involved in c-di-GMP synthesis and domains implicated in phosphate groups reception, detection of light, redox state, small molecules, quorum sensing signals, sugars, metal ions and c-di-GMP hydrolysis (EAL domains). These results suggest that these genes are involved in the biofilm production, probably through the synthesis of c-di-GMP in response to environmental and intracellular signals. In order to determine the possible diguanylate cyclase activity of proteins that encode these genes, they will be cloned and expressed in *E. coli* analyzing the production of curly fiber. These results will be present at the congress.

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Biochemical characterization of *Salmonella enterica* transcriptional regulator InvF

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Encoded in the *Salmonella* pathogenicity island I (SPI-1) is one of the two type III secretion systems (T3SS) required for this bacterium virulence. SPI-1 and other genes distributed or not in other SPIs are necessary for the initial invasion, escape from the *Salmonella* containing vacuole (SCV) and spread to other cells. SPI-1 transcriptional regulation is tighly regulated by multiple transcriptional regulators organized as a cascade. The last regulator in this system is InvF, an AraC/XylS like transcriptional regulator that activates genes coding for T3SS-1 effectors and secretion components. InvF has been shown to interact with the chaperon SicA and both seem to transcriptionally activate their cognate genes. The aim of this study was to determine the role of InvF as a classical activator, find possible interactions with the RNA polymerase (RNAP) and identify amino acid residues relevant for this regulator function. Our results showed that InvF acts as a classical regulator as the expression of InvF-dependent genes did not occur in an *invF* and *hns* double mutant. It also corroborated that SicA has no role in DNA binding. As for other AraC/XylS like transcriptional regulators, the 3' region is necessary for the expression of transcriptional fusions. Moreover, by using a purified version of InvF we were able to identify interactions with subunits of the RNAP. Furthermore, the interaction of InvF with the RNAP alpha subunit was corroborated by using negative dominants and proten-protein interactions. In summary, InvF is shown to be a classical transcriptional regulator able to interact with the RNAP.

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CreR, an EIL domain-containing protein, positively regulates the expression of the *ecp* fimbrial operon in *Citrobacter rodentium*

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Citrobacter rodentium is a bacterium that causes colitis and transmissible murine crypt hyperplasia, which shares 67% of its genes with enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC), both causal agents of human diarrheal diseases around the world. These pathogens produce the A/E (attaching and effacing) lesion on the surface of enterocytes, which is mediated by the gene products encoded within the locus of enterocyte effacement (LEE). Surface structures called fimbriae or pili, often mediate adherence of these bacteria to host epithelial cells. The *E. coli* common pilus (ECP), present in commensal and pathogenic *E. coli* has been shown to play a role in pathogenic *E. coli* interactions with environmental reservoirs and host epithelial cells.

As for *E. coli*, the *C. rodentium ecp* operon is a cluster of five genes (*ecpABCDE*) encoding proteins involved in the assembly of the fimbria, which expression is favored in static DMEM cultures at 26°C. Using an ecp-cat transcriptional fusion, mutant strains, and 3XFLAG-tagged derivates, we found that, in contrast to *E. coli*, CreR, a novel protein with a conserved cyclic-di-GMP phosphodiesterase domain, is essential and specific for *ecp* activation. The *creR* gene is located downstream of the ecp operon and co-transcribed as part of it; however, its expression is also directed by a putative *creR* promotor, which responds to the same growth conditions than *ecp*. Single and double mutants carrying the *ecp-cat* fusion and Electrophoretic Mobility Shift Assays showed that the global regulators IHF and H-NS control *ecp* expression positively and negatively, respectively. CreR and IHF were still needed even in the absence of H-NS.

Moreover, a regulatory motif, named Distal Regulatory Element (DRE), is essential for CreR-mediated activation and perhaps the binding site of a positive regulatory protein responding to c-di-GMP levels. Using a synthetic chromogenic substrate, we found that CreR is a functional PDE; however, point mutations in conserved amino acids for PDE activity have only a partial or null effect in the CreR-mediated activation of *ecp* expression. We hypothesized that CreR has an additional novel regulatory function, which is essential to activate *ecp* expression in *C. rodentium*.

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A member of ANR family modulates the expression of genes regulated by PerA in enteropathogenic *Escherichia coli*

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Enteropathogenic *E. coli* (EPEC) is one of the main causes of diarrhea in children in developing countries. EPEC infections are characterized by two distinctive phenotypes: localized adherence (LA) and formation of attaching-and-effacing lesions (A/E). The genetic components required for these phenotypes are encoded mainly within the EAF plasmid (EPEC adherence factor) and the pathogenicity island LEE (locus of enterocyte effacement). The regulation of EPEC virulence genes are under the effect of diverse regulators, one of which is PerA, a protein belonging to the AraC/XylS family. PerA activates its own expression and that of the *bfp* operon involved in the formation of the bundle-forming pilus (BFP) that mediates the LA phenotype; PerA also promotes PerC expression, which in turn stimulates LEE activation through the LEE-encoded regulator Ler.

In 2014, Santiago *et al*., reported the existence of a new family of anti-virulence regulators which they called ANR (AraC-family Negative Regulator) that suppress the effects of AraC/XylS members in Gram-negative. Aar (AggR-activated regulator), a prototype member of this ANR family in enteroaggregative *E. coli* interacts with AggR, a AraC type regulator that activates several virulence genes expression; this interaction prevents AggR from binding to DNA, avoiding its function as an activator.

In this study we evaluated the effect of the *orfRS14140* (a homologue of *aar*) on the expression of virulence genes regulated by PerA in EPEC. For this aim, we examined in EPEC wild type the effect *orfRS14140* overexpression on *perA*, *bfpA*, *perC* and the genes *espA* and *escJ* encoded in LEE by qRT-PCR. Our results suggest that *orfRS14140* represses expression of *perA*, *bfpA*, *perC*, *espA* and *escJ.* At the same time, we analyze overexpression effect of this putative ANR on secreted proteins profile by SDS-PAGE and on the BfpA and EspA expression in total extracts by western blotting. *orfRS14140* represses the secretion of proteins of LEE and also the expression of BfpA and EspA whereas GroEL, a protein used as a control was not affected. Also we evaluated the expression of the *orfRS14140* under the induction and repression conditions of the virulence genes of EPEC by End-point RT-PCR. *orfRS14140* is expressed in all evaluated conditions.

The results of this work suggest that *orfRS14140* modulates negatively the expression of virulence factors regulated by PerA. The existence of this anti-virulence protein in EPEC indicates the importance of this ANR family in control of virulence attenuation in order to favor the survival of the pathogen.

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Effect of the two-component system CpxRA on the expression of the pathogenicity island 2 of *Salmonella enterica* serovar Typhimurium

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Introduction. *Salmonella enterica* serovar Typhimurium (*S*. Typhimurium) is a Gram-negative bacterium that causes gastroenteritis and localized diarrhea in humans. The main virulence factors of *S*. Typhimurium are encoded on two pathogenicity islands called SPI-1 and SPI-2. Both SPI-1 and SPI-2 code for Type 3 Secretion Systems (T3SS), which are necessary for the invasion of intestinal epithelial cells and for its survival and replication within macrophages, respectively. CpxRA proteins form a two-component system that responds to stress and at the same time is related to proteins that are integrated and secreted through the inner membrane. It has been shown that CpxR negatively controls the expression of both SPI-1 and SPI-2 genes when *S*. Typhimurium is grown in rich medium (LB), mainly affecting the positive auto-regulation of HilD. Unpublished data in our group show that CpxR also represses the expression of SPI-2 in N-minimal medium, a condition where HilD is not required to activate SPI-2, suggesting a direct mechanism of CpxR on SPI-2. The objective of this work is to evaluate the effect of the CpxRA system on the expression of SPI-2 of *S*. Typhimurium during its growth in N-minimal medium. **Methodology.** *S.* Typhimurium WT, Δ*cpxR*, Δ*cpxA* and Δ*cpxRA* strains will be grown in N-minimal medium and the expression of SPI-2 genes (*ssrA, ssrB, ssaB, sseA, ssaG, pipB, sseA, sseJ* and *sifA*) will be quantified. Intracellular replication assays will be performed infecting macrohages with *S.* Typhimurium WT and *cpx* mutants. Finally, the interaction between CpxR and the regulatory region of *ssrAB* will be analyzed by EMSA. **Results.** The design of qPCR primers for *ssrA, ssrB*, *ssaB, sseA, ssaG, pipB, sseA, sseJ* and *sifA* genes was performed by using the Primer 3 Plus program. The relative expression of 8 genes (*ssrA, ssrB, ssaG, ssaB, pipB, sseA, sseJ* and *sifA*) was determined in the WT strain and in the *ΔcpxA*, where it revealed a significant decrease in the expression of the 8 genes of the strain *ΔcpxA* compared to the WT strain: *ssrA* (7-fold)*, ssrB* (11-fold)*, ssaG* (8-fold)*, ssaB* (13-fold)*, pipB* (11-fold)*, sseA* (14-fold)*, sseJ* (50-fold), *sifA* (13-fold). Also, the replication rate of the WT and *ΔcpxA* strains was determined in human macrophages, in which the *ΔcpxA* mutant showed a replication rate 5 times lower with respect to the WT strain.
Preliminary conclusions. Relative expression results indicate that the absence of CpxA represses the genes of SPI-2 in minimal medium as it happens in LB medium, but this effect was HilD-independent. The absence of CpxA affects the replication of *S*. Typhimurium inside macrophages.

The GacS/A pathway regulates positively the motility and flagella synthesis in *A. vinelandii ATCC*

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Abstract

Azotobacter vinelandii is a motile bacterium that possesses an unusual pattern of peritrichous flagellation into the *Pseudomonadaceae* members. Although FleQ is the master regulator of motility in *Pseudomonas spp*, in *A. vinelandii* FlhDC is the motility master regulator and is under σ^E and CydR negative control. In some members of the families *Enterobacteriaceae* and *Pseudomonadaceae* the GacS/A-Rsm pathway is another important factor in motility regulation. This work reports the GacS/A-Rsm pathway study in the *A. vinelandii* motility control. Through motility assays in soft agar plates the participation of the GacS/A regulators were proved; however, a mutant in the sRNA *rsmZ1* cannot prove the participation of the Rsm system in the motility control, then an overexpression strategy was used and the constitutive expression of *rsmZ1* restores the motility in the ATCC-*gacA* mutant. As expected, the *fliC* expression and flagellin production is detected in this strain and there was not mRNA for *fhlDC* expression detected.

Conclusions. In *A. vinelandii* our data show that GacA positively controls motility, contrary to reported in *P. aeruginosa*, *P. fluorescens, P. protegens*, *E. coli*, *S. thyphimurium* and *L. pneumophila* in which GacA regulates negatively. To date, *P. syringae* is the sole bacterium in which it has been reported that GacS/A positively regulates swimming motility, but flagella synthesis is not related to the motility phenotype. Thus, this work is the first report of positive motility regulation through the control of flagella synthesis. In opposite to reported in *E. coli* and *Yersinia enterocolitica*, in *A. vinelandii* the master regulator FlhDC is not controlled by Gac-Rsm pathway; in agreement with this, we did not find RsmA binding boxes in the regulatory region of *flhDC* genes, but interestingly we found RsmA binding boxes in *fliA* and *flgM* operator sequences.

Regulation of the synthesis of poly-β-hydroxybutyrate by the response regulator GacA and EIIANtr in *Azotobacter vinelandii.*

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Azotobacter vinelandii, is a gram-negative bacterium that synthesize polymers of industrial interest as poly-β-hydroxybutyrate (PHB). The process through which PHB is produced, in *A. vinelandii* involves three enzymes encoded in the *phbBAC* operon which is activated by the transcriptional activator PhbR and the sigma factor RpoS. PhbR expression is also activated by RpoS. The *phbR* and *phbBAC* genes are also regulated by the two-component system GacS/A, that acts through two pathways known as Gac-Rsm and Gac-PTS^{NTR}. In the Gac-Rsm pathway GacA activates transcription of sRNA`s that bind the RsmA protein, a repressor of translation. This prevents binding of RsmA to the *phbBAC* and *phbR* transcripts allowing its translation. In the Gac- PTS^{NTR} system, GacA is necessary for the phosphorylation of the EIIA^{NTR} since in its unphosphorylated form promotes degradation of RpoS by the ClpAP chaperon protease complex resulting in a significant decrease in the transcription of *phbR* and *phbBAC*. In the absence of GacA, both negative regulators RsmA and EIIA^{NTR} will be active impairing the synthesis of PHB. Therefore, inactivation of *rsmA* and *ptsN* was expected to restore PHB synthesis in a *gacA* mutant (OP*gacA-rsmA-ptsN* strain). However, and unexpectedly, the triple OP*gacA-rsmA-ptsN* strain was unable to produce PHB. Additionally we found that in a *gacA* or a *gacA-rsmA* mutants, inactivation of *ptsN* (EIIA^{Ntr}) (E.G a *gacA-ptsN* or *gacA-rsmA-ptsN* strains) did not restored RpoS to wild type levels, whereas inactivation of *ptsN* did restore PHB synthesis in *ptsP* or *ptsNH68A* mutants (e.g. *ptsP-ptsN* or *ptsNH68A-ptsN* strains), in which EIIA^{Ntr} is present in an unphosphorylated state but are *gacA*+.These results indicate that the GacA control on RpoS by the Gac-PTS pathway is more complex than expected.

The PhoP protein of *Mycobacterium tuberculosis* activates gene expression of the MTP pilus under different growing conditions

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Mycobacterium tuberculosis is the etiological agent of human tuberculosis, the most prevalent infectious disease in the world, and is estimated that at least one-quarter of the global population have latent tuberculosis infection. The *M. tuberculosis* pilus (MTP) is encoded by the Rv3312A gene (*mtp*). MTP has been involved in formation of biofilms, and in the adhesion and invasion of lung epithelial cells in order to colonize the host tissues. Previous transcriptomic studies in *M. tuberculosis* have shown that in the absence of *phoP* gene, the MTP pilus gene expression decrease considerably, suggesting that PhoP protein activates the expression of *mtp* the gene. However, there are no studies of their regulation and gene expression in different environmental conditions. The aim of this work was to analyze the effect of the PhoP protein on the gene expression of MTP under different environmental conditions. Cultures of *M. tuberculosis* H37Rv wild-type, Δ*phoP* and complemented strains were carried out in aerobiosis and *in vitro* dormancy (hypoxia). Total RNA was extracted and purified from cultures in exponential, NRP-1 and NRP-2, and the *mtp* gene expression was quantified by RT-qPCR. The *mtp* gene expression in the Δ*phoP* strain decreased 48.3, 20.7 and 8.3 fold in the exponential, NRP-1 and NRP-2 phases, respectively, when it was compared to wild-type and complemented strains. These results support the notion that PhoP protein activates the gene expression of the *M. tuberculosis* MTP pilus during both, aerobic and hypoxic conditions.

CRISPR-Cas transcriptional regulation in *Salmonella enterica* serovar Typhi

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The CRISPR-Cas system is a mechanism in bacteria and archaea capable of providing adaptive and hereditable immunity against phages and plasmids. This system has also been implicated in biofilm formation, fruiting body development, DNA repair and virulence. Significant progress has been made regarding the functionality of this cluster in different microorganisms. However, few studies about the genetic elements and environmental signals that promote its expression are available. Hence, in this work we analyzed CRISPR-Cas transcriptional organization and regulation in the human pathogen *Salmonella enterica* serovar Typhi (*S.* Typhi). The results showed that this locus contains five transcriptional units: two of them, the *cse1-cse2-cas7-cas5-cas6e-cas1-cas2*-CRISPR (*cas*-CRISPR operon) and s*cse2* (sense *cse2* RNA) are transcribed from the sense strand, whereas as*cse2-1* (antisense RNA of *cse2* to *cse1*) and as*cas2-1* (antisense RNA of *cas2* to *cas1*) are present on the antisense strand. Additionally, the *S*. Typhi *cas3* gene is transcribed as an independent unit divergent to the *cas*-CRISPR operon. The transcriptional activity of the *cse1-cse2-cas7-cas5-cas6e-cas1-cas2*-CRISPR polycistronic mRNA is induced by LeuO whereas the nucleic associated proteins H-NS and Lrp participate in its negative regulation. In the case of *cas3* and as*cse2-1* units, the role of H-NS in silencing their expression was also demonstrated. Even more, the five transcriptional units identified are expressed in N-minimal medium and up-regulated at pH 7.5. Therefore, multiple global regulators and specific conditions modulate the CRISPR-Cas transcription in *S.* Typhi.

Genetic elements involved in pH resistance in *Salmonella enterica* serovar Typhi

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Salmonella enterica serovar Typhi is the ethiological agent of typhoid fever. *S*. Typhi survive stress conditions such as oxygen depletion, low osmolarity, acid and alkaline pH during its infection cycle. Genetic determinant involved in acid and basic pH resistance are considered pathogenic factors, since allows *S*. Typhi survive in stomach, macrophages and in the human intestine and gallbladder respectively. Thus, survival depends on the presence of adaptive mechanisms that sense an acid or alkaline environment and coordinate an appropriate molecular response.

To identify *Salmonella enterica* serovar Typhi genetic elements involved in pH resistance, we evaluated the growth of 40 strains with specific genomic deletions in LB medium (Luria-Bertani), at acidic (pH 4.5, 5, 5.5, 6 6.5) and alkaline pH values (6.5, 7, 7.5 and 7.8).

The results shows different *S*. Typhi bacterial strains unable to growth efficiently in pH 4.5, 5.5, 6.5, and 7.5. The genes responsible for these phenotypes correspond to: thiamine transport system, NADH-dehydrogenase, putative transporter genes and major outer membrane lipoproteins respectively. These results suggest that transport systems are essential mechanisms in the adaptive response to acidic and basic environments. Therefore, we can concluded that *S*. Typhi utilized different genetic strategies and cellular mechanisms to specifically survive in each pH value during its infection cycle.

Study of *retS* mutants in *Azotobacter vinelandii* strain AEIV

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Azotobacter vinelandii is a Gram-negative bacterium, Proteobacteria from the gamma subdivision, belonging to the *Pseudomonaceae* family. It has industrial relevance for its ability to synthesize biopolymers like alginate and PHB. The alginate is a polysaccharide composed of two monomers, the mannuronic acid and guluronic acid. In *A. vinelandii* the alginate production is regulated by the Gac/Rsm pathway, that is integrated by the Gac two-component system, which includes the histidine kinase GacS and the response regulator GacA; while the Rsm system is composed by a mRNA binding protein called RsmA and small non coding RNAs (sRNAs) RsmZ1-RmsZ7 and RsmY.

In *Pseudomonas aeruginosa* the phosphorylation and activation of the Gac system is regulated by two hybrid histidine kinases, RetS and LadS. RetS having a repressor activity while LadS being a positive regulator. In *A. vinelandii* by a bioinformatic search a homologous to RetS was found, but although there is a significative identity of 57% between the *A. vinelandii* and *P. aeruginosa* protein sequences, mutants in this kinase decrease the alginate synthesis, meaning that RetS could be a positive regulator contrary of how it regulates in *P. aeruginosa*.
In a previous study two mutants were generated, using a cassette of antibiotic resistance as interposon, the orientation of the insertion of the interposon generates a polar and non-polar mutants. While the non-polar mutant presents a significant decrease in the alginate production; the polar mutant presents a partial phenotype.
In order to explain this previous result, we search the genome context of *retS* and we find a gene downstream of the *retS* loci, an Hsp70 homologue, that could have some effect in the polar mutation.

To assess whether the differences observed in alginate production are due to the polar effect, two strategies were developed to complement the mutants; a *cis*- and a *trans*-complementation. The structural gene *retS* with its regulatory region was cloned in an integrative vector utilized in *A. vinelandii* to produce a *cis*- complementation, while the pBRR1MCS-5 was used to clone the same region in order to obtain a *trans*-complementation strain. We are currently performing the complementation experiments.

We expect that by returning the structural gene to *retS* mutants the phenotype will be restored. Whilst in case of not recovering it could be considered if *retS* is part of an operon along with that hsp70 homologue.

The type III secretion system of *Pseudomonas aeruginosa* PAO1 is regulated positively by the Rhl *quorum sensing* system.

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Pseudomonas aeruginosa is an environmental and opportunistic Gram-negative pathogen capable of causing a variety of infections in plants and humans. Their virulence properties are multifactorial and comprise adherence factors, biofilm formation, antibiotic resistance, and exotoxin secretion by the type 3 secretion system (T3SS) which regulation is finetuned controlled by the quorum sensing (QS) response at the transcriptional level. *P. aeruginosa* possesses three QS systems named Las, Rhl, and PQS which are arranged in a complex hierarchal regulatory cascade. In the Rhl system, RhlR is a transcriptional regulator that binds to N-butyryl-homoserine-lactone (C4-HSL), synthesized by RhlI, to activate the transcription of several genes involved in different cellular process.

It has been reported that the Rhl system negatively regulates the T3SS expression since the transcription and secretion of ExoS, an effector of the T3SS, increase when *rhlI* gene is inactivated. Moreover, the addition of exogenous C4-HSL restored ExoS expression to the wild-type levels. However, a recent study demonstrated that in PAO1 strain with *lasR* and *rhlR* inactivated is still able to secrete exotoxins. Furthermore, experiments carried out in our group showed that the inactivation of *rhlR* decreases the protein levels of ExoS and ExoT compared with the wild-type suggesting a positive role of the Rhl system on the expression of the T3SS.

In order to determine the regulatory effect in T3SS by the Rhl system, we constructed transcriptional fusions with the *lux* reporter genes and the promoter region of the *exsCEBA, exsA, exoS, exoT* and *spcS* genes from the PAO1 strain. These plasmids were used to electroporate PAO1 WT, *rhlR* mutant and *rhlR* mutant strain complemented with the *rhlR* gene expressed from pGMYC, a multicopy plasmid. Bacteria were cultivated in induction medium. Expression of *lux*-based reporters and bacterial growth were evaluated using Synergy HT Plate Reader. The analysis was realized by triplicate at OD_{600}=4.0. Results showed that the *exsCEBA* promoter activity was notably decreased in the *rhlR* mutant strain and this expression was restored to the wild-type levels when the mutant is complemented with pGMYC. Similar results were obtained for *exoS*, *exoT*, and *spcS* transcriptional fusions; however, the *exsA* transcriptional fusion activity was not affected by the *rhlR* inactivation. These results indicate that the expression of the T3SS is controlled positively by the Rhl *quorum sensing* system.

Regulation of the *E2348C_1013* gene by GrlA in enteropathogenic *Escherichia coli*

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Enteropathogenic *Escherichia coli* (EPEC) is a human diarrheal pathogen that belongs to the A/E (Attaching and Effacing) family of bacterial pathogens, which hallmark is the intimate adhesion to enterocytes, the elimination of microvilli, and the formation of an actin-rich pedestal-like structure on the surface of enterocytes. The genes involved in this phenotype are encoded within the LEE pathogenicity island (Locus of enterocyte effacement). The global regulator H-NS represses the expression of the LEE while the LEE-encoded regulator (Ler) antagonizes H-NS-mediated repression. GrlA and GrlR are regulators also encoded within de LEE. GrlA binds to the spacer sequence located between the -35 and -10 boxes of the *ler* promoter, which contains an ATGT motif essential for the activation mediated by this regulator.

A preliminary analysis of the GrlA transcriptome suggested that GrlA regulates the *E2348C_1013* (*E1013*) gene. This gene is annotated as a hypothetical protein, but its promoter region shares high identity with the *ler* promoter, including the presence of the ATGT motif in the spacer region. Complementation experiments in *E. coli* K12, containing transcriptional fusions of the *E1013* promoter (*E1013p*) to the promoterless *cat* gene, with plasmids encoding different EPEC regulators, showed that GrlA, and the regulatory region spanning positions -43 to +26, are sufficient to activate *E1013p* expression. The mutation of the ATGT first base dramatically reduces GrlA-mediated *E1013p* activation, confirming this motif's functionality. In turn, the untranslated region (UTR) negatively regulates its expression independently of H-NS.

In contrast, PerC has a very modest effect on *E1013p* activation compared to that of GrlA and contrary to what PerC shows for *ler*. Although GrlA activates the *E1013p* in a heterologous background such as *E. coli* K12, the activity in wild type EPEC is barely measurable with *cat* fusions, both in LB and in DMEM, unlike a *LEE* promoter that is activated in DMEM. However, expression in minimal medium with glycerol, but not glucose, significantly favors the expression of both promoters even more than DMEM, suggesting that the carbon source, when EPEC grows in MM, is a regulatory determinant of the LEE and other GrlA regulated genes. These results are allowing us to look into variations of the GrlA action mechanism at different promoters, as well as into the role of the carbon source in LEE regulation.

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Participation of a putative operon encoding a two-component system involved in motility and biofilm formation.

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Plant-microbe associations play a vital role in plant health and crop productivity. The ability to detect and respond to environmental changes in the vicinity of bacteria is essential for their survival and growth. A variety of mechanisms the bacteria have evolved by which cells sense their environmental changes and respond appropriately. *Azospirillum brasilense* has been identified and characterized as a Plant Growth Promoting Rhizobacteria (PGPR), which promotes important beneficial effects in plants such as nitrogen fixation, auxin biosynthesis, nitric oxide synthesis, and biofilm formation. However, before the bacterium exerts its beneficial effects an intimate bacteria-plant interaction is essential to colonize the root plants. The colonization processes require the bacterium moves towards an environment by external stimuli such as amino acids, carbohydrates and other carbon sources, this process is known as chemotaxis. Chemotaxis provides bacterial motility toward specific compounds found in root exudates. These compounds can cause chemo-attraction or chemo-repulsion responses giving a wide advantage in the colonization of ecological niches. In addition, when the motile bacteria find a favorable ecological niche, changed their lifestyle from planktonic to sessile condition and, form a biofilm structure a critical process essential for successful colonization. The complex association between motility and biofilm formation involves the use of a particular structure for different functions at different stages and requires the precise integration of environmental and cellular signals involving the two-component systems (TCS). Most bacterial TCSs consist of two proteins, a sensor histidine kinase (HK) and a response regulator (RR). In this study a putative operon comprising five genes: Two hybrid histidine kinase (HkhB and HkhC), and three different response regulators; a diguanylate cyclase response regulator (CdgE), a chemotaxis response regulator (CheY-Like) and a transcription factor response regulator (LuxO-Like) was identified in *A. brasilense* Sp245 strain. We have shown an important role of this putative operon in motility and biofilm formation by constructing four mutants, three deletions (mutants *ΔdgcE*, *ΔhkhB,* and *ΔluxO-like*) and one insertion (mutant *cdgE::gusA-Km*^{R}). As the preliminary data indicate that this operon is involved in motility and biofilm formation, further studies are running to unravel the contribution of this operon in colonization to root wheat.

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Analysis of the expression and regulation of the *pmf* and *tsf* chaperone-usher fimbrial operons in *Citrobacter rodentium.*

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Citrobacter rodentium is a specific mouse pathogen that causes transmissible murine colonic hyperplasia. It belongs to a family of enteric pathogens that colonize the host gastrointestinal tract producing the attaching and effacing (A/E) lesion on the epithelial cell surface. This family includes the human pathogens enteropathogenic *Escherichia coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) for which there is no animal model of disease, making of *C. rodentium* an excellent model to study *in vivo* the pathogenesis of this bacterial family. A/E lesions are characterized by the effacement of the brush border microvilli and the formation of pedestal-like structures under the adherent bacterium; however, prior to this intimate interaction, the initial adherence to the host enterocytes is often mediated by non-motile filamentous structures called fimbriae. The *C. rodentium* genome harbors 19 fimbriae operons, of which 13 belong to the chaperone-usher (CU) family. Nevertheless, there is limited knowledge about these operons, and only the role of two of them during infection has been studied: *kfcHGFEDC* and *gcfFGABCDE,* which encode the KFC and GCF fimbriae, respectively.

Using transcriptional fusions between the putative regulatory regions (PRR) and the promoterless *cat* gene, we evaluated the expression of two uncharacterized CU fimbrial operons herein named *pmf* and *tsf*. Fusions were evaluated in the *C. rodentium* wild type strain DBS100 and its isogenic mutants in genes encoding H-NS-like proteins (Δ*hns*, Δ*stpA*, Δ*pehH*, Δ*cicR* and Δ*ler*) under different growth conditions. Both the *pmf* and *tsf* PRRs are not active in the WT strain but showed significant activity in the Δ*hns* mutant, indicating the presence of functional promoters that are strongly repressed by H-NS. Interestingly, the *pmf* promoter was similarly active in the absence of StpA, suggesting a cooperative role with H-NS in the negative regulation of this operon. We also observed that PehH positively regulates the expression of *pmf,* while for *tsf* only a moderate role was observed for StpA and Ler in negative and positive regulation, respectively. A series of transcriptional fusions carrying fragments that span different sections of the *pmf* and *tsf* PRRs were generated and evaluated in the WT and Δ*hns* strains. The minimal regulatory region of *pmf* was located up to position -98 with respect to the transcriptional start site, which was determined by *primer extension.* The minimal regulatory region of *tsf* was delimited between positions -324 to +120 with respect to the start codon ATG.

pmf is an interesting CU operon because it lacks a gene encoding a pilin subunit; instead it contains a gene encoding a hypothetical protein. RT-PCR assays showed that this gene, *pmfC,* is co-transcribed with the *pmfAB* genes coding for the chaperone and usher, respectively. Consistently, both the *pmfA* and *pmfC* transcripts are translated into proteins, as shown by western blot. The role of these fimbriae in intestinal colonization and their expression *in vivo* will be evaluated.

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Characterization of the replicator region of the *Acinetobacter baumannii* plasmid pAba3207a carring a carbapenem resistence gene (*bla*OXA-58).

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Acinetobacter baumannii is a Gram-negative bacterium that has become a serious human pathogen and is responsible for a wide variety of infections in intensive care units. *A. baumannii* can acquire antibiotic resistance easily. So the ability to treat *A. baumannii* infections has become challenging. *A. baumannii* isolates with resistance to “last resort” antibiotics such as the carbapenems or colistin are now commonly seen, and pan-resistant strains have been reported with increasing frequency. This has prompted the World Health Organization to list *A. baumannii* as a number one priority for the research and development of new antibiotics. *A. baumannii* acquires antibiotic resistance by through mobile genetic elements like transposons and plasmids.

In this study, we show that *A. baumannii* 3207 strain possesses a blaOXA58 gene responsible for its carbapenem resistance. These genes are encoded in a 13.47 kb plasmid that we named pAba3207a. To understand the mechanism or replication of this plasmid we found that the replicator region of plasmid pAba3207a is contained within a 2088 bp fragment. This region can confer replication abilities to a suicide vector (pBBR1MCS3) when transformed into *Acinetobacter baylyi* ADP1. The different elements involved in the stable replication of this plasmid were identified.

The pAba3207a 2088 bp replicator region contains two genes. One of them is similar to other replicator proteins of *A. baumannii* plasmids. The other one is a gene encoding an HTH protein. These genes are organized in an operon. The first gene, *rep1,* is essential for replication, because plasmid derivatives carrying in frame deletions of this gen are incapable to replicate. In constrast, when we eliminated the HTH gene the resultant construct was able to replicate stably. Upstream of the *rep1*gene, we found a putative promoter region and three short tandem repetition sequences (iterons, usually important for plasmid replication and /or copy-number control). Close to the Iterons, we found a region rich in AT, that points a possible origin of replication (*oriV*). When one or more iterons were eliminated, the ability to replicate of constructs containing these deletions were lost, indicating that iterons are essential for replication. 300 bp upstream of the start codon of *rep1* we deleted 50 bp region, the resulting construct is capable to replicate, but their stability is decreased. When we deleted 81 bp or more in the same region, the replication capacity was eliminated

Creation and characterization of a thermo-inducible prophage of *Pseudomonas aeruginosa* for RNA-Seq analysis

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Pseudomonas aeruginosa is an opportunistic pathogen of humans, animals and plants and it has the capacity to adapt to environmental stresses resulting in a ubiquitous distribution, distribution that shares with its infecting phages. Therefore, studying the molecular mechanisms of the phage-host interactions can help to understand insights into phage biology, host evolution and phage-derived antimicrobial strategies derived from phage-bacteria interactions. To understand and characterize the molecular program of a bacteriophage genome expression in its bacterial host, as well as to identify essential genes during the lytic and lysogenic cycles, we devised a thermo-inducible system in strain PAO1 lysogen for Fc02. However, PAO1(Fc02) lysogens, unlike *Escherichia coli* lambda lysogens, are not inducible by *recA*-dependent procedures based on inflicting damage to bacterial DNA. In order to characterize gene expression in the transit from lysogenic to lytic cycles of phage development, we introduced a thermo-sensible mutation in the repressor gene of a Fc02 lysogen. The resulting lysogen, PAO1(Fc02rts), was evaluated at two temperatures; 30°C where it remains repressed, and 40°C which induces the lytic cycle. We determined that the latent period of the phage at 40°C is 60 minutes, upon this temperature shift phage titer rose from $2x10^6$ to $8x10^9$ UFP/ml. We did a qualitative assay to assess the mRNA expression of some phage genes, and concluded that 5, 10, 20 and 40 minutes, after the shift to 40°C will be adequate time-points to take samples for RNA extraction and further analysis through RNA-Seq. We will discriminate phage genes expressed in the lysogen from those expressed upon thermo-induction of the lytic cycle, and attempt to correlate the expression of phage genes with changes in the expression of host genes.

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Transcriptional regulation of *ipdC* gene involved in the indol-acetic acid (IAA) production in *Azospirillum brasilense.*

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The Plant growth-promoting rhizobacteria *Azospirillum brasilense* Sp7 produces the phytohormone indole-3-acetic acid (IAA), a signaling molecule involved in bacteria-plant interaction. IAA biosynthesis in *Azospirillum* occur mainly through the indole-3-pyruvic acid (IPyA) pathway. The key enzyme phenylpyruvate decarboxylase (PPDC) encoded by the indole pyruvate decarboxylase (*ipdC*) gene catalyzing the reaction of IPyA to IAdh and further produce IAA. It was shown in previous studies that promoter elements are required for *ipdC* transcription in *A. brasilense*, however, until now, trans-acting transcriptional factors involved in *ipdC* expression have not been yet identified. To identify trans-acting transcriptional factors regulating *ipdC* expression, a DNA-affinity chromatography and mass spectrometry were performed to isolate and identify proteins interacting with the *ipdC* promoter. Two putative transcriptional regulators with helix-turn-helix domains were identified that bind to *ipdC* promoter: A LuxR family and a MarR family transcriptional regulators, which were named LibR (LuxR-family indole-3-acetic acid biosynthesis regulator) and MibR (MarR-family indole-3-acetic acid biosynthesis regulator), respectively. A *libR* deletion mutant, named as *A. brasilense* 2113, showed a 4-fold lower level of *ipdC* transcripts compared to wild-type strain. The level of *ipdC* transcripts was slightly lower in the *A. brasilense* 2114 *mibR* deletion mutant, albeit it was not statistically different from the wild-type strain. Interestingly, the *A. brasilense* 2115 *libR-mibR* double mutant showed 7-fold decrease level in *ipdC* transcripts compared with wild-type strain. In addition, *A. brasilense* 2113 IAA production was decreased until 35% as compared to wild-type strain and slightly lower in *A. brasilense* 2114, albeit it was not statistically different from wild-type strain. Interestingly, the level of IAA production in *A. brasilense* 2115 was decreased to 51% as compared to wild-type strain. Bioinformatic analysis of *ipdC* promoter revealed a potential LuxR binding site as well as a MarR binding site. Such sites overlap an identified inverted repeat upstream of the putative sigma 54 binding site. All data obtained here, suggest that both LibR and MibR coregulate the *ipdC* expression, LibR acts as an activator, while MibR acts as an antiactivator, probably binding simultaneously or independently to *ipdC* promoter. Both protein have been purified and further electrophoretic mobility shift assay (EMSA) will confirm this hypothese. These results provide novel insights, on the genetic regulation of *ipdC* and contribute to our understanding about the regulatory mechanism controlling IAA biosynthesis in *A. brasilense*.

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"Evaluation of the open reading frame RSWS8N_09490 in the function of Fla2 of *Rhodobacter sphaeroides*"

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Rhodobacter sphaeroides is an α-proteobacterium with a versatile metabolism that has two different sets of flagellar genes. The expression of the *fla1* set is constitutive under the growth conditions commonly used in the laboratory, whereas the expression of *fla2* is dependent on the activation of CckA-ChpT-CtrA, a two-component system that was first described in *Caulobacter crescentus* and that is widely distributed in other α-proteobacteria. Also, in *R. sphaeroides*, this system seems to be regulated by other components such as the proteins Osp and DivL.

In order to establish what other genes are regulated by the CckA-ChpT-CtrA system, the *R. sphaeroides* regulon was recently characterized in the laboratory. It was determined that CtrA regulates the transcription of 321 genes in *R. sphaeroides*, and probably 175 are directly controlled. Interestingly, it was found that CtrA positively regulates its own transcription, as well as that of the genes *cckA, divL* and the chemotactic operon cheOp1, which controls Fla2 rotation. In addition, within this group of positively regulated genes, a gene was found that encodes a protein with a REC domain of unknown function, so in this work we aim to characterize and determine if this protein participates in any point of the regulation of the biosynthesis and functionality of Fla2 or in some related process, such as chemotaxis.

In a search for conserved domains only the putative REC domain was found, although the sequence has a carboxyl terminal region of approximately 95 amino acids. A homology search showed that this protein is conserved in the Rhodobactaraceae family, but none has been yet characterized. To assess if the absence of this protein impacted Fla2 motility, we isolated a mutant in which the wild-type gene was replaced with a resistance cassette. We found in soft agar swimming plates that the halo was considerably reduced in the mutant strain compared to the parental strain. The phenotype was restored when this ORF was expressed from a low-copy-number plasmid. A Western blot analysis showed the presence of the hook and filament proteins in the mutant strain, and the presence of flagella was confirmed by transmission electron microscopy. Nonetheless, observation of free swimming liquid cultures under the optical microscope revealed that most of the cells were unable to swim.

The effect de PqsE in virulence factors production by *Pseudomonas aeruginosa* INP43.

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Pseudomonas aeruginosa is an opportunistic pathogen and is the main cause of mortality for patients with cystic fibrosis or patients who have a compromised immune system. It produces different virulence factors as pyocyanin, elastase and rhamnolipids, the expression of these factors depends on the quorum sensing response. (QSR) The protease elastase is regulated by the lasR and rhlR system, rhamnolipids are regulated by the rhlR and pqsR system, and pyocyanin is regulated by the rhlR system and the pqsE protein. In recent years this protein has been widely studied and the molecular mechanism in the production of pyocyanin and other virulence factors is still unknown. It is known that PqsE is crucial for the production of pyocyanin in PAO1 and PA14 strains since their pqsE mutant does not produce pyocyanin, decreases the production of elastase and its virulence, but the effect that it has on the production of rhamnolipids is still unknown. All studies have been carried out on the type strains, but the effect of this protein on strains that lack or do not express one of the quorum sensing systems is unknown. The objective of this work is to study the effect of the PqsE protein in strain INP43, a pediatric isolate that although lacking the LasR system produces the mentioned virulence factors. To accomplish this objective, the IPN43 pqsE mutant was constructed by conjugation and the production of pyocyanin, elastase and rhamnolipids was evaluated. It was surprisingly found that the production of pyocyanin is reduced but not completely absent, while elastase production is completely abolished. These results show that in INP43 strain, PqsE is more important for the production of elastase than for pyocyanin synthesis.

Phenotypes of *Azotobacter vinelandii* strains carrying mutations in genes involved in ribosome rescue

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Protein synthesis is carried out by the ribosome in a process called translation. It is divided into four stages: initiation, elongation, termination and recycling of ribosomes. During this process the mRNA may undergo some deterioration resulting in the formation of a “non-stop” translation complex. This complex arises when the ribosome is stalled at the 3´ end of the mRNA owing to the absence of a codon in the decoding center.

Of the bacteria that have been studied, all use a pathway known as trans-translation as the main mechanism for rescuing ribosomes that form non-stop complexes. This system consists of a specialized RNA molecule (namely, transfer-messenger RNA (tmRNA)) and small protein B (SmpB). However, some bacterial species also have protein-based pathways that can function as backup systems for trans-translation. These systems are known as alternative ribosome-rescue factor A (ArfA) and B (ArfB) (1).

Azotobacter vinelandii is a Gram-negative bacterium that produces poly-β-hydroxybutyrate (PHB), a polymer of industrial importance in the production of bio plastics. A BLAST search using the *E. coli ssrA, smpB, arfA* and *arfB* genes as query allowed us to identify that the *A. vinelandii* genome possesses genes encoding for the RNA and proteins involved in the three mechanisms of ribosome rescue so far identified in bacteria. Unexpectedly, two homologues of the *E. coli arfA* were identified as avin08960 (*arfA1*) y avin08930 (*arfA2*). Among 431 bacterial strains analysed for *arfA* homologs by Shaub *et al,* 2012 (2) only *A. vinelandii* DC strain and a Gram-negative *Eikenella corrodens* strain possessed two copies of *arfA*.

E. coli strains deficient in trans-translation have several phenotypes related to stress responses as *ssrA* or *smpB* mutants are more sensitive to high temperatures, antibiotics and oxidative stresses among others (3). In order to determine the involvement of genes identified in mechanisms of ribosome rescue we constructed strains UWssrA, UWarfA1 UWarfA2, and UWarfB that carry mutations in *ssrA, arfA1, arfA2* and *arfB* respectively. Results of the effect of high temperature to assess the susceptibility of *ssrA* as well as *arfA1, arfA2, arfB* mutants on their growth will be presented.

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A novel MarR-like transcriptional regulator of *Klebsiella oxytoca* represses expression of genes involved in biosynthesis of tilivalline

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Klebsiella oxytoca is part of the intestinal microbiota, however in some patients treated with penicillin antibiotics, rapid proliferation of this organism results in antibiotic-associated hemorrhagic colitis (AAHC). The cytotoxin associated with AAHC is a pentacyclic pyrrolobenzodiazepine known as tilivalline. The genes that code for these proteins are part of a pathogenicity island (PAI). There are two operons in the PAI involved in the biosynthesis of tilivalline: "*aroX*- and NRPS-operons", being *aroX* and *npsA* the first genes of each transcriptional unit, respectively. Furthermore, one gene that codes for a putative MarR-like transcriptional regulator has been found in such PAI. In order to investigate if MarR could be related to transcriptional regulation of the PAI in the cytotoxic strain *Klebsiella oxytoca* 09-7231, we determined the expression of some genes in the wild-type and Δ*marR* strains cultured *in vitro*. Total RNA was isolated and purified from cultures in exponential phase, and gene expression was quantified by RT-qPCR. In addition, cytotoxicity assays were tested on HeLa cells. The expression of *aroX* and *npsA* genes increased more than 400-fold in the Δ*marR* strain with respect to the wild-type strain. Finally, was found a greater cytotoxic effect on HeLa cells when these cells were inoculated with the supernatant of the Δ*marR* strain. In summary, we found that MarR represses the transcriptional expression of genes involved in the tilivalline biosynthesis, and the cytotoxicity phenotype coincided with the results of gene expression. Our data suggest, that in the Δ*marR* strain more tilivalline is produced due to transcriptional derepression of the genes involved in biosynthesis of tilivalline, and in turn, such strain produces more cytotoxicity. These findings may shed further light on the host-pathogen relationship of the cytotoxin-producing *K. oxytoca*.

Dominance in *tcdC* alleles, the anti-sigma factor of the PaLoc in *Clostridium difficile.*

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Recently, *Clostridium difficile* emerged as an important gut pathogen. This pathogen is responsible for intestinal diseases which symptoms range from mild diarrhea to pseudomembranous colitis. The main virulence factors of *C. difficile* are two toxins that are codified in a pathogenesis locus called PaLoc. This island also contains a sigma factor (TcdR) and an anti-sigma factor (TcdC). The activity of TcdC as an anti-sigma is not clear yet. Previously reports identify that hypervirulent strains (strains that produce higher amount of toxins) possess mutations that generate a truncated and unfunctional version of TcdC denominated $TcdC_{\Delta 117A}$. This supports the possibility that the lack of a functional TcdC allow the overproduction of toxins making hypervirulent strains more aggressive. However, a later report demonstrated that deletion of the gene *tcdC* in the 630Δerm strain do not affect toxin levels production.

The aim of this work is analyze the *tcdC* allele present in 41 clinical strains isolated from patients in Mexico. We identified 5 different alleles, being the most common the truncated version $TcdC_{\Delta 117A}$. After classification of the strains based in *tcdC* allele, we made *in silico* TcdC models to identify the mutations present in each allele. We overproduce the $TcdC_{\Delta 117A}$ protein and analyzed the effect on toxin levels. After that, we analyzed the dominance of this allele on a wild-type background. As a product of these, we may have elements to clarify the role of $TcdC_{\Delta 117A}$ as an anti-sigma factor.

STUDY OF THE EFFECT OF THE PROMOTERS OF THE *leuO* GENE IN *Salmonella enterica* SEROVAR Typhi

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Salmonella enterica serovar Typhi is a Gram-negative bacterium, facultative anaerobe and intracelular pathogen that belongs to the enterobacteriaceae and the ethiologic agent of typhoid fever. Multiple genetic factors have been associated to virulence in Typhi, including transcriptional regulators. Such is the case for LeuO, a dual transcriptional regulator that has been implicated in the regulation of genes involved in the survival under stress conditions and in virulence, in several pathogens. In Typhi, LeuO positively regulates genes such as *ompS1*, *ompS2*, *assT* and *crispr-cas*; and represses *tpx* y *ompX*. The precise signal that triggers the expression of *leuO* is unknown in *S*. Typhi. However the expression of *leuO* has been studied in other bacteria and has been associated to the entrance to stationary phase, to the activity of the heterodimer RcsB-BglJ and to the (p)ppGpp alarmone. In addition, the *leuO* gene is required in virulence of several pathogens such as *S.* Typhi*, S.* Typhimurium*,* Enterohemorrhagic *E. coli, Y. enterocolitica* and *V. cholerae.*

The regulation of *leuO* in S. Typhi has been interestingly complex. In our laboratory, this regulation has been studied through the analysis of various segments of its regulatory region fused to *lacZ.* In addition, with the study of mutants to regulators such as H-NS and Lrp, we postulate that these proteins bind to the *leuO* regulatory region acting as repressors. We have postulated the existence of at least five forward promoters that would participate in its transcription, and two reverse promoters within the same intergenic region. In this work, we have observed a differential regulation by (p) ppGpp on *leuO* promoters P3, P1, P2, P5, and P4. This analysis suggests that such regulation depends on a previous step of derepression by antagonizing H-NS and Lrp action. Hence, we intend to use the *hns lrp* strain to analyze the effects on expression of each promoter through their individual mutagenesis; in order to determine if there is cooperativity between them, and what is their contribution towards the synthesis of the LeuO protein and the regulation of the target genes.

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Evaluation of the fimbrial protein 0024 expression conditions of Enteroaggregative *Escherichia coli*

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Enteroaggregative *Escherichia coli* (EAEC) is an emerging intestinal pathogen causing acute and persistent diarrheal disease in humans worldwide. The strains of EAEC are very heterogeneous in their pathogenicity mechanism although the prototype strain on which various studies have been based is EAEC 042 (O44:H18). The main mechanism of EAEC pathogenicity is the stage of initial attachment to the intestinal mucosa which represents an essential step in the colonization and production of the disease. The adherence of EAEC requires expression of aggregative adherence fimbriae (AAF) that favors the formation of biofilms composed by aggregates of bacteria in association with a thick mucus layer, this pattern of adherence called aggregative adherence (AA) allows the bacteria to persistently colonize in the intestinal tract. Epidemiological data indicate that only 10 to 15% of strains isolated from different regions of the world produce some of the AAF fimbriae, suggesting the existence of other uncharacterized adhesins that could play an important role in most strains of EAEC. In this sense, the analysis of the EAEC 042 genome revealed the existence of different fimbrial operons, in addition to those already characterized, one of them is in the position 21439-25167 of genome and its organization suggests that putative fimbria is assembled by the way of the chaperone-usher (CU). The aim of this work was to evaluate the expression of the 0024 fimbrial protein and its role in EAEC biofilm formation. For this, the *orfEC042_0024* (fimbrial protein) was cloned in pBAD-myc-his A, the recombinant protein was purified by affinity chromatography and inoculated in New Zealand rabbits. The antibodies obtained were used in western blotting assays to evaluate the 0024 protein expression under the following growth conditions: Luria-Bertani (LB), Dulbecco´s modified Eagle medium (DMEM), pleuropneumoniae-like organisms (PPLO), trypticase soy broth (TSB) and Brain Heart Infusion (BHI) at 30ºC and 37°C. Finally, biofilm formation assays were performed on polystyrene plates in presence and absence of antibodies α-0024. The results suggest that 0024 protein is expressed when EAEC is cultured in all media tested at 30°C and to a lesser extent at 37ºC. While biofilm assays indicated that antibodies α-0024 decrease adherence and biofilm formation of EAEC. These dates suggest that fimbrial protein 0024 has an important role in the EAEC biofilm formation process. Future studies will characterize and evaluate the role of this new fimbriae in the disease generated by EAEC.

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Internalization of wheat root by PGPR *Azospirillum brasilense* Sp245 is regulated by second messenger c-di-GMP

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Plant-growth promotion by *Azospirillum brasilense* Sp245 is mediated throughout the induction of expression of plant defense genes, biological nitrogen fixation and synthesis of phytohormones leading to the root system modification, which increases the uptake of nutrients and water. To establish a successful and prolonged bacteria-plant interaction, microorganisms tend to form biofilms, which are bacterial communities embedded in a matrix formed by extracellular polymeric substances. Biofilm formation is a complex process regulated by multiple factors, being cyclic di-GMP (c-di-GMP) one of the most studied. This molecule is synthetized by diguanylate cyclase enzymes. Several reports have demonstrated the role of c-di-GMP in a plethora of cellular process such as motility, bacterial virulence, cellular cycle and biofilm formation. The genome of *A. brasilense* Sp245 encodes for 35 predicted proteins involved in c-di-GMP metabolism. In this study, a gene that encodes for a putative diguanylate cyclase, denominated CdgC, was found by bioinformatics. To evaluate c*dgC* function, a gene-deleted mutant strain derived from *A. brasilense* Sp245, named *A. brasilense* 59C and a complemented strain were constructed. The biofilm formation was analyzed with the strains tagged with the enhanced Green Fluorescent Protein (eGFP), while the fluorophore calcofluor-white was used to elucidate the components of this matrix due to it shows affinity towards polysaccharides with β-1,3 and β-1,4 bonds in their structure. Biofilm formation and exopolysaccharide production were evaluated with an inverted confocal microscope (CLSM). In the wheat root colonization assays, the plants were inoculated with *A. brasilense* Sp245 or *A. brasilense* 59C tagged strains. After 7 days of post inoculation, roots were macerated and colonizing bacteria was determined by colony counting. In addition, root sections were examined using a CLSM to evaluate colonization patterns of these strains. Qualitative analysis of biofilm formation using confocal microscopy not shown significant differences in the biofilm three-dimensional architecture, however, quantitative analysis of biofilm-matrix proved a decrease in exopolysaccharide production with calcofluor-white affinity. Moreover, wheat root colonization experiments demonstrated that *A. brasilense* 59C was unable to internalize into the root as compared with wild type strain. This data suggested that c*dgC* mutation caused a decrease in calcofluor-binding exopolysaccharide production that could lead to a weak and transitory bacteria-plant interaction, lessening to bacteria internalize into wheat roots.

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Quorum sensing systems regulation in low phosphate conditions in *Pseudomonas aeruginosa*

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Pseudomonas aeruginosa is a Gram negative opportunistic pathogen of plants and animals, its intrinsic resistance to many antibiotics make it one of the main cause of nosocomial infections leading to high mortality and morbidity rates. *P. aeruginosa* virulence is due to the variety of extracellular factors that produces such as elastase, rhamnolipids, pyocyanin, among others. The synthesis of these factors is regulated transcriptionally by the quorum sensing system (QSS), which is a process involving cell-density-dependent accumulation of signal molecules that together with a regulator protein modulate the expression of specific genes. This species has three QSS; two of them based on the detection of N-acyl-homoserine lactones (*las* and *rhl* systems), and one based on the detection of 2-alkyl-4(1H)-quinolones (PQS system), they are correlated and it has been proposed that are regulated hierarchically being *las* the system on the top.

When *P. aeruginosa* establishes an infection, low phosphate is a common stress that this bacterium faces, and this condition is even more pronounced on immune-compromised patients. It has been reported that in this condition *P. aeruginosa* shows a more virulent phenotype by overproducing some virulence factors (VF). The objective of this project is to understand how the regulation of the QSS that control the VF production is restructured in this condition.

Pyocyanin, rhamnolipids and elastase production were evaluated in wild type (WT) and isogenic mutants on every of the three QSS, in low and high phosphate conditions. We confirmed that all of the evaluated VF were overproduced on the low phosphate condition. Also it was possible to determine that the *las* system is only needed to turn on the other two systems and thus control the VF production on high phosphate conditions. However, this transcriptional regulator is not necessary in low phosphate conditions, as the *las* system mutant was able to produce VF.

In order to determine the expression of the QSS genes in low and high phosphate conditions, transcriptional fusions were evaluated in the WT in both conditions. The *las* system expression is higher in high phosphate than in low phosphate conditions, which suggests that this system function is more important in non-stressed high phosphate condition. Conversely, the *rhl* system expression is higher in low phosphate conditions.

Taken together these results agree with the hypothesis that the QSS regulation is structured differently in the two conditions. While in high phosphate the *las* system is the master regulator, in low phosphate the *rhl* system takes more relevance and the *las* system is dispensable. These results are relevant since for years the efforts to block QSS have been targeting *las* system, and here we show that it is not the best target.

Do the SPFH-containing proteins affect secretion in enteropathogenic *Escherichia coli* in association of membrane microdomains?

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Membrane lipid rafts have been broadly studied on eukaryotic cells. These membrane structures have been associated with diverse cellular processes such as signal transduction and protein transport. Interestingly, a set of proteins, all of which have the SPFH domain (Stomatin, Prohibitin, Flotillin, HflK/C), is always associated with the eukaryotic lipid rafts. Accordingly, disruptions of lipid rafts or SPFH-protein mutants are associated with a large variety of diseases. Nevertheless, it was not until recently that such membrane microdomains were reported to exist in bacteria, and a method for their isolation from *E. coli* inner membranes was reported.

Here, we present our results aiming at elucidating whether mutants of the SPFH-containing proteins affect the assembly and function of the enteropathogenic *E. coli* type III secretion system.

Expression of the *LsPDR1* gene - orthologous strigolactone transporter in mycorrhized tomato plants

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Exudation and transport of strigolactones (SLs) require a protein called PhPDR1, an ABC type transporter that has recently been identified as a cellular exporter of SLs in Petunia hybrida. *PhPDR1* gene expression in Petunia roots was induced by phosphorus deficiency, arbuscular mycorrhiza colonization and treatment with GR24 (a synthetic strigolactone). The objective of this work was to study whether this SL transport protein is involved in the interaction between ethylene and phosphorus during mycorrhizal symbiosis. For this, we analyze the expression of the orthologous gene *PhPDR1* in tomato. The design of the primers consisted of a BLAST type alignment using the tomato genome database. We use the sequence of the *PhPDR1* gene to obtain the nucleotide sequence of the hypothetical orthologous gene of the PDR1 transporter in tomato, LsPDR1. Specific primers were designed to analyze the expression of this gene by qRT-PCR in wild tomato plants and mutants altered in their ability to perceive and respond to ethylene and grown under conditions of high phosphorus availability. The Ailsa Craig genotype (Rin wild type) showed a similar expression of the *LsPDR1* gene under conditions of mycorrhization in high level of assimilable phosphorus and in conditions without treatment of Pi (no significant differences). On the other hand, in the corresponding mutant, Rin (impaired in their ability to respond to the presence of ethylene), the phosphorus repressed the gene expression at 100 ppm Pi, although its expression slightly increased to 300 ppm Pi above the control value (up to once) In the case of the VFN8 genotype (Epi wild type), gene expression increased directly and significantly with phosphorus concentration. Similarly, in the Epi mutant genotype (overexpression of the response to ethylene), gene expression was positively regulated by the application of Pi, and expression levels were above those observed in the wild genotype (up to 5 times at 100 ppm Pi and up to 7.5 times at 300 ppm Pi). This data suggests that the Epi mutation interacts with phosphorus to enhance the expression of the *LsPDR1* gene, and probably, with the transport of strigolactones in mycorrhized tomato plants.

Spatiotemporal regulation of the BarA/UvrY two component signaling system.

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The BarA/UvrY two-component system mediates adaptive responses of Escherichia coli to changes in growth stage. At late exponential growth phase, the BarA sensor kinase senses and responds to acetate, leading to its auto-phosphorylation and transphosphorylation of UvrY, which activates transcription of the CsrB and CsrC noncoding RNAs. CsrB and CsrC, in turn, sequester the RNA binding protein CsrA, which posttranscriptionally regulates translation and/or stability of its target mRNAs, antagonizing its regulatory functions.
Curiously, CsrA appears to be required, although indirectly, for the activation of BarA. Therefore, it was suggested that, in addition to acetate, other factors, whose expression depends on CsrA, might be involved in the control of BarA activity.

Here, we provide results of experiments demonstrating that CsrA negatively controls expression of the *hflK* and *hflC* gene products, and that the HflKC complex plays a pivotal role in the control of BarA, by recruiting it to the cell poles and inactivating its kinase activity during the stationary phase of growth. The implications of our findings on the spatiotemporal regulation of the BarA kinase activity will be discussed.

Characterization of coliphage mEp021 protein Gp17

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The bacteriophages or phages are the most abundant biological entities in nature. mEp021 is a “non lambdoid” temperate phage, its development depends on host Nus factors, and is part of human microbiota. The genome of this phage contains many ORFs related with hypothetical proteins, so it is important to characterize these proteins to know the regulatory mechanisms that phage present. For this reason, the aim of this project was to know the Gp17 function. Firstly, we cloned gp17 gene in an expression vector pKQV4 to evaluate the infection of mEp021 on different *nus* mutant strains. The infection assays was made on the following strains: *nus*A1, *nus*B5, *nus*C60, *nus*D26, *nus*E70 transformed with pK-gp17. Every mutant strains that overexpress Gp17 with 0.5 mM of IPTG was able to allow the development of mEp021.

To determine if Gp17 is essential to phage development, *gp17* mutant was constructed by recombineering. The mutant was not able to produce viral particles (non-viable), this indicated that gp17 is essential for a successful development. Gp17 has an Arg-Rich domain, which is present in proteins that bind to RNA and it is characteristic of antiterminator proteins N of lambdoid phages. To know if Gp17 is an antiterminator we made an antitermination assay; for this, we used a plasmid that has a transcriptional terminator within gp21 gene, the Gp17 overexpressed at 42ºC, allowed Gp21 expression. The bacteriophages have proteins that codify for antiterminator proteins like N of λ phage, N21 of P21 phage, N22 of P22 phage, which regulate terminators at transcriptional level to allow expression of early genes. These proteins recognize a RNA sequence denominated boxB, to form a complex with Nus factors and by this way regulate the activity of RNA polymerase.

We conclude that Gp17 is essential for mEp021 development and its overexpression regulates a transcriptional terminator. In summary, we can suggest that Gp17 works like N antiterminator.

Study of the operator-promoter region of the repressor gene of mEp021 bacteriophage.

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Bacteriophages are viruses that infect bacteria. mEp021 is a temperate phage which infects *E. coli*. It has been described the mechanism that regulate “the genetic switch” between the lysogenic and litic pathway on lambda, P22 and other phages. In lambda phage the protein responsable for the maintenance of lysogenic state is the repressor CI. The λ repressor binds to its operators some of them are overlaid with its promoters pL and pR, in this way its genes are repressed. The mechanism of the “genetic switch” in mEp021 is unknown, therefore, we decided to start the study of this region. We designed primer sets to amplify the operator-promoter region of the repressor gene, the repressor gene of mEp021 and the reporter gene mCherry. We carried out two plasmids construction, one plasmid in which the operator-promoter region was flanked by two reporter genes (GFP and mCherry) in both sides, this was generated in several steps. Another one where the repressor gene was under control of the promoter pTac which is inducible by IPTG. These plasmids were transformed into the strains W3110 and W3110(mEp021). The fluorescence was measured in the Synergy ™HT. We observed that the fluorescence levels for GFP (510 nm) and mCherry (610 nm) decreased when we transformed with the plasmid that contained the repressor gene.These results suggest that there are at least two divergent promoters into the operator-promoter region, which are negatively regulated by the repressor protein. Probably the operators which are recognized by the repressor are overlaid with these promoters.

CdgB is a Hybrid GGDEF and EAL Protein Located in the Cytoplasmic Membrane of *Azospirillum brasilense* Sp245.

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Azospirillum brasilense is a plant growth-promoting that inhabits the rhizosphere and soil. This species is known to promiscuously colonize the root surfaces of a wide variety of plants, often possess endophytic ability, and is used worldwide as a commercial bio-fertilizer. These bacteria are capable of increasing the yield of important crops growing in various soils and climatic regions. The bacterium has to cope with a very competitive environment in the rhizosphere and soils. It is capable of forming biofilms on wheat root surfaces, and its c-di-GMP pathway plays an active role in biofilm formation.
Cyclic diguanylate monophosphate (c-di-GMP) is a nucleotide second messenger responsible for the control of a variety of bacterial features, most of them involved in life style transitions. In this study we identified the gene *cdgB* from *Azospirillum brasilense* Sp245, which encodes a multi-domain predicted protein (CdgB) with architectural domains containing three MHYT modules in tandem, considered a transmembrane domain and a PAS/PAC domain both located at the N-terminus and, coupled to the GGEEF and EAL domains located at the C-terminus. Mutant Δ*cdgB* analysis showed that the gene is involved in biofilm and pellicle formation, exopolysaccharide production, and motility, indicating that both GGEEF and EAL domains function as diguanylate cyclase and phosphodiesterase. Fluorescence confocal laser microscopic analysis of *A. brasilense* cells tagged with a plasmid containing the translational fusion *cdgB::egfp* and the membrane dye FM4-64FX allowed visualization of CdgB located at the pole of the cell anchored to the cytoplasmic membrane. In addition, sequence deletion of amino acids encoding the MHYT domain reveled CdgB in the cytoplasm, suggesting that the domain interacts with and allows incorporation of the protein into cytoplasmic membrane of *A. brasilense.* Furthermore, to corroborate the MHYT deletion results, cell-free extracts of the wild-type and deleted (ΔMHYT-CdgB-eGFP) strains were analyzed by western blot with antibodies against eGFP. The truncated protein was found in the cytoplasmic extract. There are several reports regarding the hybrid diguanylate cyclase and phosphodiesterase proteins involved in exopolysaccharides (EPSs) and biofilm production. However, in spite of the important functions of EPSs and biofilm formation in *Azospirillum* strains, including, protection to stress, microbial aggregation, plant microbe interaction, and bioremediation, are already well established. Nevertheless, EPSs and biofilm regulation are poorly understood. In this study, we identified a hybrid *A. brasilense* protein involved in EPSs metabolism that is primary responsible for matrix architecture of biofilm.

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PdeA, a c-di-GMP regulated phosphodiesterase, modulates the c-di-GMP pool and swimming motility in *Vibrio parahaemolyticus*.

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Vibrio parahaemolyticus is a pathogenic bacterium capable of infecting a variety of hosts including marine organisms and humans. This halophilic bacterium inhabitant of brackish water is highly motile. *V. parahaemolyticus* can swim propelled by a single polar flagellum but can also move collectively on solid surfaces (swarming) under permissive conditions using peritrichous lateral flagella. In addition to its motile lifestyles, *V. parahaemolyticus* can form biofilms. Biofilms are sessile communities of cells encased by a polymeric extracellular matrix. These different lifestyles confer advantages for survival under specific situations, and the transition between them must be finely regulated.

The second messenger c-di-GMP plays a central role in the regulation of the transition between motile and sessile lifestyles in a variety of bacteria. Multiple proteins presumptively involved in c-di-GMP signaling are encoded in *Vibrio parahaemolyticus* RIMD 2210633 genome. Very few of these proteins have been studied in depth, leading to an incomplete picture of the building blocks that constitute the decision-making circuits that dictate the transition between motile and sessile lifestyles in *V. parahaemolyticus*. The goal of this work is to provide yet another piece to the puzzle that is c-di-GMP signaling in the marine bacterium *V. parahaemolyticus*. Here we present evidence that the gene VP1881 encodes an active phosphodiesterase (PdeA) capable of regulating the global c-di-GMP pool and swimming motility. Expression of this gene is strongly induced when the c-di-GMP phosphodiesterases PdeA or ScrG are overexpressed. A putative binding site for the biofilm regulator CpsR is located upstream of *pdeA*, hence we hypothesize that CpsR is responsible for the c-di-GMP dependent regulation of *pdeA* expression. The role of this and other effectors on *pdeA* expression is currently been analyzed by our group. Our results suggest that *pdeA* might be one of multiple switches that control the motile to sessile transition in *V. parahaemolyticus*.

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Study of the effect of the simultaneous mutation of the *rsmA* and *pycA* genes on the production of PHB in *Azotobacter vinelandii*

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Azotobacter vinelandii is a γ-proteobacteria belonging to the family *Pseudomonadaceae*. It has the ability to produce metabolites of biotechnological interest, including polyester intracellular polyhydroxybutyrate (PHB) that can be used in the manufacture of biodegradable plastics. In this bacterium, the dual component GacS/A system in conjunction with the post-transcriptional regulation system Rsm controls the biosynthesis of the PHA.

Mutants have been reported in rsmA, which promote the accumulation of PHB, and it was also observed that this mutation affects and disfavors metabolites of the Krebs cycle by directing or generating the accumulation of acetyl-coA. On the other hand, mutant *pycA* has been studied; as the flow of acetyl-CoA in the TCA cycle depends on its condensation with oxaloacetate, acetyl-CoA would be available for PHB synthesis. So, taking into account the carbon fluxes and mutants already reported, it would be interesting to study the synergy between the control points of the Rsm system and the metabolites of the Krebs cycle to direct and increase the production of PHB. It was therefore proposed to study the role of the Rsm system together with the enzyme pyruvate carboxylase on the production of PHB in *Azotobacter vinelandii.*

A double mutant of the AEIV strain (E*algD*-) *pycA-rsmA* was created for this purpose. The *pycA* mutation was made by cloning the gene into a pJET 1.2/blunt mutagenic plasmid and then generating the mutation by inserting the Km resistance cassette from the pBSL98 plasmid. The resulting pJET*pycA*::Km plasmid underwent the transformation into competent cells of the E*algD*- strain of *A. vinelandii*, recovering transformants that established the homologous double recombination; these candidates were verified by PCR. Once the mutants were obtained in *pycA*, we proceeded to work with *rsmA*. For the double mutant with *rsmA*, two plasmids were taken (pUC*rsmA*::ΩSm and pGEMΔ*rsmA*::Km) that were previously constructed in the laboratory but that due to incompatibility of markers with the strain and with the mutant in *pycA*, first the change of the resistance cassettes was made**,** replacing them with the gentamicin cassette (Gm), taken from the vector pBSL141. The resulting plasmids were transformed into competent cells of the E*pycA*- strain, the obtained transformants were verified by PCR. With the double mutants obtained, finally, the production of PHB in PY medium with different carbon sources will be quantified.

ANTIMICROBIAL RESISTANCE AND ANTIMICROBIAL AGENTS

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Brominated Furanones with low molecular weight decrease quorum sensing in *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa is an opportunistic pathogen affecting immune-compromised patients. This bacterium has high levels of antibiotic resistance so that it is difficult to treat. In this situation, the WHO released a list of bacteria which new treatments are needed. In answer to this, a new alternative is decrease bacterial virulence through quorum sensing system. It has been reported that furanone C30, a furanona with 2 bromine atoms ($C_4H_2O_2Br_2$), decrease quorum sensing system products on *P. aeruginosa*. Therefore, the objective of this work is to evaluate furanones with different substituents that can inhibit quorum sensing in *P. aeruginosa*.

In order to evaluate the furanones, we used two strains of P. aeruginosa, a reference strain PA14 and a multidrug-resistant strain INP64. Furanones were dissolved in DMSO and were added at beginning of culture. The cultures started at OD 0.05 in LB medium and incubated at 37°C/200 rpm during 18 h. Later the culture was harvested to measure growth, pyocyanin and biofilm. The measure of swarming motility, was achieved when bacterial strains was inoculated in BM2 plates and incubated during 18 and 36 h. The β-galactosidase assay was performed in PA14 strain, the strain was incubated for 4 h in LB medium. The DMSO and furanone C30 was used as negative and positive control respectively. Our results show that the C-30 type furanones A1, A2 y A3 at 100 μM are capable to decrease pyocyanin production, biofilm formation and swarming motility in both strains as furanone C30. Besides these furanones diminish transcription of genes involved in quorum sensing as rsaL, rhlA, pqsA and phz1. However, furanones with one benzene substituent B2, B6 and those with two benzene substituents C1, C3 and C7 decrease pyocyanin production and biofilm formation at 500 and 1000 μM in PA14 strain, whereas on INP-64 only diminish the biofilm formation.

In conclusion, furanones without substituents can interfere in quorum sensing of *P. aeruginosa* strains while those with aromatics substituents have slightly effect indicating that the size of furanones are important to action.

The transcriptome and drug susceptibility of *Mycobacterium tuberculosis* in a lipid-rich dormancy model.

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The World Health Organization estimates that up to one-third of the world's population has latent tuberculosis (LTB), and most active tuberculosis (TB) cases arise because of the reactivation of LTB. Lipids are abundant molecules surrounding the dormant *Mycobacterium tuberculosis* (*Mtb*) in the granuloma and *in vitro* studies better mimicking its actual metabolic state during LTB are needed. Although TB treatment is dependent on the implementation of drug susceptibility testing (DST) of clinical isolates, and molecular detection of gene mutations associated with drug resistance, treatment failure remains a challenge. Some studies have suggested that treatment failure may be due to the survival of dormant mycobacteria. **Our objective** was to determine and analyze the global gene expression of *M. tuberculosis* and to evaluate its anti-TB drug susceptibility in an *in vitro* model of dormancy to which a combination of lipids was added. **Methods:** *Mtb* H37Rv was grown in Dubos medium supplemented with lipids (palmitic, stearic and oleic acids plus cholesterol), in aerobic conditions, as well as in hypoxic conditions to perform the *in vitro* latency model. The transcriptome of *Mtb* was analyzed by RNAseq using the Illumina platform. Also, *Mtb* was cultured until reaching exponential and stationary phases of growth as well as NRP1 and NRP2 hypoxic stages of dormancy in the presence of lipids. Once the mycobacteria entered each experimental phase, the mixture of antibiotics, R-MX-A-MZ or R-MX-A-PA, was added. The antibiotic activity was evaluated at 7, 14, and 21 days by estimating mycobacterial survival in solid and liquid media. **Results:** Lipids significantly induced the expression of 368 genes of *Mtb*. A main core lipid response was observed involving efflux systems, toxin-antitoxin systems, iron caption, and sulfur reduction genes, which may act coordinately to prepare the machinery conferring drug tolerance and increasing a persistent population. Also, we demonstrated that the presence of lipids confers *Mtb* some tolerance against the mixture of antibiotics tested, and this tolerance could be even higher during dormant stages. Since current DST is based on aerobic cultures with dextrose as a carbon source, the implementation of a DST in clinical strains that includes lipids as carbon source could potentially lead to a better treatment strategy. Our findings could be useful to tag relevant pathways for the development of new drugs, vaccines, and new approaches to control TB.

Transference Of Antibacterial Resistance From Environment To Agricultural And Healthcare Workers .

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Background:
Antibacterial resistance is recognized as an important public health problem, related to emergence of bacterial pathogens, increased costs of hospital stay, and morbi-mortality; the root factor in the transfer of antimicrobial resistance might be contact with selecting environments. According to the Center for Disease Control and Prevention (CDC) these could include among others: hospitals, farm animals and pets (Centers for Disease Control and Prevention, 2017). Despite the importance of the hands as a vector for the transmission of pathogens, the role of occupational exposure, carrier status and transfer of resistant strains in agricultural workers and healthcare personnel has been little studied (Edmonds-Wilson, Nurinova, Zapka , Fierer, & Wilson, 2015), therefore, the aim of this research was to determine isolation incidence of ESKAPE members in handwashing samples of subjects with known exposure, and to asses risk factors for ESKAPE isolation.**Material and methods**:
A case-control study was conducted among farmers, healthcare personnel, and subjects without environmental exposure to antibacterial resistance (IRB; FM/DI/046/2017). We quantified the number of CFU / mL of hand wash samples in chromogenic, selective media (Chromoagar® (ORIENTATION, KPC, ESBL, VRE and mEA) to asses for resistance against vanciomycin, β-lactams, carbapenems. The results were analyzed using the SPSS Software (Version 21.0, August 2012) to evaluate significance differences between groups by U Mann-Whitney. Finally, logistic regression model was explored to characterize association of exposure pathways, of specific resistant microorganisms.**Results:** The results show significant differences among the number of CFUs of ESKAPE members and resistance, present on farmer's hands who irrigate crops with sewage (enterobacteria and *Pseudomona spp*), vs. those who use well water.**Conclusions:**
Although the isolation of resistant microorganisms was a common finding, the differences between study groups could be the reflection of the sources and the carrier status a first step to draw vectors between the source and susceptible subjects.

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Pseudomonas aeruginosa with mutations in *pmrA/B* two-component system isolated from a Pediatric Patient with Cystic Fibrosis in Mexico is Higher-Resistant to Colistin and Polymyxin B and virulent in the murine model.

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Abstract: Cystic Fibrosis is a complex genetic disease. Patients that suffer CF, once they are born, start rapidly developing in their lungs a progressive respiratory disease associated to recurrent chronic infections that results in the formation of bronchiectasis and lead to respiratory failure, which is the leading cause of death in these subjects. The affectation in CF is related to decreased chlorine transport through epithelial cell membranes caused by mutations in the Cystic Fibrosis Transmembrane Conductance Regulator (CFTR). *Pseudomonas aeruginosa* (*Psa*) is an opportunistic Gram-negative bacterium that causes chronic lung infections to persist until the end of the patient's life. During chronic lung infections, *Psa* is able to adapt to the host environment without causing overwhelming lung injury. In Mexico, children with CF are detected less frequency (1/8500). Between 2016 and 2018, we studied 52 pediatric patients with CF, in these patients we isolated 158 bacterial pathogens, 48.2% of these (76/158), were *Psa*. The antibiotic susceptibility shows that the 17.1% (13/76) of these isolates were resistant to colistin and 4.0% (3/76) to polymyxin B. In particular, the isolate FQ-2016-053 present's a higher-resistant to Colistin and Polymyxin B (MIC ≥128 µg/ml in both cases). The objective of this study was to identify genes associated to polymyxins resistance and virulence by sequencing of the whole-genome of FQ-2016-053. The results show a punctual mutation in *pmrA* a response regulator for Lipid-A modification and seven punctual mutations in *pmrB* a histidine protein kinase sensor. In addition, this strain carrying the *pmrL* gene that codes for a phosphoethanolamine transferase that modifies the structure of Lipid A with 4-amino-4-deoxy-L-arabinose (Ara4N), the *pmrB* gene that codes a histidine protein kinase sensor also involved in the modification of the structure of Lipid A. In addition, this clone carrying several genes associated to virulence. In the murine model of pneumonia, we detected that this clone is lethal in comparison with *Psa*-PAO1. The lungs of infected mice show an intense infiltration of polymorphonuclear cells into the peribronchiolar space. In conclusion, in this study, we detected in Mexico for the first time a *Psa* with punctual mutation in *pmrA* that also carrying genes associated to modification of structure of the lipid A that confers a higher resistance to colistin and polymyxin B to this bacterium, antibiotics used as a last resort for treatment of critically ill CF pediatric patients.

Study of the antibacterial effect of silver nanoparticles in multidrug resistant strains of *Pseudomonas aeruginosa*

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Abstract

Pseudomonas aeruginosa is an opportunistic pathogen in patients with burns, cystic fibrosis and immunosuppressed. Its epidemiological importance lies in its remarkable ability to develop resistance to multiple antibiotics, due to intrinsic or acquired mechanisms [1]. Silver has been used for thousands of years, and among its most important applications has been as an antimicrobial agent. At nanometric scale (nanoparticles), silver has some interesting characteristics that make it an object of study in bionanotechnology. The mechanism of action of silver nanoparticles (AgNPs) in bacteria, is based on their ability to penetrate the cell, release Ag^+ ions and generate reactive oxygen species, as well as interacting with phosphorus and sulfur compounds present in macromolecules such as DNA, RNA and proteins, generating oxidative stress and later cell death [2]. Green (G-AgNPs) and biological (B-AgNPs) synthesized silver nanoparticles were evaluated, characterized by Uv-Vis spectrophotometry and Transmission Electron Microscopy (TEM), and their antibacterial effects were tested against six multidrug resistance strains to antibiotics, determining the minimum inhibitory concentration and minimum bactericidal concentration using the broth microdilution method and disk diffusion test. Furthermore, the toxicity of AgNps was evaluated *in vivo* on the *Galleria Mellonella* model. Silver nanoparticles exhibited an antibacterial effect on multidrug resistance strains of *P. aeruginosa,* likewise, no toxic or secondary effect was evident on the simple model of *G. Mellonella.* For this reason, this research presents silver nanoparticles as an alternative to new antimicrobial compounds, to eradicate multidrug-resistant bacteria with importance in public health.

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Antimicrobial activity of strawberry extracts of different quality against antibiotic-resistant *Staphylococcus aureus*

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Abstract

Inflammation of the mammary gland in cattle is known as mastitis, and Staphylococcus aureus is the most common causative agent, it has the ability to develop resistance to most of the antibiotics used to combat this disease, which, added to its indiscriminate use, It results in a very difficult infection to control that causes significant economic losses. That is why research for the development of new drugs that fight this condition is of great relevance. The secondary metabolites present in fruits and plants are an important source of naturally occurring antimicrobials. Strawberry is a berry with an important amount of metabolites, mainly anthocyanins, phenolic compounds and flavonoids; The beneficial potential that these compounds have on human, animal and plant health has been demonstrated. However, it is a non-climacteric fruit, with a short shelf life, so much of what is harvested that does not meet the characteristics of export quality (Extra quality), goes to the national fresh market (first quality), to industrialization (first and / or second quality) or to waste. In this sense, the objective of this work was to evaluate the antimicrobial activity of strawberry fruits of these three qualities, as an alternative proposal to add value to the strawberry that does not reach export quality (first and second quality). The Minimum Inhibitory and Bactericidal Concentration (CMI and CMB) (Seleshe et al., 2017) of strawberry anthocyanin extracts (extra, first and second) (Abdel-Aal & Hucl, 1999), was determined against *S. aureus* causative of antibiotic multidrug bovine mastitis (AMC 9, AMC 23 and ATCC 27543). The results indicated that all strawberry extracts presented antimicrobial potential. However, there were differences between the different qualities evaluated. Both the MICs and the CMBs presented by the extract of strawberry anthocyanins of second quality were significantly lower (MIC of 6.3 to 12.5 µg / ml; CMB 25 µg / ml) than those presented by extracts of Extra quality (MIC 25 µg / ml; CMB 50 µg / ml) and first quality (MIC from 12.5 to 25 µg / ml; CMB from 50 to 100 µg / ml) for all strains. These results indicate that the multiresistant strains of *S. aureus* that cause bovine mastitis are susceptible to strawberry anthocyanin extracts, in addition to a quality-dependent effect of the fruit evaluated.

Area: Antimicrobial Resistance

The enzyme Hmgr (3-hydroxy-3-methylglutaryl-CoA reductase) from *Candida auris* as a therapeutic target

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The number of cases of *C. albicans* as a cause of mycosis has decreased between 1997 and 2019. However, other species such as *Candida glabrata, Candida auris* and *Candida haemulonii* become as an emerging opportunistic pathogens of global alert that cause 30-60% of mortality in immunocompromised patients, due to the multiresistance that they present to conventional antifungals. Seeing to the important role of sterols in the plasma membrane, the biosynthesis pathway of ergosterol provides different targets for the design of antifungals. One of these proposed targets is the enzyme 3-hydroxy-3-methyl-glutaryl CoA reductase (Hmgr). In this work, the enzyme Hmgr of *C. auris* is proposed as an alternative therapeutic target. The strains of *C. auris* were donated by Dr. Javier Pemán of the "La Fe University and Polytechnic Hospital" of Valencia, Spain. *Candida* spp strains belong to the collection of the Molecular Biology Laboratory of Bacteria and Yeasts, ENCB-IPN. The molecular identification of the isolates was made by sequencing the ITS region and the 28S rRNA gene. The sensitivity to antifungals was verified by the CL27 M27-A3 microdilution method. Synthetic and natural compounds (produced by *Streptomyces* sp) were used as inhibitors of growth and the synthesis of sterols. A bioinformatic analysis of the *hmgrCa* gene was perform for cloning and heterologous expression thereof. The phylogenetic analysis allowed to verify that the strains of *C. auris* were clustered in the clade CTG. *C. auris* 8 and *C. auris* 49 were resistant to fluconazole and amphotericin B and sensitive to caspofungin. The synthetic and natural compounds tested allowed to select possible antifungals whose target is the enzyme HmgrCa. The *hmgrCa* gene has an ORF of 3059 bp and the deduced protein has 1019 aa, a PM 110,235 Da, a pI 8.96 and 7 transmembrane sequences. For the expression of the *hmgrCa* gene, there is a genetic construct that comprises only the catalytic soluble site of the corresponding enzyme.

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Molecular and genomic characterization of carbapenem-producing *Providencia rettgeri* clinical isolates

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The *Providencia* genus belongs to *Enterobacteriaceae* and it is characterized and is characterized by being carbapenemase-producing bacteria. In addition, *Providencia rettgeri* is intrinsic resistant to colistin and tigecycline. The carbapenem resistance could be encoded both chromosomally and/or in mobile elements that can be modified within this enterobacterium and transmitted to others bacterial genus by conjugation or transfection.

We have identified 27 *Providencia* spp. isolates as carbapenemase-producing obtained four different hospitals in Mexico from a total of 47 samples analyzed. The isolates where obtained in a period of 2012 to 2018; the 80% of the isolates were identified as *Providencia rettgeri* and 15% as *Providencia stuartii*. Among the carbapenemases-producing isolates, five clonal groups were identified by PFGE and corresponded to isolates from the same hospital ("Hospital Universitario de Monterrey"). Plasmid analysis determined that 100- and 80-kb plasmids are the most common among carbapenemases-producing isolates. Ten isolates were selected by their origin and date of isolation, as well as for their carbapenem resistance for genome sequencing by Illumina. The genomic analysis showed the β-lactamases PER-7, OXA-1, IMP-27 and NDM-1; he last two enzymes corresponds to metallo-β-lactamase; being NDM-1 the most frequent. Virulence factors such as *ureE, ompC, fliO, flaA* and *rtxE* were identified by BLASTp in data bases that contains reports of possible virulence factors of *Providencia* spp. The incompatibility groups identified were; Col3M, IncA/C2, ColRNAi and T among the isolates from genomes sequenced. The genomes were annotated and in average 4,000 genes were identified, of which only an 60% have a known function. The phylogenetic analysis identified from one to three taxonomic groups with a core genome range of 356 to 3,444 genes. As well as, the average nucleotide identity (ANI) analysis support the identification of *P. stuartii* and variations between *P. rettgeri* isolates.

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Área: Resistencia antimicrobiana y Genómica.

Actinomycetes isolated from Mexican jungle soils with activity on diverse physiological capacities of human antibiotic-resistant pathogenic bacteria.

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Abstract

Antibiotic-multiresistant pathogenic bacteria have become one of the most important problems in public health, particularly within the intra-hospital environment. The failure of antibiotic therapy causes the natural development of the disease, increase the severity of the case and the time of hospital stay, and and compromises the lives of patients. In 2017, the WHO launched a global alert to draw attention to the problem of extending the phenomenon of multiresistance to antibiotics and summoned scientists to restart the search for new antimicrobial molecules. The actinomyces are bacteria with a very broad secondary metabolism that maintain a potential interest for the discovery of new antimicrobial activities.
The aim of this work was to search for inhibitory metabolites into supernatants of an actinomycetes collection isolated from jungle Mexican soils. New proposed molecular or physiological targets of antibiotic-multiresistant pathogenic bacteria were chosen. Several lyophilized supernatants exhibited antimicrobial activity; anti-biofilm formation in *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Staphylococcus aureus*; anti-quorum sensing inhibition in *Serratia marcescens*; and inhibition of iron acquisition in *Salmonella* Typhi. All actinomycetes with biological acivities were identified as new species of *Streptomyces* genus.

"Typing of Virulence Factors and Class 1 and 2 Integrons in the Epidemic clone O25b of Multidrug-resistant Uropathogenic *Escherichia coli*"

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Urinary tract infections (UTIs) are a public Health problem, in Mexico they are associated in more than 65% with Uropathogenic *Escherichia coli* (UPEC). In 2008, a clonal group O25b of multidrug-resistant UPEC (MDR-UPEC) was characterized and distributed in 3 continents, with a virulent genotype associated with the phylogenetic group B2 and related to Health Care Associated infections (HCAI). In Mexico, the clone was reported in 2011; however, there are a few studies in clinical strains associated with UTIs in pediatric patients. The aim of this work was to identify MDR-UPEC O25b clinical strains by *papB* gene specific allele PCR in pediatric patients, typify phylogenetic groups (A, $B2_1$, $B2_2$, D_1 and D_2) and genotype to 17 virulence genes (*papG*I*, papG*II *, papG*III*, bcsA, fimH, csgA, ecpA, iutD, fyuA, hlyA, tosA, satA, cnf*1*, chuA, fliC, motA and motB*). In addition, identify the resistance associated to class 1 and 2 integrons class 1 and 2 by multiplex PCR. One hundred and forty MDR-UPEC clinical strains were obtained by amplification of the specific *papB* gene, for the classification by phylogenetic group we obtained 62.14% of the phylogroup $B2_2$, 14.28% of D_1, 10% of D_2, 9.28 % of $B2_1$, and 8.4% of phylogroup A. The distribution of the fimbrial genes was 100% for *ecpA*, 94.28% for *fimH*, 92.85% for *csgA*, 81.42% for *papG*II, 2.85% for *papG*III and absence of *papG*I. The genes associated with flagella were obtained with the following frequencies: 100% of *motA*, 99.28% of *motB* and 31.42% for *fliC*. The *bcsA* gene coding for cellulose was found in 11.42%. In addition, the genes coding for iron scavengers like *chuA*, *iutD* and *fyuA*, were identified in 97.85%, 87.85% and 61.42% respectively. The genes that code for UPEC secreted proteins were observed in 83.57% of *satA*, 42.14% of *hlyA* and 32.85% of *cnf*1. Finally, 48.57% was obtained for the integrase 1 gene, of which 25% was positive for the amplification of the variable region, for the integrase 2 gene, a 2.14% frequency was obtained, being the 100% positive for the amplification of the variable region. In conclusion, the clone O25b is highly related to phylogroup $B2_2$, as well as to virulence and resistance factors in the pediatric population.

Determination of virulence and antimicrobial resistance profile of *Escherichia coli* producing Shiga toxin (STEC) isolated from river water and farm animal feces in Culiacan, Sinaloa.

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Area Resistencia Antimicrobiana. Presentación Cartel.

Shiga toxin-producing *Escherichia coli* (STEC) divide in *E. coli* O157 and non-O157. STEC are zoonotic enteric pathogens associated with human gastroenteritis worldwide, ranging from diarrhea to life-threating diseases such as hemorrhagic colitis and hemolytic uremic syndrome (HUS). The aim of the study was to determine the virulence profile and antimicrobial susceptibility of STEC strains from river water and farm animal feces nearby rivers of the Culiacan, Sinaloa, Mexico Ninety-four samples of river water and from animal feces (Cattle, sheep and chicken), were collected from October 2016 to January 2017 (54) and June to September 2017 (40). The presumptive STEC isolates based on distinctive colors were examined by PCR for presence of O-antigen (O157) and H-antigen (H7). STEC isolates were analyzed by multiplex PCR for the presence of *stx1*, *stx2*, *ehxA*, *eae* genes which are associated with human illnesses. The antimicrobial resistance profiles were examined in STEC strains by the Kirby-Bauer method. A total of the 24% environmental samples were positive for presumptive STEC strains. Multiplex PCR results revealed twenty-eight (90.3%) strains were positive for the gene *stx2*, nineteen (61.2%) for the genes *stx1* and *exhA*, and three (9.6%) strains for *eae* gene. The PCR results showed that two STEC strains were positive for the serotype O157:H7. Different profiles of resistance from one to eight antibiotics were identified in STEC strains. The results confirmed the presence of STEC in river water. The presence of these pathogens in river water and farm animal feces could result in the dissemination into the environment and pose risks to humans by affecting recreational and food production activities.

Thanks to Jenny Juárez-Torres and Dr. Ignacio Contreras for their technical support.

The effect of *Thymus vulgaris* on the growth and the respiratory activity of uropathogenic *Escherichia coli*

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Introduction:
The essential oils of plants have been considered as natural antibiotics which can be used in the production of new agents with antimicrobial activity for the pharmaceutical and food industry. In this context, the essential oil obtained from *T. vulgaris* is a potent inhibitor of Gram-positive and Gram-negative bacteria. As is known, uropathogenic *E. coli* is the causal agent most frequently isolated in urinary tract infections, representing a global public health problem. In this work some data of effect of *T. vulgaris* essential oil on the growth and the respiratory activity of uropathogenic *E. coli* are shown.

Objective:
To determine the effect of *T. vulgaris* on the growth and the respiratory activity of uropathogenic *Escherichia coli.*

Methodology:
Uropathogenic *E. coli* was grown in tryptic soy broth at 37°C for 24 hours. The effect of *T. vulgaris* on growth from uropathogenic was determined using the plate diffusion test, adding different concentrations of essential oil. The plates were incubated at 37°C for 24 hours. To test the effect on respiratory activity it was used an oximeter equipped with a Clark electrode and different concentrations of the essential oil were added. The reaction mixture contained: 20 mM phosphate buffer pH 7.0 and a cell suspension (the optical density at 560 nm was adjusted to 0.1). Respiratory measurement was initiated by the addition of 10 mM glucose. The temperature was kept constant at 37°C.

Results:
The effect of *T. vulgaris* on growth of uropathogenic *E. coli* was determined. The results indicated that the essential oil tested had a strong inhibitory effect on the growth of uropathogenic *E. coli*. In addition, a notable decrease in the respiratory activity of the bacteria was observed.

Conclusion:
The essential oil of *T. vulgaris* inhibited the growth and respiratory activity of uropathogenic *E. coli.*

Genetic analysis of resistance to β-lactams and carbapenems in *Acinetobacter baumannii* isolated from three hospitals in Mexico.

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Introduction: The most prevalent mechanism of resistance to β-lactams is associated with enzymes that hydrolyze them (β-lactamases of class A, D and B according to Ambler's classification). Therefore, the genetic analysis of the genes that code for these enzymes is of great importance.

Objective: To identify the genes that code for extended-spectrum β-lactamase (ESBL) and carbapenemases in isolates of *Acinetobacter baumannii* that caused infections associated with health care in three hospitals in Mexico.

Materials and methods: Isolates of *A. baumannii* from patients of the Hospital Civil de Guadalajara (HCG *n* = 202), Hospital Reg. Gral. Ignacio Zaragoza ISSSTE (HRGIZ *n* = 42) and the Pediatric Unit of the Hospital General de México (HGM-P *n* = 8) were studied during the years 2015-2016. Oligonucleotides for detection of bla_{VIM}, $bla_{NDM,}$ $bla_{IMP,}$ bla_{OXA-72} and bla_{KPC} genes were designed for the PCR-multiplex, $bla_{TEM,}$ $bla_{CTX-M-2-type}$ and bla_{SHV} genes were amplified with primers previously reported. ISABA1 and ISABA1::bla_{OXA-51} collinearity was amplified with primers previously reported. The confirmation of the genes was carried out by nucleotide sequencing. The profile of plasmids and megaplasmids was performed by Eckhardt gels. Horizontal transfer analyzes were performed with electroporation with *E. coli* DH5α and conjugation assays with *E. coli* J53-2.

Results:OXA-24 and TEM-1were the most prevalent carbapenemase and ESBL in *A. baumannii* from the three hospitals. The most frequent resistance genetic profiles were bla_{OXA-24} and bla_{OXA-24}+bla_{TEM1}. *Isaba1*::bla_{OXA-51} collinearity was not detected. Ten representative strains analyzed did not present mega-plasmids, only plasmids ≤15,000 bp, these plasmids did not transfer resistance by conjugation or transformation.

Conclusion: The presence and prevalence of bla_{OXA-24} gene in carbapenem-resistant *A. baumannii* isolates is the first report in Mexico. The results showed that bla_{OXA-24} is not transferable suggesting a chromosomal location. bla_{OXA-51} did not play an important role in carbapenem-resistant in *A. baumannii* isolates from this three hospitals in Mexico.

Moringa oleifera flowers are prospective source of antimicrobial agents against multidrug-resistant *Staphyloccoccus* spp strains isolated of common Canary (*Serinus canaria*).

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Background: Phytochemicals found in *Moringa oleifera* have diverse therapeutically activities. Extract from *M. oleifera* have been reported to have antibacterial and antifungal effects against pathogens cause several human infections. However, despite these findings there has been little work done on the effect of *M. oleifera* flowers extracts on multidrug-resistant *Staphyloccoccus* spp strains of veterinarian importance. Canaries are regarded by cage-bird fanciers to be their hardiest species, although several losses can be sustained by bacterial infection. The most common gram-positive bacterial pathogens are *Staphylococcus aureus*, *S. intermedius*, *Clostridium*, *Enterococcus*, *Streptococcus*, and *Rodhococcus equi*. **Aim**: The increased microbial drug resistance and the side effects associated with the use of conventional drugs limit the use of antimicrobial agents in canary (*Serinus canaria*). Therefore, this work evaluates the antimicrobial potential de phytochemicals extracted from *Moringa oleifera* flowers against diverse multidrug-resistant *Staphyloccoccus* spp strains isolated from *S. canaria*. **Methods and results**: Flowers of *M. oleifera* were grinded to powder and its phytochemicals were extracted using water and 80% methanol, part of the methanolic extract was sequentially partitioned to fractions with different solvents (hexane, chloroform, and ethyl acetate) The antimicrobial activity of each *M. oleifera* flowers extracts was tested using both the disk-diffusion agar method and agar well diffusion method, with sulfamethaxazole used as a positive control. Seventeen *Staphyloccoccus* sp strains exhibited resistance to at least two antibiotics. Ethyl acetate extract and chloroform exhibited a higher degree of antimicrobial activity compared to the aqueous, and hexane extracts, which are comparable with a zone of inhibition exhibited by sulfamethoxazole. Minimum inhibitory concentration was ranged from 5-100 mg/mL. This reveals that flowers of *M. oleifera* could be an alternative for the control of infections caused by *Staphyloccoccus* spp strains in birds. **Conclusion**: the study showed that *M. oleifera* flowers ethyl acetate extract possess inhibitory properties thus can serve as an alternative therapy for bacterial infections in birds. Further phytochemical analysis of ethyl acetate extract will be helpful for elucidation of lead molecules.

Keywords: *Moringa oleifera*, flowers, Staphylococcus spp strains, canary birds.

Hypermucoviscosity and biofilm production frequency in multidrug resistant *K. pneumoniae* isolates.

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Introduction: Multidrug resistant (MDR) *K. pneumoniae* has become an important health care-associated pathogen, being the causative agent in 14-20% of hospital infections (De Rosa *et al.,* 2015). A variety of virulence factors allows itto survive and evade the immune system during infection. Biofilm formation constitutes a physical barrier against antimicrobials, promoting its persistence in tissue epithelial cells and on surfaces of medical devices (Ramos et al., 2019); in addition to this, the production of a hypercapsule whose phenotype is characterized by hypermucoviscosity, protects against macrophage phagocytosis, the action of the complement system and decreases the bactericidal effect of antimicrobials (Clegg and Murphy, 2016). **Objective:** To describe the frequency of biofilm production and hypermucoviscosity phenotype in MDR *K. pneumoniae* isolates. **Methodology and results:** 48 *K. pneumoniae* isolates from two Public Hospitals at the city of Querétaro were analyzed; the resistance profile was determined by the Vitek2 system and the disk diffusion method; the strains were grouped as susceptible (12.8%), MDR (72.3%) and XDR (14.9%). The isolates with the ability to form a viscous cord of > 5mm in length were defined as hypermucoviscous (HV), of which 44.1% of the MDR isolates and 28.6% of the XDRs were positive. The biofilm production was determined by quantitative analysis in 96-well polystyrene plates in LB medium, 73.5% of the MDR and 57.1% of the XDR isolates showed moderate / high production. **Conclusions:** this is the first work that provides information analyzes the association of virulence factors in MDR *K. pneumonia* in Queretaro´s region. Biofilm production and hypermuscoviscocity are more frequent in MDR isolates compared to XDR K. pneumoniae, suggesting that as a mean of energy saving and based in its high resistance level, the latter express less virulence phenotypes. This type of studies could support the treatment and control of the infection in its early stages, since MDR strains can be more virulent when they produce biofilm and hypermucoviscosity.

The Effect of Essential Oil of *Thymus vulgaris* on the Growth of Bacterial Environmental and Clinical Isolates

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Introduction:
The essential oil of *Thymus vulgaris* as an antioxidant agent for a long time has been used. *T. vulgaris* inhibits the growth of some pathogenic microorganisms such as *Candida albicans*, *Legionella* sp., *Mycobacterium* sp., *Pseudomonas aeruginosa*, and others. Some of these bacteria have shown to be resistant to the action of antibiotics and they are also capable for forming biofilm on both abiotic and biotic surfaces. In this context, it has been reported the presence of pathogenic bacteria forming biofilm inside water pipes commonly used, as well as in catheters of patients hospitalized for long periods of time and who suffer from chronic urinary tract infections. A common problem in water distribution networks is the formation of bacterial biofilms inside them. So the bacterial growth in the water pipes has been of interest in many countries, especially because the pathogenic microorganisms to humans can grow forming biofilm. In the present study, the effect of *T. vulgaris* on growth of bacterial environmental and clinical isolates was studied.
Objective: To determine the effect of *T. vulgaris* on the growth of bacterial environmental and clinical isolates.

Methodology: The essential oil of commercial *T. vulgaris* was used for this study. The environmental bacterial strains were previously isolated from drinking water pipes; as clinical isolates, the uropathogenic *E. coli* strains were used. The bacteria were grown in broth and nutrient agar plates at 37°C for 24 hours. The effect of *T. vulgaris* on the growth of bacteria was determined in nutrient agar plates, placing different concentrations of the essential oil on paper discs (plate diffusion test). The plates were incubated at 37°C for 24 hours

Results:
The results showed that the growth of bacterial strains from environmental and clinical isolates was completely inhibited at the different concentrations of *T. vulgaris* tested.

Conclusion:
In the present study was possible to conclude that the essential oil of *T. vulgaris* was a potent inhibitor of growth from clinical uropathogenic *E. coli* and environmental isolates strains.

Resistance to last-resorce antimicrobials in ESKAPE isolates obtained from three public Hospitals of the state of Queretaro.

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Bacterial species included in the acronym ESKAPE are a global health problem, due to its rol in the development of Health-care Acquired Infections (HAI). Their main characteristic is multidrug resistance (MDR); this can comprise resistance to last-resort antimicrobials, which are not evaluated as part of the rutinary susceptibility tests. Yet, some of these last-resort antimicrobials have increasingly been used for the treatment of infections for which there are no other therapeutic options, due to MDR. Thus, the aim of this study was to identify the most frequent ESKAPE pathogens and its resistance to last-resort antimicrobials in public health hospitals at the state of Querétaro. After authorization of the protocol, clinical ESKAPE isolates were collected, during the first quarter of 2019, from three public hospitals in the state of Queretaro. Species identification and rutinary susceptible test were performed by automatized systems, while the expanded resistant profile was completed by Kirby Bauer and microdilution techniques. Gram negative pathogens were, by far, the predominant species identified in the following order: *K. pneumoniae* (*Kp*, n=38), *A. baumanni* (*Ab*, n=25) and *P. aeruginosa* (*Pa*, n=17). Eighty one percent of the isolates were from 2 of the hospitals and the distribution and predominant species in each was *Kp* (16/35) and *Ab* (14/35) in hospital A, and *Kp* (12/29) and *Pa* (11/29) at hospital B. Regarding last-resort antimicrobial resistance, BLEEs phenotype was found in 33/38 *Kp*; carbapenem resistant in 3/33 *Kp*, 22/25 *Ab* and 14/17 *Pa*, polimixyn resistance in 13/29 *Kp*, 18/25 *Ab* and 15/17 *Pa*. Noteworthy, 46% *Pa* and 82% *Ab* were classify as extreme drug resistant, while 6% *Ab* were pandrug resistant. In summary, *Kp*, *Ab* and *Ps* were the mainly ESKAPE pathogens identify, expressing diverse last-resort resistance, particularly to carbapenem and polimixyn cathegories. It is well known that recognition of the particular hospital microbiota and its resistance profile is essential to establish effective management and prevention strategies.

Genomic characterization and pathogenicity determination of the classical, hypermucoviscous and hypervirulent *Klebsiella pneumoniae* isolates

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K. pneumoniae is one of the main pathogens causing IAAS and in recent years it causes infections in the community. It is highly resistant to antibiotics and its pathogenicity has increased, which is classified as classical *K. pneumoniae* (cKpn) and hypervirulent (hvKpn). Infections caused by hvKpn are related to liver and kidney abscesses, necrotizing fasciitis and meningitis. Likewise, metastases have been described. The objective of this study was determining the genomic, antimicrobial resistance and pathogenicity characteristics of the phenotypes and genotypes of *K. pneumoniae.* A collection of 392 isolates were collected from 8 hospitals (2016-2017). The bacterial species was identified by VITEK and by PCR-multiplex. The phenotype hypermucoviscous was determined by string test, virulence factors by PCR, production of extended spectrum β-lactamases (ESBL) by Kirby-Bauer, carbapenemases by CarbaNP, resistance to colistin by Polymixin-NP. Its pathogenicity was determined in murine model (MM), serum resistance (SR) and neutrophil phagocytosis (NF). The genomes were sequenced by Illumina, and phylogenetic analyzes were performed and virulome and

SCCmec types in *Staphylococcus aureus* and *Staphylococcus* coagulase SCN negative of three hospitals in Mexico

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Abstract. Methicillin-resistant *Staphylococcus* MRSA impacts morbi-mortality and can acquired in the community *(CA-MRS)* and acquired in hospital *(HA-MRS).* Gene *mec*A is in mobile element as *"Staphylococcal cassette chromosome mec" now* SCCmec Types (I - XI). classify as HA-MRS and CA-MRS, also associated with Panton Valentine leucocidin *PVL.***Aim.** To determinate HA-MRSA and CA-MRSA and classify *SCCmec* types + Panton Valentine leucocidin PVL in three hospitals (A, B, and C) strains in Mexico +. **Material and Methods**. Blood culture samples must of them Beta-lactamase producer of *Staphylococcus spp. Test* with Cefinase®, and cefoxitin discs. Polymerase chain reaction for *mecA* gene, *SCCmec* types and gene *luk-PV* in 100 strains isolated from 3 hospitals were determined. Pulsed field gel electrophoresis (PFGE) with restriction enzymes *ApaI, SmaI*, and *XhoI.* **Results.** 21 isolates were analyzed, 47.61% *S. aureus* and 52.38% were SCN from A hospital; 24 isolates 62.5% were *S. aureus* and 37.5% SCN at B hospital; and. 55 from C hospital, 34.6% were *S. aureus* and 65.4% were SCN. Methicillin-resistant strains corresponding to A hospital, 90% of *S. aureus* and 45.5% of SCN. For B hospital, 62.5% of *S. aureus* and of SCN 37.5% was obtained, All had *mecA* gene at C hospital 63.1% were *S. aureus* and 91.66% were SCN only two MRSA were negative for *mecA.* The strains were grouped as HA-MRS and CA-MRS, classiffied SCCmec+LPV, type II was predominant for HA-MRS and type IV was for CA-MRS. 4 strains had more than one SCCmec type, finding the combination of types III & IV, II &IV. Of 100 strains studied gene lukS-FPV were positive by PCR only in 3 *S. aureus,* 6.81%. Any SCN had *luk-PV* gene. The dendogram PFGE reveal that there is no clonal diversity on CA-MRS and HA-MRS. The analysis of the three hospitals concluded that they had similar population essencially on B and C hospitals, regardless of the geographic location of each hospital apparently there were no outbreaks. **Conclusion**. SCCmec type II was the most frequent in the three hospitals, but there were also community types, IV and SCCmec V. The lukPV gene was identified in 3 isolates of *S. aureus*, this virulence factor and the SCCmec identify CA-MRSA strains, then there are *Staphylococcus spp*. causing IAAS that presenting community bookmarks.

Trimethoprim sulfamethoxazole (SXT) resistance in clinical isolates of *Stenotrophomonas maltophilia*.

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Abstract.

S. maltophilia is a Gram-negative bacilli related with nosocomial infections and intrinsically resistant to diverse antibiotics, but only trimethoprim sulfamethoxazole (SXT) is the option for treatment. Nevertheless, SXT^R strains appeared causing intrahospitallary infection. **Aim.** To establish whether the strains isolated from the last outbreak from 2016-2018 at one Hospital could be related phylogenetically. We performed multi-locus sequencing typing (MLST) on these *S. maltophilia* multidrug-resistant strains. **Material and methods.** We studied 106 clinic isolates identify by Vitek 2 as *S. maltophilia* from November 2016 to October 2018. Additionally we automatically determined antibiotic resistance by Vitek 2. Furthermore, DNA was purified by standard methods and resistance genes *sul*1, *sul*2 and *int*1 were detected by PCR using *S. maltophilia* ATCC 17666 as positive and *Escherichia coli* ATCC 25922 as negative controls. For MLST we amplified *mut*M, *nuo*D, *pps*A, *rec*A, *gua*A, *gap*A and *atp*D. Sequences from PCR amplicons were edited and compared with the Sequence Type data base at https://pubmlst.org/smaltophilia/. **Results.** It was demonstrated that 38.7% (41/106) from blood culture and 23.6% (25/106) from sputum. Many strains were isolated from ICU and Hematology than other services. We found that 10.4% (11/106) of the isolates were SXT resistant. However, all SXT^R isolates were positive to the SXT resistance genes *sul*1, *sul*2 and *int*1. Three out eleven isolates were randomly selected for MLST and their sequences corresponded to the ST84 and ST23 and one is not recorded. In summary, we detected that patients from ICU and Hematology departments were more susceptible to the infection of *S. maltophilia*. Also sputum was the common source for isolation of this bacterial species, which confirms its association as etiologic agent for nasopharyngeal infection. Furthermore it may suspect that one outbreak was cause by *S. maltophila* occurred from February to March 2018. **Conclusions.** Not all strains were phylogenetically related because all three sequences belonged to different sequencing types. We are currently performing further analysis to determine whether the strains without unknown ST may represent a new variety.

Study of the production of extended spectrum β-lactamases and Quinolone resistance in strains of *Escherichia coli* isolated from urinary infections

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Urinary Tract Infections (UTIs) constitute the group of bacterial infections that most frequently affect the population worldwide. *Escherichia coli* is the main pathogen responsible for UTIs. β-lactams and quinolones continue to be the antibiotics of choice for these types of infections; however, the production of Extended Spectrum β-lactamases (ESBL) enzymes and the expression of plasmid genes (*qnrA, qnrB, acc(6 ')-Ib-cr, qepA, oqxA/B*) that confer quinolone resistance, reduce their effectiveness and advance the appearance of multi-resistant strains. In this study, 73 isolated strains of urine cultures from the 3rd level hospital of Puebla were collected during the period from January to September 2017, which were identified by biochemical tests (Cowan and Steel method). Sensitivity to 20 antibiotics from different families was tested (AMP, AMC, FOX, CTX, CAZ, CRO, FEP, ATM, MEM, IMP, GM, AMK, CIP, NAL, C, SXT, COL, TE, FOS, NIT) by Kirby-Baüer (CLSI, 2018). Phenotypic detection of ESBL production was performed using the double disc diffusion test and 61 strains (CTX^R) were genotypically characterized by amplifying the genes encoding ESBL CTX-M, TEM, SHV, OXA, AmpC; as well as the quinolone resistance genes *acc(6')-Ib-cr, qnrA, qnrB, qepA, oqxA and oqxB*, by conventional PCR. We found that over 50% of the isolates were resistant to β-lactams and over 70% were resistant to quinolones, and only 5 strains gave positive phenotype to the production of ESBL. The prevalence of *bla* genes was: bla_{CTX-M} 42.6%, bla_{TEM} 36%, bla_{SHV} 0%, bla_{OXA} 40.98%; only 1 strain producing CMY; and the quinolones resistance genes was: *aac(6')Ib-cr* (24.59%), *qepA* (1.63%), *qnrA* (3.27%), *qnrB* (31.14%), *oqxA* (22.95%) and *oqxB* (14.75%). The results show the presence of multidrug-resistant strains isolated from UTI patients of the 3rd level hospital in the city of Puebla, Puebla, Mexico.

AiiM lactonase strongly reduces quorum sensing controlled virulence factors in clinical strains of *Pseudomonas aeruginosa* isolated from burned patients

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Pseudomonas aeruginosa is an opportunistic bacterium associated with healthcare infections in intensive care units, ventilator-associated pneumonia, surgical site infections and burns. This bacterium causes 75% of death in burned patients, since it can develop a persistent biofilm associated with infections, by expressing several virulence factors and activating resistance mechanisms. Some of these virulence factors are proteases as elastase and alkaline protease, or toxins as pyocyanin, also, is one of the few microorganisms able to produce cyanide, which inhibits the cytochrome oxidase of host cells. These virulence factors are controlled by quorum sensing. In this work, 30 clinical strains of *P. aeruginosa* isolated from burned patients in a tertiary hospital in Mexico City were studied. Susceptibility patterns were done, and virulence factors (elastase, alkaline protease, HCN and pyocyanin) were determined in presence of an N-acylhomoserine lactonase, AiiM. Purified AiiM showed hydrolysis of a wide spectrum of Acyl homoserine lactones and was able to reduce significantly the activity of elastase, alkaline protease, and the production of pyocyanin and HCN in all producer strains. However, AiiM did not inhibit the secretion of effector toxins through the type III secretion system. Our work suggests that AiiM treatment may be an effective therapy to combat *P. aeruginosa* infection in burn patients.

Inhibition of Lecithin-dependent Hemolysin from *Vibrio parahaemolyticus* by phenolic compounds

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Vibrio parahaemolyticus (*Vp*) is widely distributed around seas and also as microbiota in many marine species, including cultivable species as oyster and shrimps. *Vp* has been associated with various infection processes in shrimp aquaculture as early mortality syndrome and systemic vibriosis, which causes losses in shrimp production. *Vp* contains several infection mechanisms as hemolysins. Which include human pathogenic markers as thermo-direct (TDH) and thermo-related (TRH) hemolysins. Additionally, a third less studied lecithin dependent or thermolabile hemoysin (*TLH*) that has phospholipase activity. All shrimp-pathogenic *Vp* strains are positive for TLH but could be negative for *TDH and TRH*. *Vp*LDH is an atypical phospholipase B/A_2 activity, which produces lysophospholipids that lysates, both sheep erythrocytes and shrimp hemocytes; and also was lethal for shrimp by intramuscular injection (2 mg/gr shrimp weight). Therefore, we evaluate natural compounds as phenolics aced and flavonoids as possible inhibitors for *Vp*TLH. This enzyme was overexpressed as inclusion bodies in *E. coli*, purified under denaturing conditions (urea 8M) and dialysis-refolded by removing chaotropic agent. Both enzymatic and hemolytic activity were measured by spectrophotometric method using *p*-nitrophenil laurate or human erythrocytes, respectively. Phenolics compounds as gallic, prothocateic, vanillic acids at 30 and 100 uM cause 20% inhibition, while chlorgenic acids only diminish 15% of VpTLH activity at 100uM. However, rutin (quercetin rutinoside) at concentration of 1-20 μM did not inhibit the enzyme. Meanwhile, quercetin at 1, 5 and10 uM was able to inhibits the enzyme from 20, 40 and 60 %, respectively. Increasing quercetin concentration (20 μM) causes same results as 10 μM. Similar results were obtained when morin was used by diminish enzymatic activity to 30% compared to *Vp*TLH in absence of inhibitors. Additionally, the IC_{50} values were calculated using dose-response model for both quercetin (4.514 μM) and rutin (9.914 μM). These results show that flavonoids here evaluated are inhibitors for *Vp*TLH and could be used as alternative (with antibiotics or alone) to reduce *Vp* infection in shrimp aquaculture.

Genome sequencing of two environmental *Escherichia coli* strains from agricultural drainage ditch water in Sinaloa, Mexico.

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Escherichia coli a rod-shaped, Gram-negative bacterium belonging to the family *Enterobacteriaceae*, inhabits the lower intestinal tract of humans and warm-blooded animals and is often discharged into the environment through feces or wastewater effluents. The presence of *E. coli* in environmental waters has long been considered as an indicator of fecal pollution. The survival and growth of *E. coli* strains in the environment raise concerns since the contamination with pathogenic ones may cause intestinal and extra-intestinal infections. Additionally, the antimicrobial resistance (AR) dissemination is a healthcare matter by the antibiotic treatment failure it causes worldwide. An *E. coli* collection recovered from water in a section of the agricultural drainage ditch "La Michoacana" in Sinaloa Valley (Mexico) was assembled. Phenotypic and molecular characterization of these isolates revealed the presence of multidrug-resistant (MDR) and diarrheagenic *E. coli*. In the present study, genomic sequences of a MDR [EC-ADD167] and an enterotoxigenic *E. coli* (ETEC) [EC-ADD183] strains from the collection were analyzed to gain insight in its antimicrobial resistance and pathogenic fitness, respectively. Genomic DNA was extracted using a ZymoBIOMICS DNA Miniprep Kit and sequenced on Illumina Miniseq platform using a 2x150 paired-end approach. *De novo* assemblies by SPAdes and A_5 assemblers were concatenated to generate final draft assemblies using Mix tool, which then were scaffolded using Medusa server; while genome annotation was performed with prokka. Multilocus sequence typing (MLST), acquired antimicrobial resistance, and virulences genes were determined using MLST v2.0, ResFinder v3.2, and VirulenceFinder v2.0 web-tools available in CGE server. According to Achtman's MLST scheme EC-ADD167 and EC-ADD183 strains were classified as sequence type (ST) ST617 and ST4, respectively; both ST belonging to ST10 clonal complex. Regarding AR, EC-ADD167 harbored diverse genes conferring resistance to aminoglycosides, β-lactams, macrolides, sulphonamides, tetracyclines, and trimethoprim; however, only a multidrug efflux pump-encoding sequence was identified in the EC-ADD183 genome. Moreover, point mutations in *gyrA*, *parC* and *parE* genes were identified. Although ST10 clonal complex strains are generally devoid of the virulence-associated genes required for pathogenesis, VirulenceFinder analysis in both genomes revealed the presence of the Enteroaggregative *Escherichia coli* heat-stable enterotoxin 1 (EAST-1). Besides, the EC-ADD183 strain harbors heat-labile enterotoxin (LTa) and ETEC autotransporter A (EatA). The genomic data evidenced the basis of AR and the pathogenic potential of these *E. coli* strains, highlighting the presence of strains with AR and virulence traits in the environment.

Antimicrobial activity of extracts of medicinal plants of traditional use in Mexico

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Antibiotic resistance is a growing problem globally, so the search for new alternatives is a priority. Recently, the appearance of various bacterial strains resistant to 3rd and 4th generation cephalosporins, piperacillin and ciprofloxacin has been reported in several hospitals in Mexico. A pharmacological alternative for the treatment of some infections is the use of plant extracts with antimicrobial activity.

In this work the antimicrobial activity of the extracts of *Artemisia absinthium* (wormwood), *Artemisia ludoviciana* (estafiate), *Dysphania ambrosioides* (epazote), *Mentha piperita* (mint), *Datura ferox* (toloache), *Rosmarinus officinalis* (rosemary) and Ruta graveolans (rue) were evaluated against ten bacterial strains of medical importance (EPEC, ETEC, UPEC, DAEC, *Staphylococcus aureus, Shigella flexneri, Enterobacter cloacae, Citrobacter koseri, Klebsiella pneumoniae* and *Clostridioides difficile*). Using the Kirby-Bauer method, the inhibitory concentration of the extracts was established, their effect on membrane integrity, alteration of protein synthesis and DNA degradation were subsequently evaluated. In addition, qualitative tests were carried out to determine the presence of alkaloids, polyphenols and saponins that could be responsible for the activity of the extracts against the microorganisms.

The results obtained suggest that a mixture of different extracts would allow better activity on various bacterial genera. The plants analyzed in this study provide a promising source of antimicrobial compounds that can be applied in the pharmaceutical industry as antibiotics, antiseptic and disinfectants.

"Drug resistance dynamics and virulence factors of *Escherichia coli* uropathogen strains (UPEC) from different years in Chihuahua city"

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Introduction. The worldwide misuse of antibiotics, has led the causing bacteria of infecctious disease to develop resistance The resistance and virulence genes are transferred between bacteria, spreading and complicating their elimination. In 2017 the WHO published the list of bacteria for wich new antibiotics are urgently needed. The first of three categories includes antibiotic multiresistant bacteria, dangerous in hospitals, as *E.coli*, the most common pathogen in urinary tract infections (UTI) which has high rates of resistance. **Objective.** Determinate the drug resistance dynamic, and virulence factors of *E.coli* uropathogen strains (UPEC) from different years in Chihahua city. **Methodology and Results.** Three collections of UPEC strains from 2014, 2017 and 2019 were analyzed. Susceptibility to cefotaxime, ceftriaxone, ceftazidime and cefepime was determined by the Kirby-Bauer technique, in 2014 12.82% resistance to ceftriaxone was found, 84.62% in 2017 and 94.44% in 2019. For cefotaxime the resistance was 20.51%, 92.31% and 100%, for 2014, 2017 and 2019 respectively;10.26% resistance to ceftazidime of was found in 2014, 69.23% in 2017 and 68.52% in 2019; the resistance for cefepime was 10.26% in 2014, 76.92% in 2017 and 55.56% in 2019. The cefinase test was used for the beta-lactamases production, finding that 41% (2014), 69% (2017) and 76% (2019) of the strains were producing them. Alpha-hemolysin production (Beutin, 1989) was found in 100% of the 2014 and 2017 strains, 91% produced alpha-hemolysin and 9% of 2019 strains produced beta-hemolysin. Tube and plate biofilm formation (Stepanovic, 2000) showed 38% (2014), 15% (2017) and 35% (2019) of moderate adherent strains, while 41% (2014 and 2019) and 46% of 2017, adhered weakly in tube. Moderate plate adhesion was observed in 46%, 31% and 52% of the 2014 strains, 2017 and 2019 respectively, and strong adhesion in 8% (2014), 69% (2017) and 35% (2019) of UPEC strains. **Conclusions.** The progressive cephalosporins resistance increase and beta-lactamases production; alpha hemolysin producing strains prevalence and the presence of beta hemolysins in the last year; as well the moderate and strong adhesion biofilm formation, indicate the virulence dynamics of UPEC strains through the years and the need for adequate therapeutic strategies to delay, or even prevent the evolution of these strains towards more virulent and difficult to attend genotypes.

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Antifungal activity of poly(2-(dimethylaminoethyl)methacrylate) with different alkyl halides and quaternization degrees

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The historic monuments located at the south of Mexico whose main component is calcareous rock, are colonized by various types of microorganisms mainly by fungi due to their environmental conditions such as high humidity, temperature and solar radiation which promotes their accelerated growth. Fungi causes an aesthetic damage originated by melanin, physical damage through their hyphae that penetrates the rock, also chemical damage which dissolve the calcium carbonate, producing acids that weaken the rock causing the appearance of cracks and fissures. Therefore, one of the challenges in the conservation of cultural heritage is to propose new materials with antifungal properties that are friendly to the environment, and contribute to minimize their biodeterioration. This study evaluated the antifungal effect of 21 polymers of p-DEMAEMA [Poly(2-(dimethylaminoethyl)methacrylate)] grouped in 3 lots according to their molecular weight (47.7, 17.8, and 9.7 kDA), with different alkyl halides (iodomethane, iodobutyl) and degrees of quaternization (5%, 12.5% and 25%) and the polymer without quaternization. Minimum inhibitory concentration (MIC) was determined by the microdilution method against five biodeteriorant fungi: *Aspergillus niger Penicillium oxalicum, Phoma eupyrena, Pestalotiopsis maculans* and *Curvularia lunata* in a 96-well microplate, making serial dilutions of the different polymers in concentrations of 5 mg/ml to 0.001 mg/mL in potato dextrose broth (PDB) and inoculated with a spore suspension of $1x10^5$/mL. The microplates were incubated for 24 to 48 h and the MIC was determined with the help of an optical microscope, then 50 µL of each well was transferred to microplates with PDB, they were incubated and the minimum fungicidal concentration (MFC) was determined. Of the three lots, the best results were observed in polymers with lower molecular weight and with the iodobutyl halogen. At higher degree of quaternization, a lower MIC was required (2.5 to 0.019 mg/mL) to cause irreversible spore germination (fungicidal effect) for all fungi, except against *C. lunata* that generally required a higher concentration of the polymers (5 mg/mL) with a fungistatic action mode. This is the first report of p-DEMAEMA as a fungicide; the effective capacity to inhibit spore germination suggests the use of these polymers in the control of the various fungi that colonize the cultural heritage built with calcareous rock.

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Surveillance of carbapenem-resistant *Pseudomonas aeruginosa* in a tertiary care pediatric hospital in Mexico City

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Background. *P. aeruginosa* is an opportunistic pathogen, is one of the major bacteria causing hospital acquired-infections. Carbapenems are one of the last-line antimicrobials for treatment; however, the emergence and spread of carbapenem-resistant strains decreases the therapeutic options. In Mexico, information about carbapenem resistant *P. aeruginosa* causing infections in pediatric patients is limited. **Objective.** To surveillance carbapenem-resistant *P. aeruginosa* strains obtained from pediatric patients.

Material and Methods. During a 14 months period (January 2018-February 2019), we collected 62 carbapenem-resistant *P. aeruginosa* obtained from clinical infections in patients of INP. The identification and susceptibility profile were determined using a semi-automated System. Phenotypic detection of carbapenemases was performed by CarbaNP test and Modified Carbapenem Inactivation Method (mCIM). Molecular detection of carbapenemases genes (*bla*VIM, *bla*IMP, *bla*KPC, *bla*NDM, *bla*OXA-48 and *bla*GES) was performed by PCR and sequencing. The clinical and epidemiological data were collected.

Results. The susceptibility profile of the isolates was: ceftazidime 33%, cefepime 38%, imipenem 2%, meropenem 8%, piperacillin-tazobactam 41%, ciprofloxacin 52%, levofloxacin 47%, amikacin 55%, gentamicin 44%. CarbaNP test was positive in nine strains and mCIM was positive in 12 strains. Carbapenemases genes were detected in 11 isolates, *bla*IMP was the most common (n=7), we also found *bla*VIM y *bla*GES in two strains, respectively. Coexisting of *bla*IMP and *bla*GES was detected in two isolates. Carbapenemase gen was not detected in one strain. Eighty-nine percent of patients had some underlying disease; 97% received meropenem and cefepime as empirical treatment. All the infections were health-care associated, which pneumonia was the more frequent (40%). Eighty-seven percent of patients had complications. The mortality in this study was 18%, the cause of death was septic shock.

Conclusion. Carbapenemase-production was not the main carbapenem-resistance mechanism in *P. aeruginosa*. The frequency of carbapenemase-producing *P. aeruginosa* was 19%. The surveillance system allowed us to detect the emergence of GES carbapenemases. *P. aeruginosa* AMR strains limit therapeutic options, however; it still observes isolates with susceptibility to antibiotics such as ceftazidime and piperacillin-tazobactam as the last line treatment.

Effect of Sodium Hexanoate on Planktonic Cells and Biofilms Formation of *Klebsiella pneumoniae*

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Klebsiella pneumoniae, a Gram-negative bacterium, is a natural inhabitant of the microbiome of the gastrointestinal tract of healthy humans and is an opportunistic pathogen of both humans and animals. This pathogen causes urinary and respiratory tract infections and septicemia, especially in immunocompromised individuals. The ability of *K. pneumoniae* to form biofilm result in persistent infections and dissemination of disease through medical devices. Biofilms are resistant to antibiotics and to the host immune system. According to World Health Organization, *K. pneumoniae* is considered a critical priority pathogen due to its multidrug resistance (MDR). For this reason, it is urgent to discover/develop new antimicrobial agents. In this sense, the use of natural compounds such as fatty acids (FA) is a very attractive alternative. Among the advantages of using FA are that (i) their microbicidal activity occurs in low concentrations (µM-mM), (ii) the resistance selection of FA is low, and (iii) FA are chemically stable and naturally degrade. In this sense, sodium hexanoate (NaH, C6) is an important energy source present in foods as triglycerides (i.e. butter, cheddar, coconut oil). Besides, this FA is present in fat´s goat milk. It is known that NaH can modulate the innate immune response in epithelial cells which is related to a decrease in bacterial internalization. However, little is known about the direct antimicrobial effect of sodium hexanoate (NaH, C6). For this reason, the aim of this study was to evaluate the effect of NaH on (i) planktonic cells and (ii) biofilm formation of *K. pneumoniae*. For this, five strains of *K. pneumoniae* MDR were used (KL-08, KL-10, KL-147-III, KL-1006009851 and ATCC 700603). The strains biofilm formation capacity was determinate by microtiter plate test and with violet crystal method. The antimicrobial activity of NaH (1-100 mM) *on K. pneumoniae* was monitored (24, 48 and 72 h) in terms of optical density (OD_{600}). The NaH effect on biofilm formation was assessed on microtiter plate and crystal violet method (24 h). Our data showed that NaH (50-100 mM) possess antimicrobial activity on all strains of *K. pneumoniae* MDR. In addition, three strains (KL-08, KL.10 and 1006009851) were found to be strong biofilm formers. Finally, NaH (75 mM) possess anti-biofilm formation effect due to it reduces biofilm formation of KL-08 and KL-10 strains (74 and 80%, respectively). Further, this FA (75 mM) only inhibits 42% the biofilm formation of 1006009851 strain. Taken together, these results suggest that NaH is a novel antimicrobial molecule with potential utility against MDR pathogens.

Live Attenuated *Salmonella enterica* Expressing Cell-permeable Bax BH3 Peptide Elicits Antitumor Activity in a Murine Xenograft Model of human B Non-Hodgkin´s Lymphoma.

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Drug-resistance represents an obstacle to completely eradicating tumor cells in patients with Non-Hodgkin Lymphoma, therefore it is necessary to find new antitumor therapies that can completely eradicate transformed cells. Chemotherapy-resistant cancer cells are characterized by the overexpression of members of the antiapoptotic Bcl-2 proteins family. Recently we have shown that peptides derived from the BH3 domain of the proapoptotic Bax protein may antagonize the antiapoptotic activity of the Bcl-2 proteins family, restore the apoptosis and induce chemosensitization in tumor cells. In this work, we investigated the feasibility of releasing this peptide to the tumor microenvironment using a live attenuated *Salmonella enterica* serovar Typhimurium SL3261, which has been proved to be an ally in the therapy of cancer due its high affinity for tumor tissue, ability to activate the innate and adaptive antitumor immune response and its potential use as delivery system of heterologous molecules. Recombinant Salmonella strains that carry or release the cell-permeable Bax BH3 peptide on their surface were obtained by recombinant DNA Technology, and the expression of the recombinant proteins was evaluated by SDS-PAGE, WB and Immunofluorescence. The viability of the tumor cells infected with the different recombinant Salmonella strains, was measured by trypan blue, and the induction of apoptosis was evaluated by active caspase 3 and TUNEL. The antitumor effect of recombinant *Salmonella enterica* strains, was analyzed using a murine xenograft model of human B non-Hodgkin Lymphoma. Thus, we expressed and released the cell-permeable Bax BH3 peptide from the surface of *Salmonella enterica* through the MisL autotransporter system. We demonstrated that this recombinant bacterium significantly reduced the viability and increased the apoptosis of Non-Hodgkin Lymphoma cell line. Indeed, the intravenous administration of this recombinant *Salmonella enterica* elicited antitumoral activity and extended survival in a murine Xenograft model of human B Non-Hodgkin´s Lymphoma. Our results confirmed that Live Attenuated *Salmonella enterica* serovar expressing and releasing cell-permeable Bax BH3 peptide may represent an eventual alternative to treat relapsed or refractory Non-Hodgkin's Lymphoma.

Mycobacterium tuberculosis biofilms susceptibility to compounds with antimicrobial activity

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One third of the world population is infected with the tubercle bacilli, potentially leading to the development of highly contagious tuberculosis active form. Studies suggest that *Mycobacterium tuberculosis* (MTB) and non-tuberculous mycobacteria (NTM) can develop biofilm microcolonies in animal and human tissues. These microbial matrices provide bacterial persistence and resistance to drugs, possibly hampering the elimination of latent infection in healthy household contacts and patients. Therefore the development of new inhibitors or disruptors of biofilms is considered as an alternative to increase the efficiency of current drugs, as well to reduce the period of treatment and consequentially its toxicity. In this study we evaluated the effect of recently isolated flavonoids from *Rhynchosia precatoria* roots on biofilm growing bacteria, to potentially be used as co-adjuvant in latent tuberculosis treatment. To achieve this *M. bovis* BCG Pasteur (a member of *M. tuberculosis* complex) was used as a model for MTB since antimycobacterial drugs (rifampicin, isoniazid and ethambutol) demonstrated similar minimal inhibitory concentrations (MIC), as well as the bioactive compounds from *R. precatoria* by the fREMA method. Likewise, biofilm formation evaluated by crystal violet staining showed the mycobacteria's ability to proliferate and develop biofilm in presence of inhibitory and super-inhibitory drug concentrations. Of the bioactives compounds tested, Pre-A proved to delate the biofilm development and in a lesser degree compared with the untreated control. Cell distribution on biofilms analyzed by CFSE/IP staining and Confocal Microscopy displayed diverse morphologies possibly associated to different action mechanisms. Moreover, the pre-treated biofilms with Pre-A exhibited less roughness than untreated and rifampicin controls, as analyzed by Atomic Force Microscopy. In conclusion, Pre-A a compound with antimicrobial activity against MTB planktonic growth, is also able to induce morphological changes in BCG biofilms, however its action mechanism is yet to be described.

Bacteriostatic and cytotoxic effect of methanol extracts of *Echeveria craigiana*, *E. kimnachii*, and *E. subrigida* on six diarrheagenic *Escherichia coli* pathotypes

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Diarrheagenic *E. coli* (DEC) pathotypes are *E. coli* strains that have acquired diverse virulence factors by horizontal gene transfer, based on these factors and their pathogenic mechanisms DEC are classified in six pathotypes: enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), diffusely-adherent *E. coli* (DAEC), and shiga-toxin producing *E. coli* (STEC) that encompasses the enterohaemorrhagic *E. coli* (EHEC) group. DEC pathotypes are the main aetiological group isolated from children with acute and persistent diarrhoea in less developed regions of Latin America, including Mexico and developing countries from Asia and sub-Sahara Africa. Also, several food items from these regions harbour DEC strains in enough quantifies to cause foodborne illness. Some of these DEC-strains have shown antimicrobial resistance and multidrug resistance profiles. Consequently, effective and accessible therapeutic alternatives are required, natural products and their components could be a good option. The study aim was to evaluate the bactericidal activity of methanolic extracts of three *Echeveria* species (*E. craigiana, E. kimnachii,* and *E. subrigida*), collected in Sinaloa, against six DEC reference strains (EPEC E2348/69, ETEC H10407, EAEC 042, EIEC E110124, DAEC C1845-A and EHEC EDL933). Bactericidal activity was determined by a tube assay developed by use 1.5 mL tubes containing 50 μL of 300 CFU (at mid-log growth) in DMEM-High Glucose media and 50 μL of methanol extracts at concentrations of 200, 20 and 2 μg/mL, were incubated at 37 °C for 1.25, 2.5 and 5 h. Bacterial growth was determined by the drop-plate technique. Bacterial protein concentrations and death by a Bio-Rad Protein assay and propidium iodide FACS staining, respectively. Methanol extracts cytotoxicity by a Caco-2 cell assay. The three *Echeveria* extracts inhibited the growth of all six DEC strains, in a dose dependent fashion. At 5 h post-incubation, extract concentrations of 100 μg/mL inhibited bacterial growth between 95-99%, nevertheless, these bacteria were not stained with propidium iodide. Protein concentrations of treated DEC were significantly less than non-treated bacteria. At 24 h post treatment, all DEC pathotypes growth rates were similar to those of none treated bacteria. After 5 or 24 h incubation, *Echeveria* methanolic extracts did not exert any cytotoxic effect on Caco-2 cells. Methanolic extracts of *E. craigiana, E. kimnachii,* and *E. subrigida* have a bacteriostatic effect on all tested reference DEC-pathotypes but not a cytotoxic effect. *Echeverria* extracts have the potential to be used as disinfectant of food and water contaminated with DEC pathotypes.

Tetracycline resistance in *E. coli* strains isolated from surface and wastewaters in Reynosa

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Introduction. The tetracyclines are one of the most used classes of antimicrobial agents in human, veterinary medicine and agriculture due to their broad-spectrum activity and low cost. However, this family of antibiotics are not completely metabolized and are excreted into the environment in feces and urine without into wastewater, likewise, they are not eliminated during the wastewater treatment, therefore, and these residues can reach surface waters and farming. Tetracycline resistance is mainly caused by the acquisition of tet genes through horizontal gene transfer. Therefore, the objective of this work was to evaluate tetracycline family compounds resistance in *E. coli* strains from aquatic sources of Reynosa, Tamaulipas. **Materials and methods**. In this study, 50 strains of *E. coli* isolated from surface water (Rio Bravo) and residual water were collected in Reynosa, Tamaulipas, the susceptibility to tetracycline, doxycycline, and minocycline were evaluated by the disk diffusion method according to CLSI 2018. Extraction of DNA was realized by the boiling extraction method to the strains that showed tetracycline resistance, by multiplex PCR *tet*(A), *tet*(B), *tet*(C), *tet*(M), and *tet*(X) were detected. **Results**. A total of 50% (25/50) *E. coli* strains were resistant to tetracycline, 32% (8/25) to minocycline and 48% (12/25) to doxycycline. While, the tetracycline resistance gene *tet*(A) (88%) was the most prevalent, followed by *tet*(B) 16%, *tet*(M) was 1%, *tet*(C) and *tet*(X) were absent. Likewise, the presence of multiple *tet* genes was detected in the same strain, *tet*(A)/*tet*(B)/*tet*(M) and *tet*(A)/*tet*(B). **Conclusions.** Native *E. coli* strains from surface and waste waters in Reynosa have a wide resistance to tetracycline antibiotics family, and tet related genes alone or in combination can be found in those strains, indicating that horizontal antibiotic resistant transference may be a risk to public health in our region.

Inhibitory activity of plant extracts from the semi-desert of Coahuila against spores of phytopathogenic fungi.

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Introduction. In Mexico, modern agricultural production technology requires the continuous and sometimes irrational use of large volumes of agricultural inputs such as fertilizers and pesticides. The use of non-polluting disease control alternatives is of utmost importance to achieve an efficient agricultural production without environmental deterioration, with agricultural products and healthier foods, without affecting the productivity and quality of them. Plant residues and extracts with antimicrobial properties can play an important role in an integrated ecological system of agricultural production for the control of diseases. The objective of this work was to evaluate the effect of in vitro inhibition of total ethanolic extracts of *Larrea tridentata, Gnaphalium oxyphyllum and Eucalyptus melliodora* against the spore hatching of the fungi *Aspergillus flavus, Aspergillus niger and Neurospora crasa*. Methodology. Samples of 50g of milled plant were weighed, placed in an Erlen Meyer flask and added with 250 mL of absolute ethanol. The samples were left in continuous agitation for a period of 72 hours. The mixture was filtered in vacuo to remove the vegetable residues and the filtrate was concentrated in a Rotavapor at negative pressure 120 rpm and 60 ° C. Extracts of *Larrea tridentata, Gnaphalium oxyphyllum and Eucalyptus melliodora* were obtained. Each fungus was cultivated in a petri dish with 20 mL of PDA at 26 ° C for 15 days. Grown fungi were used for the preparation of a spore suspension at a concentration of 1000 spores / mL he PDA plates were prepared with each of the extracts of *Larrea tridentata, Gnaphalium oxyphyllum and Eucalyptus melliodora* at concentrations of 0, 200, 400, 600, 800 and 1000 µL / mL. With the help of a sterile capillary, they were inoculated with the spore suspension. The treatment with each of the phytopathogenic fungi were incubated at 36 ° C for 5 days. The plates were examined after 5 days of incubation and the radial growth diameter was measured in centimeters. Results The extract of *Larrea tridentata* showed greater inhibitory power in the spore emergence of the problem fungi in all its concentrations, allowing only a radial growth of the problem fungi from 0.1 to 0.8 mm. The *Eucalyptus melliodora* and the *Gnaphalium oxyphyllum* allowed a greater growth in comparison to the *Larrea tridentata*, but lower than the growth of the control fungi without treatment. Conclusion. In general, the extract of *Larrea tridentata* caused the best fungicidal effect for the fungi used in this study. Therefore, it is recommended to continue this line of research since it is very important to find new sources of control of phytopathogenic fungi for the benefit of agriculture and the environment.

Genomic and pathogenicity determination of hypermucoviscous *Enterobacteriaceae* clinical isolates

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The hypervirulent *K. pneumoniae* (hvKpn) variant is capable of causing severe community-acquired infections, affecting healthy and immunocompromised population. hvKpn displays a distinctive phenotype named hypermucoviscosity (hmv). The hmv is defined by the formation of a viscous filament. Two plasmid-borne regulators *rmpA* and *rmpA2* are involved in the hmv phenotype acting in the *cps* locus, triggering the overproduction of capsular polysaccharides. An increasing population of *K. pneumoniae* isolates displaying the hmv phenotype without *rmpA* and *rmpA2* genes associated have been reported. Other *K. pneumoniae* related species such as *K. variicola* and *K. quasipneumoniae* have been reported with this phenotype and as well the genes *rmpA y rmpA2* were absent. Some forms of immunodeficiency such as diabetes represents a greater risk for infections with classical and hypervirulent *K. pneumoniae* strains. We evaluate different hypermucoviscous *Enterobacteriaceae* isolates using healthy and diabetic mice models in order to depict pathogenicity traits and correlated with the genomic content. The tested strains belonged to *K. pneumoniae, K. variicola, K. quasipneumoniae, K. oxytoca* and *E. coli.* We performed antimicrobial susceptibility test, the plasmid profile, chemical plasmid curing, serum resistant killing assay and resistance to phagocytosis. Genomes were annotated with Prokka 1.13 in order to implement the suite ROARY for comparative genome analysis. Healthy male BALB/c mice of 7-8 weeks old were used to inoculate 10^7 and 10^8 UFC of *Enterobacteriaceae* strains using intravenous route. The hypermucoviscous strains inoculated in the healthy mice model were not able to induce mortality however, we observed that some strains caused tissue damage in the form of abscess mainly in the gallbladder and liver after 15 days post inoculation. The pan genome of hypermucoviscous strains was composed by 17,667 genes, core genome of 344 genes and the variable genome of 5,100 genes. These strains possessed classical virulence factors and 50% of the strains were multidrug resistant. One strain *K. variicola* 8917 was successfully curated, we observed that the hypermucoviscous phenotype is lost when the plasmid (pKV8917) is segregated. IncFIIB family was found associated to the plasmids. *K. pneumoniae* 3478 showed resistance to serum and *E. coli* 10270 showed resistant to phagocytosis. We conclude that hypermucoviscous strains unlike hypervirulent strains in a healthy model are unable to produce mortality but tissue damage was observed. Probably an immunocompromised status may favor the risk for infections with hypermucoviscous strains. Diabetic mice model is under evaluation. Plasmid curing showed the loss of hypermucoviscosity in one strain this suggest that the locus required for hmv phenotype is plasmid located. Virulence assays showed a heterogeneous behavior.

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Microevolution of multidrug-resistant *Pseudomonas aeruginosa* to a chronic pathogen phenotype in patients with Cystic Fibrosis in Mexico

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Abstract. Cystic fibrosis (CF) is an autosomal recessive disorder originated by mutations in the CF Transmembrane Conductance Regulator (CFTR) gene. The patients with CF have a defective mucociliary clearance and an airway obstruction caused by cumulation of dehydrated and mucopurulent secretions. These conditions create a perfect niche for microbial colonization. Commonly, the airways of Caucasian pediatric patient are initially colonized by *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*. In Mexico, the prevalence of bacterial infections in pediatric patients with CF is not available. The purpose of the study was to identify the prevalence of bacterial infections in Mexican CF pediatric patients. In this study, we obtained samples of sputum from 52 pediatric patients with CF, the clonal relatedness was assessed by Pulse Field Gel Electrophoresis (PFGE), the antimicrobial susceptibility was determined by VITEK 2 and by MIC, as well the biofilm production was determined on a polystyrene surface and the motility was determined on 0.3% agar and finally, the susceptibility to normal human serum (NHS) was determined by reduction of CFUs. The results show that from 52 CF pediatric patient studied between 2016 and 2018, we isolated 158 bacterial pathogens, 48.2% (76/158) were *P. aeruginosa* and 32.3% (51/158) *S. aureus*. By PFGE we identified 49 clones from *P. aeruginosa* isolates, 34 clones from *S. aureus* isolates. The 78.8% (41/52) of *P. aeruginosa* shows a multidrug-resistant phenotype and 21.2% (11/52) an extreme-drug resistant phenotype. The 19.2% (10/52) of patients are colonized by clones of *P. aeruginosa* that shows a phenotype of chronical infection and 39.8% (16/52) with a transitional phenotype. Finally, 30.6% (15/49) of analyzed isolates were higher resistant to NHS activity (≥ 60%) and 24.5% (12/49) moderated resistant to NHS. The rapid identification of these bacterial pathogens in pediatric patient with CF might be important to decrease its mortality risk.

Antivirulence properties of synthetic organic compounds and plant extracts in *Salmonella enterica*.

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In the last decade, treatments against bacterial infections have been inefficient due to the rapid acquisition of antibiotics resistance. In this work we propose the use of antivirulence compounds as a complementary treatment of antibiotics. Antivirulence are based on blocking virulence factors, those that participate in the pathogenesis and are not indispensable for the viability of the bacteria. The objective of this work was to analyze the antivirulence potential of synthetic organic compounds, as well as plant extracts, using models such as the human pathogens *Salmonella enterica* serovars Typhimurium and Typhi. The antimicrobial and antivirulence potential of synthetic organic compounds, fibrate analogues and atorvastatin analogues were analyzed; as well as plant extracts such as hibiscus. It was found that compound DAM2-352 and DAM-3891 partially inhibits the secretion of the proteins through the type III secretion system 1 of *S.* Typhimurium. Additionally, several plant organic extract were analyzed for their antimicrobial and antivirulence properties. Our results showed that the hibiscus organic extract inhibits bacterial mobility of *Salmonella* Typhi and the fraction with activity obtained by column chromatography was identified and it will be used for further studies. In conclusion, compounds and extracts that inhibit *Salmonella* virulence factors were found and currently are being characterized.

Antimicrobial Activity of the Essential Oil of the Regional Plant *Rhus trilobata*

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Rhus trilobata (*Anacardiaceae* family; RHTR) is considered as a medicinal plant typical of northeastern Mexico. The antineoplastic and amoebicidal capacity of RHTR extracts has been reported. However, the antimicrobial potential of its essential oil has not been studied. The objective of this work was to determine the antimicrobial activity of the essential oil on clinically relevant ATCC strains and isolated clinical strains at the General Hospital of Chihuahua "Dr. Salvador Zubirán Anchondo."

Fresh RHTR leaves were collected from a community of Namiquipa, Chih, and transported to the laboratory where subsequently, the essential oil was obtained by hydro-distillation with steam entrainment using 100 g of plant material per liter of water. For the evaluation of the antimicrobial activity, the microorganisms were recovered in trypticase soy broth (TSB): *Klebsiella pneumonia and Salmonella typhi* and one strain of *Staphylococcus aureus* were ATCC, *Pseudomonas aeruginosa, Acinetobacter baumannii* and two strains *of Staphylococcus aureus* isolates were provided by the General Hospital of Chihuahua. Due to the RHTR essential oil density, the agar diffusion variant was implemented using a well added with 80 µL of RHTR; as controls, cultures treated with Ampicillin (AMP; 10 µg), Streptomycin (STR; 10 µg), Ceftazidime (CAZ; 10 µg) or Ertapenem (ETP, 10 µg) were included.

The results showed a mean inhibition halo for RHTR essential oil of 19.64 mm on all strains tested. In the case of the *S. aureus* ATCC strain the mean of inhibition showed no difference respect to the control with AMP, for the *K. pneumonia* and *S. typhi* ATCC strains the RHTR oil showed an inhibition significantly greater than the control with STR. As for nosocomial strains of *S. aureus* and *A. baumannii*, the essential oil of RHTR had a significantly greater inhibition than controls with AMP and CAZ, respectively. Only in one nosocomial *P. aeruginosa* strain, no difference in inhibition was detected with the RHTR essential oil and the control with CAZ.

Additionally, morphological changes were observed in *P. aeruginosa* and *A. baumannii*, which suggest a mechanism of action related to alterations in the cell wall.

These results demonstrate that the essential oil of RHTR has an important antimicrobial activity even against multiresistant isolates. Experiments are ongoing to determine the main components of the essential oil, as well as the determination of the minimum inhibitory concentration using a colorimetric microassay.

Molecular characterization of clinical isolates of *Klebsiella pneumoniae* carrying *bla*$_{NDM-1}$ and *bla*$_{TEM}$

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INTRODUCTION. *Klebsiella pneumoniae* is a pathogen that exhibits high antimicrobial resistance and belongs to the WHO critical priority group for the development of new antibiotics. Also is a bacteria that "collects" plasmids which allows it to acquire resistance genes from multiple families of antibiotics including β-lactamase enzymes genes due to their wide global spread.

OBJECTIVE: Identify molecularly the production of β-lactamases, as well as the plasmid and clonal diversity of clinical isolates of *K. pneumoniae* that cause nosocomial bacteraemia.

MATERIAL AND METHODS. *K. pneumoniae* isolates were collected during 2017 from the Hospital Civil de Guadalajara (HCG). The identification and susceptibility profile were performed using the Vitek-2 system. The clonality was obtained by performing PFGE. The type of extended-spectrum β-lactamase (ESBL) was determined by endpoint PCR and the type of carbapenemases by PCR-multiplex. Resistance transfer was determined by conjugation assays, using *E.coli* J53-2 as the recipient strain. The visualization of the transfer of plasmids in the obtained transconjugants was resolved by electrophoresis in an Eckhardt gel, and the Inc group to which the plasmids belong was determined by multiplex PCR. The isolates carrying carbapenemases were characterized by multi-locus sequence type (MLST). **RESULTS.** The 80 isolates were clustered in 69 clones with a similarity of 66%. The phenotypic production of ESBLs was 78% (35/45) and MBLs 22% (10/45). *bla*$_{TEM}$ gene was carried in 91% (32/35) of the ESBL-producing isolates and 100% (10/10) of the MBL-producing isolates carried *bla*$_{NDM-1}$ gene, of these, 20% (2/10) coexisted with *bla*$_{TEM-type}$ gene and 40% (4/10) with *bla*$_{TEM\ -type}$ and *bla*$_{CTX-M-type}$ genes. The 50% (5/10) of *bla*$_{NDM-1}$-producing *K. pneumoniae* were able to transfer resistance to carbapenems, cephalosporins and aminoglycosides by means of a conjugative plasmid belonging to the IncF family of approximately 195 Kpb. The MLST analysis determined that *bla*$_{NDM-1}$-producing isolates belong to ST661, ST683, ST1395, ST2706, ST252, ST1198, ST690, ST1535 and ST3368.

CONCLUSIONS. The results demonstrated high variability among *K. pneumoniae* clones carrying *bla*$_{NDM-1}$ and *bla*$_{TEM}$ plasmidic genes. The transfer of these genes demonstrated the consequent antimicrobial resistance acquisition. Relevantly this is the first report of different STs in *K. pneumoniae* associated with *bla*$_{NDM-1}$ in Mexico.

Characterization of Gallium resistance induced in a *Pseudomonas aeruginosa* cystic fibrosis isolate

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The repurposing of gallium nitrate, a drug used for the treatment of hypercalcemia of malignancy, as an antibacterial is a plausible alternative to combat infections by *Pseudomonas aeruginosa*, since it presents remarkable antipseudomonal properties *in vitro* and *in vivo* in animal models and human lung infections; furthermore, gallium nitrate tolerance in clinical isolates is very rare. Nevertheless, studies on the reference strains PA14 and PAO1 show that resistance against gallium nitrate is achieved by decreasing its intracellular levels and by increasing the production of pyocyanin. In this work, we induced resistance in an initially gallium sensitive cystic fibrosis *P. aeruginosa* isolate, and explored its resistance mechanisms. This isolated strain INP-58M was not a pyocyanin producer and its pyoverdine levels remained unchanged upon gallium addition. However, it showed higher activities of NADPH-producing enzymes and the antioxidant enzyme SOD when gallium was added, indicative of a better antioxidant response. Remarkably, gallium intracellular levels of the resistant isolate were higher than those of the parental strain at 20 h but lower after 24 h of culture, suggesting that this strain is capable of gallium efflux.

Detection of clonal complex of *Staphylococcus aureus* isolates obtained from blood cultures of pediatric patients admitted in a tertiary care hospital in Mexico City

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Background: Methicillin-sensitive *S. aureus* (MSSA) and methicillin resistant (MRSA) *S. aureus* are important causes of infections in both hospital and community settings. Different types of clonal complex (CC) have been described, their distribution had been associated to certain geographic regions. In Mexico, the information about distribution of CC in isolates from pediatric patients is limited. **Aim:** To detect the clonal complex of *S. aureus* isolates obtained from blood cultures in pediatric patients admitted at Instituto Nacional de Pediatria. **Material and methods:** From 2006 to 2016, we collected 245 isolates of *S. aureus*. Identity of the isolates was confirmed by the analysis of *nuc* and 16S rRNA genes. The *mecA* gene was amplified and the SCC*mec* typing was performed. The CC was detected by amplification of *sau1hsdS1* and *sau1hsdS2* genes. **Results:** We detected cefoxitin resistance in 37% of isolates; all of them had *mecA* gene: and we classified the SCC*mec* as follows: SCC*mec*-II 89%, SCC*mec*-IV 6.5% and SCC*mec*-I 3.2%. Six CC were identified in all *S. aureus* isolates. The CC distribution in MSSA isolates was: CC5 -28.5%, CC30- 22%, CC45-12.9%, CC1-2.5%, CC22-1.9%, and CC8 1.2%, 47 MSSA isolates were not typing. The CC of MRSA were distributed as follows: CC5- 60%, CC45-6.5%, CC8- 2.1%, CC30-1%, CC22-1%, and CC1-1%, 25 MRSA isolates were not typing. The MRSA-SCC*mec*-II isolates belonged to CC5, CC22, CC45, while MRSA-SCC*mec*-IV, CC45, CC8 and CC1 **Conclusion:** SCC*mec*-II was the most common in MRSA. CC5 was the most frequent in all *S. aureus*. The MSSA and MRSA had a different distribution of CC.

TAXONOMY AND SYSTEMATICS

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Comparing silico tools for metabolical prediction: Tax4Fun and Picrust

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Nowadays on the biologic science is producing a greater number of data that has become essential the development of bioinformatic tools to allow the processing all it. The metagenomic studies is an example of this. Here we will be present a case the bacterial phylogenetic and functional diversity of soil samples by analysis of metagenomic data using 16S rRNA gene. The samples where taken from Sao José Do Valle Do Rio Preto (RJ, Brazil), an important agricultural region: 2 samples were taken from a farmer that use poultry litter as an fertilizer (S1 and S2), one from a poultry houses (S3) that supply these farmers and one soil control (S0) of a natural rain forest around of the site. The total DNA was extracted and the V4-V6 region of 16S rDNA gene were sequenced by Illumina MiSeq platform (Macrogen Lab). Trough in silico data analysis was done following the pipeline described on Brito at al (2019), and OTU tables was used for a metabolically prediction by Tax4Fun and Pycrust. A total of 2,553,275 reads were obtained: 685,447 for S0, 622,641 for S1, 617,323 for S2 and 627,864 for S3, resulting in 2,699 Operational Taxonomic Units (OTUs) and 1896 chimeras. Using Simpson and Shannon-Wiener diversity indexes was estimated highest richness in soils from open environments (S0, S1 and S2), while to S3 the richness was around three times lower. The bacterial communities of the soil inside shed (S3) were hardly distinct respect to the control soil, while S1 and S2 soils showed to be more similar among them. We also used the PCA analysis to infer the relationship between environmental parameters and the bacterial biodiversity: the N, P and Pb concentrations were the parameters that most influenced the dispersion of S1 samples, while the S2 sample was more influence by Cr, CEC, Cu, Cd, Zn, OC, pH, and Mg contents and S3 was influenced more by Mn and Cd concentrations. The prediction of functional and metabolic profiles (based on KEGG database) showed that highest percentage of genes related to metabolic functions (50-56%) followed by the ones related to environmental information processing (23-26%), the functional profile genetic information processing had 5-8%, the human diseases 2-3% and to organismal systems 1%.

Burkholderia pseudomallei identification in México

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The genus *Burkholderia* is comprised by 3 bacterial groups: the *Burkholderia cepacia* complex, the *Burkholderia pseudomallei* group and a cluster of phytopathogenic bacteria. Within the *B. pseudomallei* group, exists *B. pseudomallei* the causal agent of melioidosis, an infectious disease that can be mistaken for pneumonia or tuberculosis. It forms abscess anywhere in the patient. People with diabetes mellitus are at greater risk to contract melioidosis. *B. pseudomallei* is easily isolated from soil in tropical environments. It is frequently found in the north of Australia and southeast of Asia, although it has also been found in Africa and central and south America. Recently, three mortal cases were detected in Mexico, two in Sonora and one in Oaxaca States). However, the distribution of *B. pseudomallei* in Mexico is virtually unknown. In this study, soil and sediment samples collected from Sonora, Tabasco, Chiapas, Yucatan and Coahuila were analyzed. The samples were inoculated on Ashdown plates where *B. pseudomallei* shows a typical purple wrinkle aspect. The potential *B. pseudomallei* isolates were identified by comparing the 16S rRNA sequence in the EzBiocloud database. The results showed that *B. pseudomallei* was found in Sonora and Chiapas, and potentially in Tabasco and Coahuila. In Mexico, this bacterium is poorly known and the infectious disease caused by this microorganism is completely unfamiliar in the clinical settings. A wrong diagnostic of melioidosis can lead to the patient's death in only 9 days, although it depends on the size of the inoculum and the person's resistance. Nonetheless, this infectious disease can be treated with specific antibiotics, as long as the bacterium is rapidly identified. The awareness of *B. pseudomallei* and melioidosis in Mexico have significant implications for public health. It can contribute to a suitable diagnostic and treatment, and to a favorable outcome for the patient.

Phenotypic and genotypic characterization of strains of the *Burkholderia cepacia* complex isolated from patients with pneumonia

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Within *Burkholderia sensu stricto* (s.s.), there is a group of opportunistic pathogens called the *Burkholderia cepacia* complex (Bcc). This group is known to mainly affect immunocompromised patients, although it is also important as a causative agent of nosocomial infections. One feature of the Bcc is that the species have a high phylogenetic similarity sharing up to 100% in the 16S rRNA, which has complicated its study. In Mexico, the analysis of clinical Bcc strains has been poorly studied. Therefore, we analyzed 100 Bcc strain obtained from patients from 3 different hospitals in Mexico City. So far, 86 strains were identified by 16S rRNA sequencing as Bcc species while the rest belonging to the genera *Pseudomonas, Stenotrophomonas, Providencia, Enterocccus* and *Acinetobacter.* Likewise, 10 strains of *B. pseudomallei* were also found, this is the causative agent of the infectious disease melioidosis. Strains of Bcc were identified as *B. anthina, B. territorii, B. cepacia* and *B. contaminans* (99.78-100%). In the phylogenetic analysis for the Bcc strains, it was observed that some of the strains were grouped with *B. metallica, B. vietnamiensis, B. multivorans, B. paludis, B. contaminans* and *B. lata* species. It was also observed that the strains from the three hospitals were not related among each other and there were strains that are not identified as any described Bcc species, suggesting the possibility of new species within this group.

Multilocus Sequence Analysis of Endophytic Fungi

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Fungal endophytes spend at least one stage of their life cycle within plant tissues and have steadily metabolic interactions with their host plant. This symbiotic relationship is a continuous line from mutualism to parasitism. The coevolution of endophytic fungi and their host plants allowed the biosynthesis of highly diverse natural products, which play a key role in chemical communication among the members of symbiosis, and in plant fitness and adaptation to different environmental conditions. The first step to understand the chemical profile and chemical ecology of a fungal endophyte is its identification. In this work we used a multilocus sequence analysis to identify the endophytic fungi isolated from *Psittacanthus calyculatus, Phoradendron longifolium, Phoradendron velutinum,* and *Arceuthobium* sp. Partial sequences of internal transcribed spacers regions, large ribosomal subunit, and the β-actin gene were amplified using previously reported primers. The sequence of these genes were generated through the traditional Sanger sequencing in LANBAMA. Reference sequences of all genes were obtained in NCBI using the tool Taxonomy browser. All sequences were concatenated and aligned using ClustalX and manually edited in Seaview. Phylogenic trees were built in PHYML and visualized with MEGA 10.0. In addition, trees were saved as images and edited in PowerPoint. Paired sequence identities were calculated with online tools (https://www.bioinformatics.org/sms2/ident_sim.html). Until now, P. *calyculatus* harbored members of genera *Microdiplodia, Phoma, Alternaria, Paracamarosporium Cladosporium, Notophoma.* and *Leptosphaerulina; Biscogniauxia, Epicoccum,* and *Nigrospora* have been found to be associated with *P. velutinum.* Whereas, *Xylaria, Colletotrichum,* and *Penicillium* live within the *Arceuthobium* sp., *Xylaria, Sporormiella, Nigrospora* and *Preussia* live within *P. longifolium.* Several of the fungal genera found in this work have been reported as endophytes and some of them have been reported as producers of new natural products with biological activities. The fungal endophytes of mistletoes seem to be a promising source of secondary metabolites with novel molecular structures or they could produce natural products with biological activities.