SUNDAY 1		MONDAY 2	TUESDAY 3	WEDNESDAY 4	THURSDAY 5
	7:00-9:00	BREAKFAST	BREAKFAST	BREAKFAST	BREAKFAST
REGISTRATION **11:00 – 18:00**	9:00-10:40	ORAL SESSION I Chair:	ORAL SESSION IV Chair:	ORAL SESSION VI Chair:	
	10:40-11:00	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	
	11:00-12:40	ORAL SESSION II Chair:	SYMPOSIUM Chair:	ORAL SESSION VII Chair:	DEPARTURE
	12:40-13:00	COFFEE BREAK	COFFEE BREAK	COFFEE BREAK	
	13:00-14:00	PLENARY SESSION I **GAD FRANKEL** Imperial College London	PLENARY SESSION III **VANESSA SPERANDIO** UT Southwestern USA	PLENARY SESSION V **VÍCTOR DE LORENZO** Centro Nacional de Biotecnología, CSIC. España	
	14:00-16:00	LUNCH	LUNCH	LUNCH	
OPENING CEREMONY **17:45-18:00**	16:00-17:00	ORAL SESSION III Chair:	ORAL SESSION V Chair:	PLENARY SESSION VI **JAVIER TORRES LOPEZ** IMSS	
OPENING TALK **FRANCISCO BOLÍVAR** IBT-UNAM **18:00-19:00**	17:00-18:00	PLENARY SESSION II **GISELA STORZ** NICHD, NIH. Bethesda, USA	PLENARY SESSION IV **LAURA CAMARENA** Instituto de Investigaciones Biomédicas, UNAM	Closing Ceremony 17:00-17:30	
WELCOME COCKTAIL **19:00-21:00**	18:00-20:00	Poster Session Odd Numbers	Poster Session Even Numbers	DINNER AND DANCING 20:00 h	

PLENARIAS

Biotecnología y los beneficios de los organismos transgénicos.

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La biotecnología de los organismos y plantas transgénicos se han usado desde hace muchos años para ayudar a contender con diferentes problemáticas y ayudar a producir satisfactores entre ellos están indudablemente nuevos medicamentos. Las plantas transgénica están diseñadas para contender con las plagas de insectos y simultáneamente reducir la cantidad de insecticidas químicos muchos de los cuales son carcinogénicos y recalcitrantes.

Este propósito se ha cumplido ya que en Estados Unidos y en otros países se han ido sustituyendo los cultivares tradicionales por los cultivares transgénicos. En Estados Unidos el 90% de sus cultivares son principalmente maíz, algodón y soya entre otros cultivares transgénicos.

Sus granjeros han visto las ventajas del uso de cultivares transgénicos al haber dejado la compra de insecticidas químicos, ahorrando recursos económicos y de forma importante, además ya no están expuestos a muchos de los efectos nocivos sobre la a la salud.

Se discutirá también sobre la posibilidad de emplear plantas transgénica desarrolladas en México para ayudar contender muchos de los problemas asociados con producción sustentable de alimentos y del impacto hacia el medio ambiente.

Citrobacterrodentium infection reprogrammemetabolism in intestinal epithelial cells *in vivo*

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The intestinal epithelial cells (IECs) that line the gut form a robust line of host defense against ingested pathogens, acting as a physical barrier and through detection of pathogen-associated molecular patterns (PAMPs) via pattern recognition receptors (PRRs), such as toll-like receptor (TLR) 2 and TLR4. As such, the pathogen - IEC interface constitutes the battle line between the host innate immune system and the pathogen's counteracting virulence factors. We investigated the impact of infection with the enteric mouse pathogen *Citrobacter rodentium* (a model organism to study infections with enteropathogenic and enterohemorrhagic*Eschericha coli* (EPEC and EHEC)) on IEC metabolism, using global proteomic and targeted metabolomics and lipidomics. We found that *C. rodentium* infection results in shut down of mitochondrial ATP production, a switch to aerobic glycolysis and production of phosphocreatine, which mobilises cytosolic energy.Consistently, there was a significant reduction in the levels of host high molecular weight cardiolipins, lipids essential for efficient mitochondrial oxidative phosphorylation. Using bioluminescent reporter strains we show that infection with WT type *C. rodentium* leads to increased oxygenation of the mucosal surface, supporting *C. rodentium* oxidative metabolism *in vivo* and reducing the abundance of obligate anaerobic commensals. Additionally, IECs responded to infection by activating Srebp2 and the cholesterol biosynthetic pathway. Unexpectedly, infected IECs also up-regulated the cholesterol efflux proteins AbcA1, AbcG8 and ApoA1, resulting in higher levels of fecal cholesterol and a bloom of *proteobacteria*. These results suggest that *C. rodentium* manipulates host metabolism to evade innate immune responses and establish a favourable gut ecosystem.

The Hidden Secrets of Small Genes

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Small RNAs (sRNAs) that base pair with one or more target mRNAs to regulate their translation and stability are critical to stress responses in bacteria. In a number of bacteria, the Hfq chaperone protein facilitates the limited base pairing between the sRNAs and their targets. Initially, it was assumed that Hfq-binding sRNAs are encoded by independent, non-protein coding genes. However, a number of recent observations suggest that this is not the case for many sRNAs, and the distinction between coding and noncoding is becoming increasingly blurred. First, more and more sRNAs derived from the 3' end of protein coding genes, either through processing of mRNAs or transcription from promoters within coding sequences, are being discovered. For example, the MicL RNA, which represses the synthesis of the most abundant protein in the cell, is transcribed from a promoter located within the coding sequence of the *cutC*gene. The copper sensitivity phenotype previously ascribed to the inactivation of *cutC* is actually derived from the loss of MicL. Second, more and more sRNAs thought to function solely as base pairing sRNAs have been found to encode small proteins. For example, the Spot42 RNA, which represses the synthesis of proteins required for the metabolism of non-preferred carbon sources, encodes a 15-amino acid membrane protein. Several of these small proteins modulate the activities or levels of transporters, some encoded by mRNAs that are targets of the corresponding sRNA.

"You're hot and you're cold": neurotransmitters modulation of bacterial virulence gene expression.

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Host bacterial associations have a profound impact in health and disease. The gastrointestinal (GI) tract is one of the most prominent sites in the human body where host/microbial associations are paramount. Chemical communication between microbes and their hosts underlies the basis of their associations, andthis inter-kingdom signaling mediates amicable and detrimental interactions. Both the host and microbiota produces cohorts of signalling molecules that can regulate the virulence expression of a pathogen. Epinephrine and/or norepinephrine are sensed by two bacterial adrenergic receptors to activate virulence gene expression in enteric pathogens. Serotonin is a neurotransmitter that is primarily synthesized in the GI tract by enterochromaffin cells and a subset of enteric neurons. The enzyme tryptophan hydroxylase 1 (TpH1) is responsible for the synthesis of serotonin. Serotonin signaling in the intestinal mucosa is terminated by its removal by the serotonin selective reuptake transporter (SERT). Many functional GI disorders are associated with alterations in serotonin signaling, but the effect of serotonin signaling on bacterial-mediated GI disorders remains unknown. We assessed the role of serotonin in the virulence expression of enterohemorrhagic *E.coli* (EHEC). EHEC has a pathogenicity island, the locus of enterocyte effacement (LEE) that is essential for virulence. Serotonin decreased transcription of the LEE genes through Ler, the major transcriptional activator of the LEE genes. Congruently, the levels of the LEE proteins EspB and EspA were decreased both in whole cell lysates and supernatants upon treatment with serotonin. The LEE genes are necessary for attaching and effacing (AE) lesion formation on epithelial cells, and serotonin also decreased AE lesion formation. To assess the effect of serotonin as a signalling molecule regulating LEE *in-vivo* studies using SERT knockout mice, which accumulate increased levels of serotonin in the GI tract, were performed. Mice having normal levels serotonin and those with increased levels of serotonin were infected with *C.rodentium*, which is used as a surrogate model for EHEC infection. Levels of colonization, and survival were performed. The mice with increased levels of serotonin were less susceptible to *C.rodentium* infection. Conversely, mice had increased levels of infection when the serotonin levels in the GI tract were decreased by using the Tph1 inhibitor PCPA. Thus serotonin present in the mammalian GI tract seems to serve as a signalling molecule for EHEC, and affect its virulence by regulating the LEE.

Characterization of the regulatory switch between the two flagellar systems of *Rhodobactersphaeroides**

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Rhodobactersphaeroides is an alpha-Proteobacterium that carries two copies of the flagellar genes. One copy is expressed constitutively under the growth conditions commonly used in the laboratory. The products of these genesassemble~~of~~ a single subpolar flagellum (Fla1). For several years these genes and their products were thoroughly studied and no evidence of the presence of the second flagellar system was observed. However, the genome sequence revealed the presence of ~~the~~a second complete cluster of flagellar genes, named *fla2*, but the functionality of this set remained obscure for several years. Phylogenetic analysis showed that the *fla1* genes were acquired through a horizontal transfer event, probably from a gamma-proteobacterium whereas the *fla2* genes were vertically inherited.

Using a strain unable to express the *fla1* genes, we isolated a motile mutant strain ~~able to swim using~~that used the *fla2* gene products. We found that this strain had several flagella at the cell pole. The genome sequence of this $Fla2^+$ $Fla1^-$ strain revealed a single mutation in *cckA*, which encodes a histidine kinase that together with ChpT and CtrA forms a two component system conserved among the alpha-proteobacteria. CtrA-P was shown to be required to express the *fla2* genes as well as the chemotactic genes that control its rotation.

The genes controlled by CtrA were identified through RNAseq, and from this analysis, it was possible to identify several physiological processes under the control of CtrA.

To determine if both flagella could be assembled simultaneously in the same cell, we transformed the $Fla1^-$ $Fla2^+$ strain with a plasmid that complemented the mutationthat prevented the expression of Fla1, unexpectedly the presence of Fla2 was severely reduced, indicating that a component of the *fla1* genetic system turns off the signal generated by CckA. This would explain why the Fla1 system is dominant in the wild-type strain, at least under the growth conditions commonly used.

However, challenging this view, from a library of $Fla1^-$ $Fla2^+$ mutants we identified several strains that do not carry any change in *cckA*, *chpT* or *ctrA*. In these strains, the mutation responsible for the $Fla2^+$ phenotype was identified in a gene encoding an orphan response regulator. This gene is responsible of repressing the expression of the *fla2* genes in the wild-type strain, its expression is independent of the *fla1* system, and in its absence the wild-type strain ($Fla1^+$ $Fla2^-$) is switched to a $Fla1^-$ $Fla2^+$. These results indicate that the control of the expression of these flagellar systems can probably be switched by the cell in response to an unidentified condition.

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Developing easy to use genetic tools for (de/re) constructing complex phenotypes in bacteria

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While systems biology aims to acquire a global knowledge of the physiology of the cell, synthetic biology pursues to reprogram organisms to execute new-to-nature functions. The advent of Synthetic Biology has brought about the possibility to massively manipulate bacterial genomes to efficiently program complex genetic devices within a cell.However, deep engineering tasks need to optimize two factors to maximize reprogramming efficiency. First, it is fundamental to possess a suitable genetic toolbox repertoire and second it is important to select an appropriate chassis that is easy to manipulate genetically and offers good biotechnological properties.

For that reason, we developed a toolbox collection denominated Standard European Vector Architecture (**SEVA**), that it is based on a gathering of constructs assembled in a modular fashion to effortlessly exchange the different functional parts at users' convenience[1, 2].To this end, we have expression systems, devices to stably implant genetic networks into bacterial genomes [3, 4]. And also, tools to eliminate/exchange undesired genomic regions using classical homologous recombination techniques [5]. Since classic genome engineering is still a time-consuming and laborious process, we recently adopted **ssDNA recombineering**, useof oligonucleotidescombined the protection delivered by a phage recombinase, together with the use of the **CRISPR/Cas9** system to quickly and efficiently performbacterial genome editing [6, 7]. The combination of both technologies worked with a high efficiency for entering a suite of mutations in the chromosome of *Pseudomonas putida.* These results pave the way for automated and multiplexed editing of the genome of this biotechnologically important bacterium.Nonetheless, users can interrogate the SEVA repository to find their optimal module/part combination for their desired application in their favourite microorganism through the website http://seva.cnb.csic.es.

Finally, several characteristics of *P. putida* make it an optimal choice for different environmental and biotechnological purposes. *P. putida* KT2440 is a non-pathogenic, ubiquitous bacterium with a broad metabolic versatility with a considerable tolerance to multiple organic compounds. Altogether, makes it an appealing organism to develop a chassis for Synthetic Biology. So, we used the before mentionedSEVA tools to edit the genome of *P. putida* KT2440 to create two types of chassis: (i) a **cell factory** optimized for a better functional expression of implanted heterologous DNA [8]; and (ii) a **"naked strain"** enhanced for surface display of proteins to create artificial communities or to function as an exo-reactor.

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Emergence of a new phylogeographic group of *H. pylori* in Latin American mestizos.

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For the last 500 years, the Americas have been a melting pot both for genetically diverse humans and for the pathogenic and commensal organisms associated with them. One such organism is the stomach-dwelling bacterium *Helicobacter pylori*, which is highly prevalent in Latin America where it is a major current public health challenge because of its strong association with gastric cancer. By analyzing the genome sequence of *H. pylori* isolated in North, Central and South America, we found evidence for admixture between H. pylori of European and African origin throughout the Americas, without substantial input from pre-Columbian (hspAmerind) bacteria. In the US, strains of African and European origin have remained genetically distinct, while in Colombia and Nicaragua, bottlenecks and rampant genetic exchange amongst isolates have led to the formation of national gene pools. We found three outer membrane proteins with atypical levels of Asian ancestry in American strains, as well as alleles that were nearly fixed specifically in South American isolates, suggesting a role for the ethnic makeup of hosts in the colonization of incoming strains. Our results show that new *H. pylori* subpopulations can rapidly arise, spread and adapt during times of demographic flux, and suggest that differences in transmission ecology between high and low prevalence areas may substantially affect the composition of bacterial populations.

O R A L E S

Emergent properties of bacterial interactions in a synthetic community

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Competition has been considered as one of the principal operators of the assembly of bacterial communities, however, it is largely unknown how bacteria detect the presence of a competitor, the specificity and speed of the response.

To explore the dynamics of the interactions among bacteria in a microbial community we set up a confrontation assay with three strains having different roles in an interaction network: non-antagonist and resistant (R); antagonist and resistant (A); non-antagonist and non-resistant (S). These strains belong to different *Bacillus* spp. lineages and were isolated from sediment communities of the Churince water system in Cuatrocienegas, Mexico. The experiments were performed in 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. The interaction took place over a 30 minutes period, and samples were then plated every 5 minutes. To determine the genetic response of the strains during the interaction with a competitor, we also performed a transcriptomic analysis of different combinations of interacting strains, at different time points.

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 suggest that the presence of strain R interfered the antagonism of A against S and constitutes an emergent property of interactions in a synthetic community. We will present and discuss the interaction results, the antagonism potential of the genomes, and the transcriptomic data that could explain the antagonistic and the stabilizing effects.

Analysis of a gene cluster of *Pseudomonas syringae* pv. phaseolicola containing a putative nonribosomal peptide synthetase involved in phaseolotoxin synthesis and resembling a Genomic island.

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Phaseolotoxin [N^{δ}(N´-sulfodiaminophosphinyl)-ornithyl-alanyl-homoarginine] is a reversible inhibitor of the enzyme ornithine carbamoyltransferase that catalyzes the formation of citrulline from ornithine and carbamoylphosphate in the arginine biosynthetic pathway*. Pseudomonas syringae* pv. phaseolicola NPS3121 produces phaseolotoxin in a temperature dependent manner, being optimally synthesized between 18 °C to 20 °C, while no detectable amounts are present above 28 °C. The Pht cluster, involved in the biosynthesis of phaseolotoxin, contains 23 genes that are organized in five transcriptional units. Additionally, a putative nonribosomal peptide synthetase (gene PSPPH_4550), coded outside the Pht cluster, is also necessary for phaseolotoxin production.

The purpose of the present study was to characterize the transcriptional pattern of the putative non ribosomal peptide synthetase. Analysis of the genomic context of the PSPPH_4550 showed that it is located within a group of genes that are clustered. Thus, we also determined the expression of the genes PSPPH4546, PSPPH_4547, PSPPH_4553, PSPPH_4554 and PSPPH_4555. For this, RNA was isolated from cultures of *P. syringae* pv. phaseolicola NPS3121 grown in M9 medium at 18 °C or 28 °C and Northern blot analysis were made. The hybridization results showed that these genes are transcribed at high levels at 18 °C than 28 °C, in agreement with phaseolotoxin production conditions and showing the same expression pattern as that observed for genes within the Pht cluster.

Bioinformatic analysis of the gene cluster revealed a lower G+C content (48%) compared to the rest of the chromosome (59%). Analyses in Phyre2, a protein modelling predictor, showed that PSPPH_4550 was similar to an amino acid adenylation domain from a nonribosomal peptide synthetase, whereas PSPPH_4545, PSPPH_4546 and PSPPH_4552 were also similar to nonribosomal peptide synthetase modules. On the other hand, PSPPH_4547 and PSPPH_4551, showed similarity with polyketide synthase modules. These results suggested that this region harbors genes that could be participating in the biosynthesis of polyketide and nonribosomal peptide synthesis.

This cluster is flanked by PSPPH_4539, PSPPH_4540 on the one side and PSPPH_4559 on the other side. These genes were similar to a components of the transposition system (100% confidence). According to our results, this gene cluster contains at least 18 genes distributed in at least 8 distinct operons. The presence of genetic elements involved in transposition found at the borders of the cluster and the difference in G+C content with respect to the rest of the genome, suggests that this region may be a Genomic island. According to this we propose that not only PSPPH_4550 is involved in phaseolotoxin synthesis, but also other genes coded into this cluster could be participating in the synthesis of the tripeptide moiety of phaseolotoxin.

Genetic and Functional Exploration of the BarA Periplasmic Domain

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Bacterial two-component system (TCS) signaling circuits regulate the expression of diverse genes in response of environmental cues. The BarA/UvrY two-component signal transduction system of *Escherichia coli* activates transcription of CsrB and CsrC noncoding RNAs, which, in turn, act by sequestering and antagonizing the activity of the RNA-binding global regulatory protein CsrA. BarA, which senses and responds to the presence of formate, acetate, and also to other short chain carboxylic acids, at the late exponential phase of growth, is a membrane-anchored hybrid histidine kinase with a 145 amino-acid long periplasmic domain that is predicted to fold as a PAS-like motif.

Here we present evidence, based on the substitution of the periplasmic region of BarA with the counterparts of related or unrelated sensor kinases, demonstrating that the periplasmic domain of BarA is indispensable for its regulation. We also report the identification of essential residues in the periplasmic segment of BarA that appear to be involved in direct or indirect sensing of signal molecules.

DipM is a protein of *Caulobacter Crescentus* that can recognize differences in the peptidoglycan

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The bacterial cell envelope is composed of a cytoplasmic membrane, a peptidoglycan (PG) cell wall and in gram negative bacterium an outer membrane. During cell division, the PG grows inward, forming a multilayered wall that must be degraded to allow the constriction of the outer membrane and daughter cell separation. Cell division is driven by the divisome, a multiprotein complex composed of approximately twenty proteins, which perform specific functions, some of these are PG peptidases required to separate the daughter cells.

In C. *crescentus,* DipM has been proposed as the protein in charge of digesting the PG to allow constriction, in the absence of DipM, the cells of this gram-negative bacteria form filaments. This protein has at its C-terminus a LytM domain which is indispensable for its function, the LytM domains that have been characterized in Gram-positive bacteria are catalytic proteins required for cell division, sporulation, or PG growth. In Gram-negative bacteria the LytM domains can also be regulators of PG peptidases, but it´s unclear if DipM is a catalytic o regulatory protein, our results suggest that DipM is a regulatory protein, opposite to what has been described.

At its N-terminus, DipM carries two tandems of two LysM PG binding domains; the absence of the LysM domains abolishes localization of DipM to division site. These domains appear in cell wall-hydrolases and increase the local concentration of the enzyme through by binding to the PG. This helps the enzyme to slide along the PG, but it´s unclear which are the molecular mechanism that involve localization on site division.

To understand the mechanism that allows DipM localization, we did a set of depletion strains of other divisome components to test if DipM could localize in their absence. Our results show that DipM requires all the division proteins involved in PG synthesis at the division site. Corroborating this idea, DipM failed to localize in cells with a full set of division protein but that their PG growth at the division site was inhibited.

Taking in account these results and the domain structure of DipM we propose a novel mechanism of protein localization.

Molecular and functional analysis of an operon involved in the utilization of the fucosyl-α-1,6-*N*-acetilglycosamine-asparagine glycoamino acid in *Lactobacillus casei* BL23

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L. casei is a lactic acid bacterium able to colonize several environments, including oral cavity, gastrointestinal and genital tract of humans and animals. The broad ecological distribution of *L. casei* reflects a metabolic flexibility that has allowed the widespread application of this specie in the food and health industries; different strains are employed as acid-producing starter cultures for milk fermentation and as probiotics to enhance human health. An important factor for the survival and proliferation of probiotic bacteria in the human gastrointestinal tract is their ability to use the available nutrients in that particular niche. These nutrients derive from the diet or from secretions of the intestinal epithelial cells, being the glycans or glycoconjugates a preferred source of nutrients for many microorganisms. In this work we stablished that *L. casei* BL23 is able to grow in the presence of fucosyl-α-1,6-*N*-acetilglycosamine-asparagine (Fuc-GlcNAc-Asn) as a carbon source. Fuc-GlcNAc-Asn is a glycoamino acid that forms part of the *N*-glycoprotein core of proteins from the mucosa. This glycoamino acid was synthetized in our laboratory using the transglycosylation activity of the α-L-fucosidase AlfC. We have shown that utilization of this glycocoamino acid and other fucosylated compounds by *L. casei* BL23 is due to the presence of a gene cluster (*alf2*), which encodes a permease (AlfH) and an α-L-fucosidase (AlfC), and divergently oriented, a cluster of genes that encode hypothetical proteins including an aspartate decarboxylase/aminotransferase (AsdA), a transcriptional regulator (AlfR2), a peptidase V (PepV), a glycosilasparaginase (AsnA2) and a sugar kinase (SugK). Phenotypic analyses of mutants for these proteins and transcriptional analyses suggested that the expression of the *alf2* gene cluster is repressed by AlfR2 and that the presence of Fuc-GlcNAc-Asn relieved repression. This work report for the first time the characterization of genes involved in the metabolism of a glycoamino acid in bacteria. Therefore, these results suggest that the *alf2* genes likely represent an adaptation of *L. casei* to the human gastrointestinal tract.

The bacteriophage mEp021 and its control on the cell fate: double plaque phenotype in the same progeny.

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We have investigated the molecular bases of the double plaque phenotype (clear and turbid) produced by the bacteriophage mEp021. The viral progeny from lysogenic *E. coli* W3110 (mEp021), produced an average of 0.5% lytic versus temperate phages. This frequency is about a hundred-fold that of bacteriophage lambda, and suggests that this phenomenon is intrinsic to mEp021 and not due to a regular spontaneous mutations. The clear plaque phenotype of mEp021 resulted from deletions and point mutations in the repressor gene (*rep)*. The genetic deletions of mEp021 was not exclusive of the gene *rep* since it was also observed in gene *J*, which encodes a structural protein involved in host receptor recognition. Deletions in *rep* and *J* may be due of sequence-specific recombination because the deleted segments are flanked by direct repeats of 12 and 35 bp, respectively. In order to identify possible factors in the putative molecular switch that could have an effect in produce clear plaque phenotype, the putative *rep*, *ant* (anti-repressor), *dam* (Dam methyltransferase) and *dcm1* (Dcm methyltransferase) genes and the anti-repressor operon (5 ORFs) were cloned in the vector pKQV4. It was found that gene products of *rep* and *ant* inhibit the mEp021 infection, although construction with the anti-repression operon decrease the plaque turbidity of mEp021, and also of λ. Nonetheless, none of clones appear to modify the average of clear plaque phenotype. These findings highlight the need for the identification and characterization of the molecular processes that mediate the genome variation of mEp021.

Bacteriophage endolysins as new antibacterial strategy: Aquaculture approaches

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Endolysins are enzymes coded by bacteriophages at the end of their replication cycle to degrade the peptidoglycan of the bacterial host, resulting in cell lysis. In Gram-positive bacteria due to the absence of an outer membrane, endolysins can access the peptidoglycan and destroy these microorganisms when applied externally[1]. However the expansion of endolysins as antibacterials against Gram-negative pathogens is hindered by the outer membrane. This physical barrier poses a highly effective permeability for the passage of harmful compounds[2]. *Vibrio parahaemolyticus (Vp)* is a Gram-negative halophilic bacterium that is found in estuarine, marine and coastal environments[3]. Multiple *Vp* strains carrying pVA1 plasmid (Vp_{AHPND}) that encode a toxin homologous to PirAB binary toxin produced by *Photorhabdus luminescens* were identified as the causal agent of acute hepatopancreatic necrosis disease also known as early mortality syndrome (AHPND/EMS)[4]. AHPND has generated significant economic losses in the major shrimp producing countries since 2009, spreading rapidly to Asia and Mexico[5]. In this study we evaluated a recombinant endolysin against Vp_{AHPND} called *VpEnd1*. We also evaluated an enzyme obtained by protein engineering with possible bactericidal capacity in the absence of membrane permeabilizers. Interestingly *VpEnd1* no resemble any reported endolysin. The enzymatic activity showed lytic effect against the reference and pathogenic *VpEnd1* strains. *VpEnd1* endolysins represent a novel promising class of antibacterial based on its selective cell wall hydrolysis.

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***thnR* gene regulates the immunity of *Bacillus thuringiensis* subsp. *morrisoni* against its own bacteriocin (Thurincin H)**

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Bacillus thuringiensis (Bt) is a gram-positive bacterium that produces a several number of metabolites, between them it produces small peptides with antimicrobial activity called bacteriocins. Although many bacteriocins produced by Bt have been reported, only thurincin H and thurincin CD are genetically characterized. Both of them are codified into a genetic cluster necessary to synthesis, regulation, post-translational modification, processing, and self-immunity. Producer strains of bacteriocins have the characteristic to be immune to its own peptide, *Lactococcus lactis* is a strain producing nisin, the best studied bacteriocin, and it has shown that nisFEG and nisI genes are involved in immunity mechanism. Several reports demonstrate that ABC transporters are implied into immunity mechanism: SpaFEG, SpaI genes protect *Bacillus subtilis* ATCC6633 of Subtilin; EriFEG, EriI protect *Bacillus subtilis* A1/3 of Ericin and LtnFEG, LtnI genes protect *Lactococcus lactis* DPC3147 of Lacticin 3147, among others.

Particullary *Bacillus thuringiensis* immunity mechanism has been little studied, thuricin CD and thurincin H are the bacteriocin better characterized, and there are only one work about immunity where was demonstrated that *trnFG* and *trnI* (from thurincin CD) are implicated in immunity against its own bacteriocin. Thurincin H genetic cluster has been elucidated; it was found that this cluster in *Bacillus thuringiensis* SF361 was responsible for bacteriocin activity. In this work we focus on responsible genes to give immunity into the thurincin H cluster. As other bacteriocin producer strains shows immunity with proteins of ABC transport, we examine the genes *thnD* and *thnE* and its effect on sensitive strain, several constructions carry on these genes and other upstream or downstream were proved. Interestingly we observe that these genes are really necessary to provide immunity but they need a third gene, a transcriptional regulator *thnR*. Only when three genes are present the strain acquires immunity.

Family II pyrophosphatases from photosynthetic bacteria can hydrolyze free pyrophosphate

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Abstract: The hydrolytic activity from three partially purified family II pyrophosphatases from the photosynthetic bacteria Rhodobacter sphaeroides, Rhodobacter capsulatus, and Rhodovulum sulfidophilum, as well as the recombinant cytoplasmic pyrophosphatase from Rba. sphaeroides, was tested with Mg2+–PPi, Mn2+–PPi, and PPi4-. Unlike family I pyrophosphatases that hydrolyze only the Mg2+–PPi complex like those from Rhodospirillum rubrum, all family II enzymes tested showed hydrolytic activity with Mg2+–PPi, Mn2+–PPi, and PPi4- without cation. The activity without added cation remained the same, even under exhaustive dialysis or after desalting the enzyme through a Sephadex G-25 column. However, this activity disappeared upon the addition of ethylenediaminetetraacetic acid and could not be restored by adding Mg2+. Moreover, the enzyme inactivation was not related to dissociation into lower molecular subunits as in other family II enzymes. This is the first report on pyrophosphatases that can hydrolyze pyrophosphate without a divalent cation added and that presumably contain a tightly bound divalent cation in their structure. Keywords: cytoplasmic pyrophosphatases, metal cofactors, inorganic pyrophosphate, Rhodobacter sphaeroides, Rhodospirillum rubru

Towards a large-scale comparative systems biology across bacteria: organizational landscape and evolutionary dynamics of the regulatory circuitry

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The promising field of synthetic biology aims to apply engineering principles for designing and constructing biological systems and devices. To fulfill this aim, it is crucial to understand the set of organizational principles underpinning how cellular systems interconnect, work and evolve. In synthetic biology, and even in biotechnology, a deep understanding of the alternative regulatory patterns, which have evolved as adaptations to different environments, will inspire better synthetic designs. To accomplish this, we need an inventory of systems and their properties across a large range of organisms, a key step to rendering feasible comparative systems biology approaches. The availability of databases electronically encoding curated regulatory networks, in addition to high-throughput technologies and methods to discover regulatory interactions, provides an invaluable source of data to understand the principles underpinning the organization and evolution of these networks responsible for cellular control. Nevertheless, data on these sources never goes beyond the regulon level despite the fact that regulatory networks are complex hierarchical-modular structures still challenging our understanding. We recently took the first steps towards a global understanding of the regulatory networks organization by making a cartography of the functional architectures of diverse bacteria. As a result, Abasy (**A**cross-**ba**cteria **sy**stems) Atlas (http://abasy.ccg.unam.mx) provides a comprehensive inventory of annotated functional systems, global network properties and systems-level elements (global regulators, modular genes shaping functional systems, basal machinery genes and intermodular genes) predicted by the natural decomposition approach for reconstructed and meta-curated regulatory networks across a large range of bacteria, including pathogenically and biotechnologically relevant organisms. Currently, Abasy Atlas contains systems and system-level elements for 52 regulatory networks comprising 84,956 regulatory interactions covering 42 bacteria in nine taxa, containing 3,887 regulons and 1,891 systems. All this information provides the opportunity to study the evolution and common organizational principles of regulatory networks, but at the same time pose new challenges requiring the expansion of Abasy Atlas.

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Selection of functional quorum sensing systems in *Pseudomonas aeruginosa* by environmental factors

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Pseudomonas aeruginosa uses Quorum Sensing (QS) to control the production of several energetically costly virulence factors such as siderophores and exo-proteases which provide a collective benefit for the bacterial populations and are therefore considered as public goods, interestingly public good producers can be exploited by mutants (social cheaters) that enjoy their associated benefits (iron delivery, carbon and nitrogen from usable peptides and amino acids) but that do not invest in their production, these mutants (usually defective in the main receptor of the QS signals, the protein LasR) can rise spontaneously in both laboratory settings (growing in protein as sole carbon source) or *in vivo* during lung infections, when those social cheaters emerge they usually reach stable equilibrium frequencies than when disrupted by the exogenous addition of more social cheaters could lead to a growth collapse (tragedy of the commons). Nevertheless in natural settings QS systems are well preserved, hence we speculate that mechanisms that preserve the integrity of QS should be abundant in nature. In that regard, we demonstrated that the intrinsic QS mediated tolerance against stress leads to the selection of QS proficient strains in casein as sole carbon source, and that QS-regulated toxic compounds such as the redox active pyocyanin contribute to the counter selection of social cheaters, while other had demonstrated the same for the respiratory inhibitor cyanide. Furthermore, recently we found that the temperate bacteriophage D3112 and JBD30 preferentially kill QS deficient *lasR rhlR* mutants and that are also able to select functional QS systems *in vitro and in vivo* during infections of *Galleria mellonella*, we propose that those and perhaps other selection mechanisms preserve QS systems in nature and discuss the implication of our findings in the ecological and clinical contexts.

Enhanced Cry1A toxicity from *Bacillus thiringiensis*, by interaction with Heat Shock Proteins.

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Bacillus thuringiensis (Bt) is a pathogen that produces diverse proteins that infect and kill their larval insect host[1] . The most important of them are Cry toxins that target larval gut cells by forming oligomeric structures that insert into cell membrane forming pores that burst cells by osmotic shock[2]. Accordingly, Cry toxins have been used for the control of crop pests and vectors of human diseases[2].

Molecular chaperones are proteins that assist the assembly or disassembly of other proteins. Heat shock proteins as Hsp90[3], Hsp70 and GroEL are examples of molecular chaperone proteins[4].

In our laboratory, we demostrated that mosquitos *Aedes aegypti* reduced in *hsp90* induced by gene silencing (RNAi), showed a tolerance phenotype to Cry11Aa, suggesting a positively role of this chaperone in Cry11Aa toxicity[5]

. In the present work we show evidences that chaperones as Hsp90, Hsp70 from *Plutella xylostella* and GroEL from *Alcaligenes faecalis*[6] bind to Cry1A toxins and this association is able to enhance Cry1A toxicity in a concentration dependent manner by protecting Cry1A protoxins from gut protease degradation, and/or by assisting Cry1A oligomerization.

The identification of assistants that enhance the activity of Cry toxins would help counter potential resistance and could broaden effective target spectrum.

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Characterisation of *Enterococcus faecium* vesiculogenesis. Role of cell wall-targeting antibiotics in vesicle production and cargo modification.

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The interaction of a cell with its environment is fundamental for survival and success. The phenomenon of extracellular membrane vesicle (MV) shedding is universal among living cells, suggesting that it plays a key role in the normal functioning of the cell. Despite the ubiquity of this phenomenon the dynamics of vesicle formation and release are poorly understood. In gram-positive bacteria the way vesicles pass the cell wall and reach the extracellular milieu remains mysterious. *Enterococcus faecium,* an important agent of nosocomial infections, is becoming increasingly resistant to the cell wall targeting antibiotics vancomycin and β-lactams, limiting its clinical efficacy. In this work we were interested in analyse if MV formation is modified by the cell wall targeting antibiotic penicillin, and if there are modifications of the MV protein cargo in *E. faecium* strains with different degrees of susceptibility to β-lactams. **Methodology**: We analysed MV release from *E. faecium* strains C68, D344R and CV571 treated with ½ MIC of penicillin G. MV size distribution and quantity were measured by nanoparticle tracking analysis, and MV integrity was analysed by electron microscopy. The protein profile of MV was compared by SDS-PAGE and mass spectrometry. **Results and conclusions**: *E. faecium* produces 50-200 nm rounded MV (fig1A). MV release in wild type strains (C68) increased after exposure of penicillin (fig1B). Interestingly, CV571 a mutant strain missing two cell wall synthesising proteins (PonA, PbpF) releases more MV even without penicillin exposure (fig1B). By mass spect we identified that MV from the highly penicillin resistant strain C68 were enriched in the cell wall building proteins Pbp5, PonA and Pbp2B and P_5AP. MV recovered from penicillin-exposed cells contained more Pbp5 and P_5AP, two proteins involved in β-lactam resistance (fig2). Our results show that penicillin exposure increases MV release. We consider that a weakened or modified cell wall might facilitate MV passing to reach the extracellular milieu and that MV carrying β-lactam binding proteins might help to protect the cells from penicillin exposure.

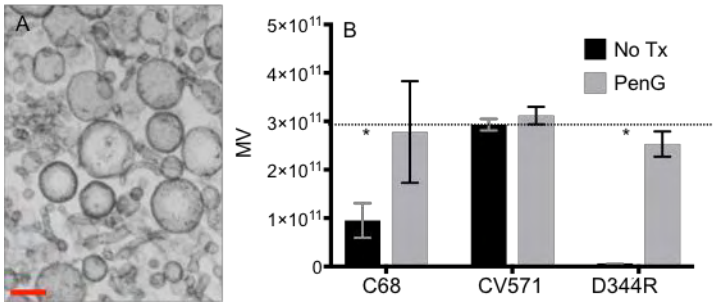

Fig. 1 **A)** EM of MV Scale bar: 100 nm. **B)** MV release in three different *E. faecium* strains. Black bars: untreated cells, grey bars: cell exposed to ½ MIC of penicillin. *p<0.05.

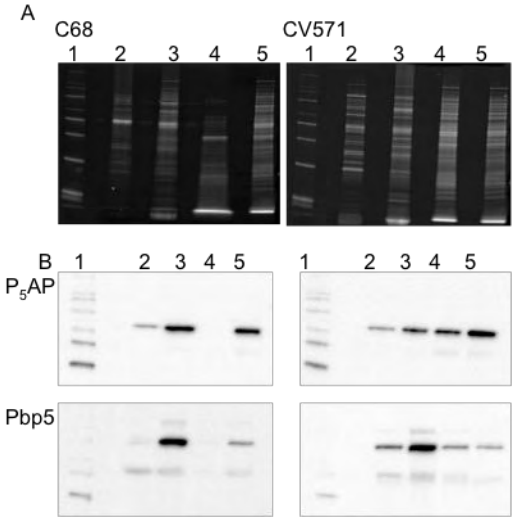

Fig. 2. Lanes 1:MWM, 2: MV No Tx, 3: MV PenG, 4: cell lysate No TX, 5: cell lysate PenG. **A)** SDS-PAGE. **B)** WB

Novel insights into the regulatory mechanism of substrate secretion in enteropathogenic *Escherichia coli*

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Enteropathogenic *Escherichia coli* (EPEC), one of the main causative agents of infantile diarrhea, exploits a Type III Secretion System (T3SS) to colonize the intestinal tract. T3SSs are complex protein-transport devices employed by several diderm bacteria to inject proteins directly into the host-cell cytosol.

T3S proteins are classified into three categories according to the order of secretion: early (inner rod and needle subunits), middle (translocators), and late (effectors) substrates. Since the translocators are required for the direct injection of effectors into the host cells, various mechanisms exist to ensure that translocators are secreted prior to effectors.

In EPEC, the SepL/SepD/CesL protein complex coordinates the orderly secretion of translocators and effectors. Since this switch in T3S has been proposed to occur in response to differences in calcium levels, we explored the link between calcium sensing and T3S regulation through the SepL/SepD/CesL complex.

Here, we demonstrated that although calcium influences effector secretion, this effect is not executed through the SepL/SepD/CesL complex, and occurs in a translocators-independent manner. Therefore, either other secretion regulatory elements are prone to calcium regulation, or calcium concentration changes does not mirror the physiological signal that triggers effector secretion *in vivo*.

Next, with the aim of identifying other secretion regulatory points, we analyzed the substrate binding capacity of different T3SS components. We identified substrate docking sites on the major export gate component EscV. Interestingly, EscV also associates with the protein SepL. Therefore, we propose that SepL might regulate the substrate docking ability of different T3SS components, aiding in the establishment of the secretion hierarchy.

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Comparative study and differential integration properties of two *P. aeruginosa* temperate phages of the group *F116virus*

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It has been proposed that *Pseudomonas aeruginosa* temperate bacteriophage F116 maintains itself as an extrachromosomal element during lysogeny (despite encoding an integrase homolog in its genome). During almost a decade the F116 genome remained as an orphan in databases, thus we deemed relevant to study homologous phages to F116 in our collection to investigate the nature of the prophage condition in the cell. Therefore, we conducted a comparative study of phages H66 and LKA5. The two genomes were similar in size, organization, sequence, and overall GC content to F116. In addition to the conserved core genome and a set of variable accessory genes, the genomes showed significant variability in core genes homologous to repressor, terminase and integrase. In contrast to the report for of F116, we showed that both H66 and LKA5 integrate their genomes in the bacterial chromosome, albeit at different sites, during lysogeny (in agreement with the different types of integrases they encode). Other homologous prophages residing in *P. aeruginosa* genomes were since found in the databanks. The sequence of the LKA5 integration site, *att*, was identical to the host *att*, but H66 *att* showed several mismatches relative to the corresponding *att* in the bacterial genome. Remarkably, we detected extrachromosomal phage genomes in both lysogens. Such elements may correspond either to genuine non-inserted episomes (as suggested for F116), or to excised replicating genomes in their way to the lytic cycle. This observation raises the question whether extrachromosomal phage genomes represent unexplored intermediate entities in equilibrium with the integrated prophage present in lysogeny.

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Understanding Acid Tolerance in *Rhizobium tropici* CIAT 899

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Rhizobium tropici CIAT 899 is a nodule-forming α-proteobacterium that displays intrinsic resistance to several abiotic stressful conditions such as low soil pH and high temperatures, which are common in tropical environments. It is a good competitor for *Phaseolus vulgaris* (common bean) nodule occupancy at low pH values, however little is known about the genetic or physiological basis of acid tolerance. In the present study we used two different approaches to identify the genes involved in this low pH response. A Tn*5* mutant library of *Rhizobium tropici* CIAT 899 was constructed. So-far we screened 18,000 mutants affected in growth under low pH conditions (pH 4.5) but unaffected under neutral pH growth conditions (pH 6.8). Among them, 18 mutants were identified and the transposon insertion sites were determined; response regulator (mutant JG9587) and antiporter (mutant JG2646) genes were selected to complementation assays. In a second approach we studied the transcriptomes of cells grown under different pH conditions. RNA was extracted from cells grown for several generations in minimal medium at 6.8 or 4.5 (adapted cells). In addition we acid-shocked cells pre-grow at pH 6.8 for 45 minutes at pH 4.5. Transcriptomes were determined by RNA-Seq. Our data provide valuable gene-expression information relevant and contributes to our still-poor knowledge of the molecular determinants of the pH response by *R. tropici* CIAT 899.

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A proposal for the organization of *Bacillus subtilis* respiratory chain.

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INTRODUCTION. *Bacillus subtilis* is a Gram-positive bacterium that has a branched respiratory chain with various dehydrogenases transferring electrons from substrates to the menaquinone 7 pool. A type 2 NADH dehydrogenase (NADH DH2) and a succinate dehydrogenase (SDH) are the main dehydrogenases. Menaquinol can be aerobically oxidized by a branch of quinol oxidases or by the b_6c and cytochrome *c* oxidase caa_3 that form a megacomplex with molecular masses between 2000 to 500 kDa. The cytochrome branch includes the membrane-bound cytochromes c_{550} and c_{551} that could be part of the megacomplex b_6c:citc_{550}:caa_3. We are interested in how the respiratory chain is organized when *B. subtilis* is grown in different media.
OBJECTIVE. Analyze the respiratory chain composition and the activity of the respiratory chain complexes in different growing media.
METHODOLOGY. The wild type (WT) 168, was grown 5, 9 and 23 h super rich medium (SRM) with 3% succinate. Membranes were isolated by differential centrifugation. Absorption spectra were made to determine cytochrome content. Activities of the respiratory complexes were measured by oximetry. To view supercomplexes, membranes were solubilized with digitonin and analyzed in clear native electrophoresis. In-gel catalytic activity staining of the respiratory complexes was made to identify changes in molecular mass. A comparison growing bacteria in LB and minimal A media (MA) was done, growing cells during 9 h.
RESULTS. Different cytochrome peaks were observed when *B. subtilis* was grown in LB, SRM and MA. At 5h growth little activity of the cytochrome branch was observed but high activity of quinol oxidases. The megacomplex b_6c:citc_{550}:caa_3 was absent in MA membranes but not in LB o SRM at 9h of growth. NADH appeared in LB and MA but SDH, was missing in MA. The megacomplex can be observed in SRM and LB from 9h of growth but not at 5h.
CONCLUSIONS. In the logarithmic phase *B. subtilis* grows with quinol oxidases and NADH or Succinate dehydrogenase. In the stationary phase it develops a cytochrome branch with a b_6c:c_{550}:caa_3 megacomplex.

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The redox state of human macrophages modulates the persistence of *M. tuberculosis* through Whib3 expression

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Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (Mtb). An essential characteristic of Mtb is the ability to survive within human macrophages. Consequently, it is vital to know the interaction between host cell and Mtb. In this regard, it has been described that the physiological state of the macrophage as; the levels of O_2, CO, reactive oxygen species (ROS) and pH may influence the metabolism and persistence of Mtb in the cell host. It is also known that Mtb responds to microenvironment conditions through various mycobacterial transcriptional regulators. Furthermore, it has been demonstrated in free-living actinobacteria (*Streptomyces)* posses a transcriptional regulator, WhiB3, that it can responds to the redox state and is capable of modulate the morpho-physiological differentiation. Recently, it was demonstrated that in the genus *Mycobacterium,* there exist homologs to WhiB3, which it promotes the synthesis of reserve lipids to survive the state of dormancy in the host. *The objective* of this study was determined if the redox status controlled by NADPH oxidase (NOX) from human macrophages modifies the WhiB3 expression and genes involved in the synthesis of reserve lipids in Mtb. *Methodology:* Monocytes were obtained from peripheral blood of healthy volunteers, differentiated into macrophages for 7 days and pre-treated with NOX modulators such as DPI (inhibitor) and PMA (activator). Subsequently, macrophages were infected at 1:10 of multiplicity of infection for 1 h, non-phagocytized bacteria were removed and cells were incubated for 1 h in the presence of DPI (ERO inhibitor) and PMA (ERO generation). We determinated that the NOX activity by NBT assay and the WhiB3 expression by RT-PCR. *Results*: The WhiB3 expression was increased in Mtb in the context of infection compared to mycobacteria cultured in vitro. Interestingly, the WhiB3 expression was less in phagocyted bacteria by macrophages treated with DPI (low ROS level) compared to the macrophages treated with PMA (high ROS level). Conclusions: The generation of ROS by NOX in human macrophages induced the WhiB3 expression, this is a mycobacterial transcriptional regulator that may activate the synthesis of reserve lipids produced to survive in the latency state in its host, which allows its persistence for long periods of time.

Phenotypic plasticity by reaction norms in *Bacillus* spp. species from wild environments from Cuatro Ciénegas Cohauila desert facing physical environmental factors

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Wild bacterial isolates of two species of *Bacillus spp*; *Bacillus cereus* and *Bacillus subtilis* from the same environmental zone in the Cuatro Ciénegas Coahuila desert were evaluated in laboratory facing environmental factors under the hypothesis that such phenotypic behaviors are species-specific. We obtained the reaction norms with the goal to evaluate the phenotypic plasticity of both species. These reaction norms evaluated growth rate versus temperature (37°C ~ 47°C), ultraviolet radiation and resistance to sodium chloride. Since genotypes are selected as the viability of the phenotype interacting with the environment. Reaction norms also reveal possible evolutionary relationships intra-species and inter-species.

The curves of reaction norms for growth rate versus temperature and resistance to sodium chloride turned out to be different inter-species and similar intra-species as was expected according to the hypothesis. This suggest the relationship between genotype and fenotype behavior beyond the evident obvious features.

The main factor of interest in environment is temperature since temperature affects all in all the whole molecular mechanisms and physicochemical parameter for every organisms. Moreover, with the goal to evaluate changes in phenotypic plasticity the strains were underwent to experimental evolution in a slow adaptation for 1000 generations (5 months) from 45°C to 48°C. Bacillus cereus strains worked out to grow and be viable at 48°C roughly whereas the ancestral strais are not able to grow at 48°C. Such results suggest a real adaptation or presence of adaptive changes in genome as product of experimental evolution.

Two essential genes on the secondary chromosome p42e of *Rhizobium etli* CFN42 participate in cell division

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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). Plasmid p42e (505 kb) is very stable, being recalcitrant to elimination. A systematic deletion analysis allowed the finding of two novel essential genes (RHE-PE00001 and RHE00024) on p42e. Thus we propose p42e as secondary chromosome (1). The essentiality of these two genes is not easy to ascertain based on sequence information. RHE-PE00001 is a hypothetical protein with a DUF1612 domain (domain of unknown function) and a helix-turn-helix motif RHE00024 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.

Disruption mutagenesis for the essential genes RHE-PE00001 and RHE00024 is not possible. In order to determine their functions we implemented negative induction systems, allowing a tight control of gene expression. Through transcriptional fusion with reporter genes, we evaluate the capacity of expression of four inducible expression systems in *R. etli*. The four systems, anhydrotetracycline (2), cumate (2), lac (3) and taurine (4) showed an inducer dose-dependent response. Integrative plasmids for the four systems were constructed, that allow the exchange of the native promoter of an essential gene for an inducible promoter. Through homologous recombination with the target gene, the native promoter is exchanged for the inducible promoter. This enables tight control of the expression of an essential gene by the amount of inducer added.

A strain carrying gene RHE_PE00024 under the control of a cumate-regulated promoter revealed a striking variation in cell morphology. In the absence of cumate, only 37% of the cells display the normal bacillary form, the remaining 63% is comprised by small, nearly spherical cells. By DAPI staining, both cell forms harbor nucleoids, although these are more condensed than in the wild type. This phenotype is partially reversed in the presence of cumate, where the corresponding strain display 62% of bacillary cells and 38% of small, nearly spherical cells. In contrast, a wild-type cell display 92% of bacilli and 8 % of small, round cells.

Similar results were observed in a strain harboring the RHE_PE00001 gene under the control of a cumate-regulated promoter. In the absence of cumate, this strain displays 25% of small, round cells and 75% of bacilli, both containing a condensed nucleoid. These proportions were unchanged upon cumate addition.

These results suggest that both genes participate in the control of cell division in *R. etli*. Efforts are underway to determine the specific steps affected, as well as to determine which genes are under control of these possible regulators.

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Characterization of the biofilm formation by *Actinobacillus seminis*

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Actinobacillus seminis is a Gram-negative bacterium, member of the Pasteurellaceae family. This microorganism causes infections in genital organs of rams, with a predilection for epididymis, a significant factor contributing to infertility and producing important economic losses to the ovine industry. The specific factors that predispose rams to *A. seminis* infection, and the virulence factors expressed by this bacterium are poorly understood. Many bacterial species survive in host tissues by forming biofilms. Biofilms are organized bacterial cells encased in an extracellular polymeric substance composed by polysaccharides, DNA, and proteins. In this work is described that *A. seminis* biofilm formation in vitro occurs optimally at 37°C in Tripticasein-soy broth after 48 h of incubation in stationary conditions. The biofilm enzymatic digestions with proteinase K, DNAseI or amylase confirm the presence of the three polymeric substances: proteins, DNA and polysaccharides, being proteins the main component in those biofilms. Reduced carbohydrates represent approximately 20% of the total exopolysaccharides. *A. seminis* biofilm microstructure was analyzed by Scanning Electron Microscopy. The immunogenic proteins of the biofilm are in process of identification.

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The mRNA levels of the genes *frpB1, frpB2* and *frpB3* of *Helicobacter pylori* are regulated under different iron sources

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Abstract

Helicobacter pylori (H. pylori), a human pathogen causes peptic ulcer and gastritis, this bacterium can survive in several environments inside the human and needs to obtain iron to maintain its cellular growth. Hb (human haemoglobin) is a metalloprotein, which is often used for pathogens as iron source during its invasive process. *frpB1* gene encodes a Hb-binding membrane protein that binds iron. In addition the *frpB2* is another gene of *H. pylori* that also encodes a protein involved in iron acquisition. *H. pylori* can maintain its cellular growth using several sources of iron available in the host, for instance Hb, hem (hemin). However, the mRNA levels remain unexplored, therefore the regulation mechanism of those genes is unknown. In this investigation *H. pylori* was cultivated in cellular media under different iron sources and then the mRNA levels were analysed by RT-PCR real time. Our results showed that the mRNA levels of *frpB1* gene were three fold change when Hb was used as only iron source. The *frpB2* gene showed a small change when hem was added to the culture media. The last gene tested was frpB3, a gene never investigated. Interestingly, this gene showed five fold change when hem was used as iron source. When *H. pylori* was cultivated under iron starvation the *frpB1* gene showed us the mRNA levels highest in relation to the others. Our overall results suggest that *frpB1* is responsible of scavenging iron from Hb, while *frpB3* gene acquires iron from hem. Finally, *frpB2* gene is perhaps a constitutive gen, which expresses its mRNA levels without changes even though hem is added. These results are the first approach in order to understand the molecular mechanism developed by *H. pylori* to acquire iron into the host.

Tracing full biosynthetic pathways in whole-genome sequenced bacterial genomes with a single hit.

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The escalation of bacterial genome data makes possible more exhaustive genomic studies. Nevertheless, requires tools that allow to handle massive data. BLAST is one of the most used tool to compare DNA or protein sequences and is available through web servers or for local application. BLAST web servers admit sequence comparisons with single or very low query sequences. On the other hand, the use of local application supports as many query sequence as needed, despite programming skills are necessary to analyze massive data. We developed a new web tool (available at www.blast-xyplot-viewer.icuap.buap.mx) that accept performing massive BLAST searches in whole-genome sequenced bacteria displaying massive results simultaneously and in an intuitive way. This tool projects the circular replicon as a line with fixed length of 360 units, which are the degrees in a circle, giving a delimited space where any replicon can be represented independently of their actual size. On this scheme, BLAST-results are depicted in an "*x,y*" plot where *x*-value corresponds to a "*relative position*" into the bacterial genome, and the *y*-value corresponds to a number previously assigned to the bacterial replicon (chromosome or plasmid). This web tool can display several thousands of BLAST-results belonging to thousands of bacterial genomes simultaneously and can be analyzed from the whole data to a particular one in real time.

Since many biological processes are encoded by gene clusters or operons, searching for full biosynthetic pathways involves multiple BLAST. All gene/proteins involved in a biological function included as query in our tool, produce an ordinate and intuitive result that shows the presence and location of possible orthologs in different bacterial genomes. Then, it is possible to trace in a single hit the presence and the completeness of any biological function in those genomes.

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Characterization of FlgP a novel protein essential for Fla1 flagellar assembly in *Rhodobacter sphaeroides*.

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Flagellar bacterial motility depends on three structural components. A large filament made of flagellin (FliC), a short hook (FlgE), and the flagellar motor (composed by many different proteins), that is embedded in the inner membrane. The assembly of the different components is accomplished following a tight controlled hierarchical pattern.

Currently, an extensive diversity of flagellar structures from different species has been observed by electron cryotomography, from these images it was observed that some species have complex structures located in the periplasmic space. These structures are collectively called the disk complex.

The flagella of *Escherichia coli* and *Salmonella* show only three rings, in the inner membrane (IM), in the periplasm, and in the outer membrane (OM); however, *Vibrio sp*, has three additional rings located in the periplasmic space, i.e the basal disk (beneath the OM), and the T and H-rings. In *Campylobacter jejuni* and other species, additional rings have been detected. The identity of the proteins that conform these structures have yet to be known.

Rhodobacter sphaeroides has two different sets of flagellar genes. The flagellar cluster 1 was acquired by horizontal gene transfer and is normally expressed under the growth conditions commonly used in the laboratory. The flagellar gene cluster *fla1* contains the genes required to build a flagellum similar to the one found in *E coli*. However, several open reading frames (ORFs) of unknown function are located in this cluster. One of these ORFs, encodes a putative lipoprotein of 177 residues. We have determined that this polypeptide is similar to the flagellar FlgP protein from *C. jejuni* and *Vibrio cholerae*. For these bacteria, it has been reported that deletion of this gene impairs motility but not flagellar assembly. Furthermore, it has been shown that FlgP is present not only in the flagellar basal body but also throughout the membrane of *V. cholerae.*

Inactivation of *flgP* in *R. sphaeroides* resulted in a paralyzed strain that was unable to swim. The swimming ability of this strain was restored when *flgP* and *flgT* (located downstream *flgP*) were expressed from a low-copy-number plasmid, but neither of the two genes expressed independently restored the wild type phenotype. We determined that the mutant strain, *flgP*::aadA does not assemble the flagellar hook or the filament. According with our results we propose that FlgP is an important structural component of the Fla1 flagellum, required for the assembly of extracellular structures of the flagellum of *R. sphaeroides*.

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Evaluation of metabolic and molecular events in *Escherichia coli* during recombinant protein production with a thermo-inducible system

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The production of recombinant proteins of pharmaceutical or industrial interest in *Escherichia coli* has been extensively studied to find strategies to increase yields and reduce costs of production at large scale. The expression system induced by temperature is one of the most studied. The thermo-inducible systems do not require the addition of chemical inducers like IPTG, which may be expensive and toxic. Other advantage of this system is the low contamination risks, minimized by handling. However, the stress generated by the temperature shift used for the induction can often affect cell growth, activating the heat shock response (HSR). These molecular events have been studied in separate contexts, but it converge in the activation of chaperones and proteases genes.

The principal aim of this study was to analyze the growth and production levels of the recombinant antigen ESAT-6 of *Mycobacterium tuberculosis* and some proteins associated with HSR in a recombinant *E. coli*, using an expression system induced by temperature. Cultures of the recombinant strain *E. coli* ATCC 53606 (rESAT-6) in 250 mL shake flasks and 1.2 L bioreactors were carried out, evaluating different induction temperatures. Growth kinetics of the recombinant *E. coli* was followed by optical density (OD) at 600 nm. Also, acetate production, glucose consumption, Dissolved Oxygen Tension (DOT) and pH were quantified. The expression of proteins was determined by SDS-PAGE and Western blot.

We found that the growth of the recombinant *E. coli* decreased in cultures at 39°C and 42°C compared to the cultures without induction (30°C), which can be attributed to the heat stress and metabolic burden. In shake flasks, glucose is not consumed completely; acetate was accumulated over time and the culture showed oxygen limitation. While in bioreactors, controlling the pH and the DOT, glucose was fully consumed and residual acetate was also consumed at the end of the culture. Finally, we found that the recombinant ESAT-6 was trapped in inclusion bodies and main chaperones like DnaK/J and GroEL/S showed a differential expression.

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Dynamics and segregation of polyhydroxubutyrate (PHB) granules in *Caulobacter crescentus*

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Polyhydroxybutyrate (PHB) is a reserve polymer that accumulates in many bacteria when the growth medium has an excess of carbon source and limited amount of other nutrient as phosphate, nitrogen, oxygen, etc. This polymer is synthesized in the cytoplasm where it forms hydrophobic inclusions that have proteins involved in the granule metabolism associated their surface. It was observed that in *Ralstonia eutropha* and *Pseudomonas putida* the granule-associated proteins PhaM and PhaF respectively, are responsible for the equal distribution of the PHB granules between the daughter cells. These two proteins possess tandem repeats of positively charged amino acids at their carboxy terminus (AKP helices), with which they interact electrostatically with the phosphate chain of the DNA. This interaction allows the segregation of the PHB granules with the chromosome when cell division occurs. *Caulobacter crescentus* is an oligotrophic alpha-proteobacteria that synthesizes PHB under low phosphate conditions; its dimorphic cell cycle and adaptation to low nutrient environments makes it an interesting model for the study of the segregation of these inclusions.

Following the distribution of PHB granules in a synchronized population showed that their position and segregation during the cell cycle of *Caulobacter crescentus* is a regulated process. Analysis of a mutant strain with impaired chromosomal segregation indicates that as it occurs in *P.putida* and *R. eutropha*, the segregation of PHB granules in this bacterium also depends on chromosomal segregation. Nevertheless no protein has been found in the genome having the properties of PhaM or PhaF. Proteome analysis of purified PHB granules (Mudpit) and co-localization assays revealed a new granule associated protein: PhaH, which has at the carboxy terminus an HhH domain which has been associated with a nonspecific interaction with the chromosome, however, there is no evidence that it has any function in the granule distribution.

In conclusion PHB granule localization and segregation in *Caulobacter crescentus* depends on chromosome segregation.

Identification of *Ehrlichia canis* by PCR in canines captured at the control center of Chihuahua City, Mexico.

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The family Anaplasmataceae of the class alpha proteobacteria includes pathogens of the genera *Anaplasma* and *Ehrlichia*. These bacteria infect different host cells such as erythrocytes, endothelial reticular cells, bone marrow cells, endothelial cells and reproductive tissue of arthropods. *Rickettsiae* are associated with a wide range of diseases and are widely distributed in different parts of the world, representing a serious public health problem. Canine Monocytic Ehrlichiosis is related to *Ehrlichia canis* infection, which is transmitted by the brown tick, some of the frequent clinical manifestations include: presence of mucosal hemorrhages and thrombocytopenia. The objective of the present study was to identified by PCR the presence of *Ehrilichia canis* in dogs captured and confined in control centers of Chihuahua City. For this, blood was collected by puncture of the saphenous or jugular vein in 123 canines: 66 females and 57 males, of different ages and breed, which had in their majority the presence of *Rhipicephalus sanguineus*. Peripheral blood leukocytes were separated from blood and the DNA was extracted using a commercial kit. The DNA was quantified and used for the PCR test. PCR primers were designed considering available sequences of GenBank from *Ehrlichia canis* of the gp36 gene regionand using different bioinformatic programs. The 435-bp positive amplicon were purified and sequenced by the Sanger method. Of the 123 canines sampled 20 were positive by PCR, 12 females and 8 males, mostly crossbreed and in the age range 4 months to 3 years. Nucleotide sequences were compared in BLAST, identifying 99% similarity with *Ehrlichia canis*, previously identified in Torreón, Coahuila.

In conclusion, this is the first molecular and genetic identification of *Ehrlichia canis* in canines from Chihuahua City, Mexico.

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LysR-type transcriptional regulator family and N-acyl homoserine lactone-type Quorum Sensing system contributes in the regulation of Swarming motility and antibiosis in a non-pathogenic *Burkholderia gladioli* strain

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***Keywords**:* AHL*;* Transposon *Himar1*; Antibiosis; Quorum Sensing; LysR regulator;Toxoflavin.

Burkholderia gladioli has emerged in the last years as a microorganism highly antagonistic against bacterium and fungi. The antimicrobial metabolites described in some strains isolated from different locations around the world include Toxoflavin, Bongkrekic acid, Enaxyloxin, Gladiolin and a Cyclic peptolide antibiotic. On the other hand, for many years *B. gladioli* has received attention since its pathogenicity to animals, plants and humans. However, the strain *B. gladioli* UAPS07070 was isolated of pineapple fruit healthy grown in Mexico that it inhabits as a saprophyte. Besides to the non-pathogenicity behavior in pineapple, a strong antimicrobial activity of UAPS07070 has been experimentally demonstrated. Three mutants of *B. gladioli* UAPS07070 selected from a random library generated with *Himar1* exhibited diminished antibacterial activity. The *loci* showed similarity to the genes: *lysR* (LysR-type transcriptional regulator family, clone BG79), *tofI* (acyl homoserine lactoene synthase, clone BG1232) and *tofR* (Quorum sensing regulator, clone BG87). Those genes were also implicated in decreased of swarming motility in comparison with the wild type strain. *tofI* and *tofR* were complemented *in trans* and showed restitution of antibiosis and swarming phenotypes. That corroborated the participation of N-acyl homoserine lactone-type Quorum Sensing system in the regulation of antagonism and swarming in *B. gladioli* UAPS07070.

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Characterization of *Salmonella enterica* isolates causing bacteremia in Lima, Peru, using multiple typing methods

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In this study different molecular typing tools were applied to characterize 95 blood isolates of *Salmonella enterica* collected from patients at nine public hospitals in Lima, Peru, between 2008 and 2013. *Salmonella* classification in serovars is based on serotyping; however, it is laborious, expensive and time consuming. Several molecular methods have been developed to complement or replace classical serotyping to classify *Salmonella* isolates. Molecular serotyping is based on the specific amplification of unique gene sequences that determine the most clinically and epidemiologically relevant O, H1 and H2 antigens. Multilocus sequence typing (MLST) relies on the comparison with a large MLST database of the nucleotide sequences of internal regions of a series of housekeeping genes common to all *Salmonella enterica* serovars, and has been proposed as an alternative system for classification. Results obtained by the combined multiplex PCR serotyping, two-loci and seven-loci MLST schemes, and IS200 amplification showed that eight different serovars (Enteritidis, Typhimurium, Typhi, Choleraesuis, Dublin, Paratyphi A, Paratyphi B and Infantis) were causing systemic infections in Lima. Among these serovars, Enteritidis, Typhimurium and Typhi were the most prevalent, representing 45, 36 and 11% of the isolates, respectively.

Accessory genetic elements are important for the adaptation of bacterial populations to changing environments, such as different hosts, and to cope with antimicrobials. We determined the presence of the *Salmonella* virulence plasmid (pSV) and integrons by PCR, as well as the susceptibility to 10 antibiotics for the 95 *Salmonella* blood isolates. Most isolates (74%) were susceptible to the 10 tested drugs; however, 35 strains showed intermediate susceptibility to ciprofloxacin (ISC). Resistance integrons were carried by one Dublin (*dfra1* and *aadA1*) and two Infantis isolates (*aadA1*). The Infantis isolates were multidrug resistant, and harbored a large plasmid, likely the pESI megaplasmid reported for other Infantis strains. Amplification of *spvC* and *spvRA* regions showed that all Enteritidis, Typhimurium, Choleraesuis and Dublin isolates carry the pSV. The effectiveness and feasibility of different typing tools to address the serovar of the isolates is discussed. We conclude that the standard serotyping method can be substituted by the multiplex PCR, and when necessary the one or two loci of the MLST sequencing scheme could complement the results.

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Identification and analysis of the *nmp* gene cluster involved in N-methylpyrrolidone degradation by *Alicycliphilus* sp. BQ1

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The molecular mechanisms underlying the biodegradation of N-Methylpyrrolidone (NMP), a common industrial solvent that produces irritation and teratogenic effects in rats, remain uncharacterized. *Alicycliphilus* sp. BQ1 is able to consume the NMP present in a minimal medium (MM-NMP) as the sole carbon source, in contrast to *A. denitrificans* K601 and BC strains, whose abilities to degrade recalcitrant compounds have been described. To identify the genes responsible for BQ1 ability to degrade NMP, a mutant clone unable to grow in MM-NMP (NMP$^-$A.IV.74) was generated by Tn5 insertional mutagenesis. The affected gene (*nmpB*) is part of a six-gene cluster (*nmpABCDEF*) absent in *A. denitrificans* BC and K601 genomes, but with high identity to similar arrangements in *Thauera* sp. and *Paracoccus* sp. genomes.

RT-PCR analysis showed that *nmpABCDEF* genes are expressed as an operon; the ability to degraded NMP was restored in NMP$^-$A.IV.74 by complementation with the wild type *nmpABCD* genes. Based on the proteins encoded by *nmpABC* and *F* genes, we propose a novel NMP degradative pathway where NMP is hydrolyzed by a putative N-methylhydantoinase (NMPA/NMPB) to 4-aminomethylbutyric acid. This compound could be either deaminated to form succinic-semialdehyde (SSA) or demethylated to produce γ-aminobutyric acid (GABA) by a putative aminoacid oxidase (NMPC); GABA could be transformed to SSA by GABA transaminase (GABA-AT) and finally, SSA is converted to succinate by a putative succinic semialdehyde dehydrogenase (SSDH) (NMPF).

Demonstration that this metabolic pathway is responsible of metabolize NMP was obtained when *E. coli* MG1655, unable to grow in NMP, acquired the ability to degrade NMP after functional complementation with *nmpABCD* genes. This metabolic gain of function was not affected after deleting the two GABA-AT genes. In addition, BQ1 cultures growing in NMP exhibited intracellular levels of SSA, but not of GABA. These results provide strong evidence that NMP is degraded by the enzymes encoded by *nmpABCDEF* cluster and that in this pathway, 4-aminomethylbutyric acid is deaminated to SSA instead of being demethylated to produce GABA.

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A novel Alcohol Dehydrogenase from N_2-fixing bacteria

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The activation of kinetically inert molecules, such as N_2, N_2O, or O_2, usually requires sophisticated catalytic machinery, with unique transition metal centers at the active site. To cleave the N,N triple bond of N_2, up to 16 ATP are needed to produce two molecules of NH_3 and one molecule of H_2 by nitrogen-fixing bacteria [1,2]. In the case of *Gluconacetobacter diazotrophicus* a set of dehydrogenases is overexpressed when grown under nitrogen-fixing, energy demanding conditions. Among these is the PQQ-dependent enzyme alcohol dehydrogenase (ADH) which is located in the cytoplasmic membrane of the microorganism. ADH (consisting of subunits I and II) is oriented towards the periplasm and transfers electrons to membrane-bound quinones. *Ga. diazotrophicus* can oxidize CH_3CH_2OH to CH_3COOH in two consecutive reactions, using ADH and a molybdenum-dependent aldehyde oxidase as catalysts.

In this contribution, biochemical, spectroscopic, and structural properties of this metabolically important catalyst will be presented. ADH of *Ga. diazotrophicus* is a novel multi-heme enzyme, with one PQQ, four heme *c* groups, and one [2Fe-2S] cluster as documented by low temperature EPR spectroscopy. The oxidation-reduction potentials Em (pH 6.0/SHE) of the four heme centres range from -64 to + 210 mV (spectroelectrochemistry), compared to – 250 mV for the [2Fe-2S] cluster, and -210 mV for the PQQ/PQQH2 couple (EPR spectroscopy)[3]. A structural model for the membrane-bound ADH of *Ga. diazotrophicus* will be proposed showing the intra- and intermolecular electron pathways. SU I binds the PQQ cofactor, the [2Fe-2S] cluster, and one heme *c* group, SU II harbors three heme-c groups. In conclusion, ADH provides a perfect electron transfer route to the quinones located in the cytoplasmic membrane which is a key for an efficient energy conserving system required by the nitrogen-fixing organism.

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Production of lipopeptide biosurfactants by *Bacillus subtilis* in solid state fermentation

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Keywords: lipopeptide, fermentation, Bacillus.

Introduction. Biosurfactants are amphipathic molecules produced by microorganisms. Lipopeptides (LP) as surfactin are one of the most efficient. In general, lipopeptides are produced in submerged fermentation with low productivity because of foaming, production of exopolysaccharides, and low oxygen transfer (1). The solid-state fermentation does not present these problems. However, there are few reports of this bioprocess and all reports use agro-industrial residues as the culture medium for LP production. The objective of this study was to enhance the nutrient mineral medium to improve the surfactin purification, production, and productivity.

Methodology. *B. subtilis* ATCC 6633 was inoculated into 30 mL flasks containing 1 g perlite (solid support) and 2.35 mL of liquid mineral medium (inoculum size, 10% v/v). Effect of different concentrations of Mg^{2+}, K^{2+}, Fe^{2+} and Mn^{2+} was evaluated (16 mediums, Fig.1). Incubation was carried out at 30°C for 48 hours.

M	Mg	K	Fe	Mn	M	Mg	K	Fe	Mn
1	-	-	-	-	9	+	-	-	-
2	-	-	-	+	10	+	-	-	+
3	-	-	+	-	11	+	-	+	-
4	-	-	+	+	12	+	-	+	+
5	-	+	-	-	13	+	+	-	-
6	-	+	-	+	14	+	+	-	+
7	-	+	+	-	15	+	+	+	-
8	-	+	+	+	16	+	+	+	+

Fig. 1. Full factorial design matrix. "-"and "+" is the lowest and highest concentration respectively.

Lipopeptides concentration was indirectly determined from measuring emulsification activity (2) using a standard curve with known surfactin lipopeptide concentrations.

Results. The highest lipopeptide production (estimated as the equivalent of surfactin) was obtained with medium No 14 (Fig.2). Furthermore, a statistical analysis showed significant differences between mediums and the interaction of 4 nutrients was significant. A mass spectrometry analysis revealed peaks that are characteristic surfactin isoforms (3).

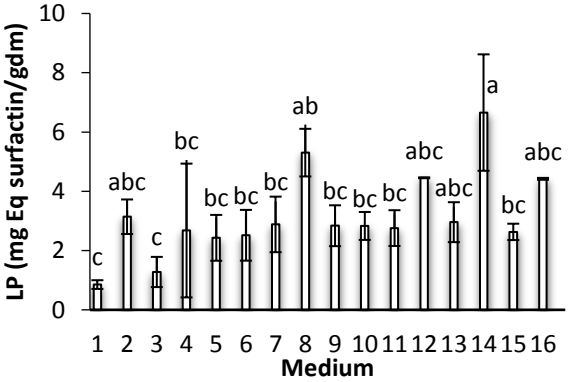

Fig. 2.Concentration of lipopeptide by different mediums. Error bars indicate standard deviation from duplicates. Different letters indicate significant differences (∝=0.05, Tukey's test)

Conclusion. The medium improvement because of change in concentrations of Mg^{2+}, K^{2+}, Fe^{2+} and Mn^{2+} led to a higher lipopeptide production.

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Alkaline effects in *Escherichia coli* cultivation on recombinant protein production and aggregates formation

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During heterologous protein overexpression using *E. coli*, an inefficient folding could occur, promoting the aggregation. Those aggregates are dynamic reservoirs that contain a large amount of recombinant protein, with importance in bioprocess as a raw material. We demonstrated that aggregates formed in *E. coli* BL21 producing a Sphingomyelinase D (33.1 kDa; pI 6.04) in complex medium under conditions of increase of alkalinity to pH 8.5, aggregates were less amyloid structured and more soluble compared with those formed at pH 7.5. Furthermore, our most recent objective was to evaluate the effect of pH change on the production of a recombinant phospholipase A2 (rPLA2) (14.7 KDa, pI 6.80) and on the properties of the aggregates caused by its overproduction. We evaluated the *E. coli* Origami producer of rPLA2, cultured in bioreactors with minimal medium and four pH strategies: pH shift from 7.5 to 6.5 or 8.5, without pH control (NC), and constant at pH 7.5. The chemical induction was performed after 5 h of culture. The production of total proteins and rPLA2 was quantified, as well as, the glucose consumption and organic acids production were determined. To define the structural characteristics of the aggregates resistance to degradation and amyloid structures were evaluated. Our results showed that cultures at pH without control and 7.5 achieved a maximal biomass (Xmax) of ~3 g / L after 11 h. While at pH 6.5 only 66% of Xmax was reached, and close to 75 % of Xmax was obtained compared to cultures at pH 7.5 and NC. In the cultures at pH 8.5 the basic pH favors the exit of acetate from the cells, the yield of acetate on biomass was> 25% compared to the other conditions. At pH 8.5 the total protein yield and productivity of rPLA2 all in insoluble form was two fold compared to the other conditions. This could be due to a favor in protein synthesis associated with the cell acetate output.
Interestingly, aggregates produced at pH 8.5 were more susceptible to proteolytic degradation by proteinase K, and bound less dye thioflavinT indicating a less β-sheet and amyloid structure. Similar to the results previously observed with Sphingomyelinase D, under different genetic background. In conclusion, cultures at pH 6.5 and 8.5 showed the lowest values of Xmax, reflecting a stressful environment. Nevertheless, pH 8.5 favored the secretion of acetate avoiding the inhibition of protein synthesis, favoring the accumulation of rPLA2. Furthermore, the aggregates formed at pH 8.5 had a more relaxed conformation and less amyloid content. This information could improve protein recovery and the use of aggregates in downstream bioprocess.

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A novel regulator of the two component system CckA-ChpT-CtrA

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Rhodobacter sphaeroides is an alpha-proteobacteria that possesses two cluster of flagellar genes denominated flagellar system 1 (Fla1) and flagellar system 2 (Fla2). Both codify for functional and mutually exclusive flagella. The flagellar system 1 is regulated by the enhancer binding protein FleQ and is expressed constitutively under laboratory growth conditions, while the second system is regulated by the two component system CckA-ChpT-CtrA, being only expressed after a selection process from *fla1*- strains, in which is common to isolate constitutive mutants in CckA.

The CckA-ChpT-CtrA pathway is widely distributed in the alpha-proteobacterium and regulates different bacterial processes like cell division, motility, symbiosis, gene transfer mechanisms, among others.

In this work we describe a novel negative regulator of the *fla2* genes that is capable of turn off their expression, named *ant*.

The *ant* gene codifies to a protein of 120 amino acids that shows homology to phosphate receiver domain of the two component system response regulators, but it does not appear to participate in a phosphorelay pathway. We found that a null mutant of *ant* in a Fla1- strain is enough to confer a Fla2+ phenotype. Additionally, the expression in *trans* of *ant* in a Fla2+ strain down-regulates the expression of the flagellar and chemotactic genes, both regulated by phospho-CtrA. Taking together these results we propose that Ant interferes with CtrA phosphorylation. This negative control could be at level of the hybrid histidine kinase CckA or over the phosphotransferase ChpT.

To explore if this protein plays a role to control the interplay between the expression of Fla1 vs Fla2 in the wild type strain. We evaluate the presence of Fla1 and Fla2 in the WS8Δ*ant* strain. By electron microscopy and Western blot, we determined that this strain is able to assemble the Fla2 flagella. Therefore, in the absence of Ant, the *fla2* genes are activated even in the wild type strain.

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The genomic basis of resistance and host adaptation in *Stenotrophomonas*

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The genus *Stenotrophomonas* (*Gammaproteobacteria*) currently comprises 12 species found in diverse natural habitats such as soil, sediments and water bodies. *S. maltophilia* is the best known one, because it is an emerging multidrug-resistant (MDR) opportunistic nosocomial pathogen. It is the third most prevalent Gram-negative, non-fermenting infectious agent recovered from lung and blood infections, after *Acinetobacter* spp. and *Pseudomonas aeruginosa*. This species is referred to as *S. maltophilia* complex (Smc) in the literature, due to its huge genetic, phenotypic and ecological diversity. We generated a collection of 108 environmental *Stenotrophomonas* spp. isolates from the water column and sediments of rivers in Morelos affected by contrasting levels of residual contamination. The isolates were characterized by advanced phylogenetic and population genetic methods based on multilocus sequence data, revealing that the Smc lumps multiple cryptic species that are genetically, ecologically and phenotypically differentiated[1]. Only the clade that we defined as *S. maltophilia sensu stricto,* comprising clinical and environmental isolates, contains MDR strains that express metallo-beta-lactamases[1]. Based on these findings we postulated that the genus *Stenotrophomonas* represents a good, nearly unexplored model to study the evolution and genomic basis of resistance and host adaptation in environmental bacteria. We selected 41 isolates representative of the diversity in our collection for genome sequencing. We could close 23 of them, representing complete genome sequences. The remaining 18 are high quality draft genomes with ordered scaffolds. We performed phylogenomic and comparative genomics analyses of our 41 plus 33 reference genomes downloaded from GenBank using our open-source get_homologues[2] and get_phylomarkers[3] pipelines. We uncovered an evolutionary pattern of gain of specific efflux pumps (EPs) along the phylogeny of the genus, consistent with the empirically determined resistance, which increases towards the most recently evolved species, being maximal in *S. maltophilia.* Furthermore, we identified a specific accumulation of type II and type Vb secretion systems, as well as of particular fimbriae in the latter species. We show that *S. maltophilia* encodes for two T2SSs. The *gsp*-T2SS locus, which is linked to a T5bSS (two-partner secretion system; TPS) is up-regulated under P-limitation via a cell-surface signaling system (CSS), similar to the PUMA3 CSS from *P. aeruginosa*. We are currently evaluating biofilm formation and virulence phenotypes of *gspD*, *xpsD*, *smf-1* (major fimbria) and large exoprotein (TpsA) mutants in the *Galleria mellonela* infection model. We conclude that the specific accumulation of EPs, SSs and of certain fimbriae have been key evolutionary events that largely account for the increased resistance and virulence displayed by *S. maltophilia,* as compared to the more basal species.

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Prevalent role of homologous recombination in the repair of double-strand breaks in the genome of *Rhizobium etli*

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Double-strand breaks (DSBs) are the most dangerous injuries for a genome. When unrepaired, death quickly ensues. Depending on the species, bacteria have two pathways for repairing DSB namely, homologous recombination (HR, leading either to a crossover or to gene conversion) and direct ligation of broken ends (Non-homologous end joining, NHEJ). While DSB on a chromosome have to be repaired for the cell to survive, we were interested on understanding how DSB are repaired on non-essential replicative DNA such as plasmids. For this reason we used *Rhizobium etli* as a model, since one-third of its genome is arranged in six megaplasmids. Unlike *Escherichia coli*, this bacterium contains active systems for HR and NHEJ, allowing us to evaluate the relative contribution of these pathways.

To generate an *in vivo* DSB, we used a modified version of the symbiotic plasmid (264 kb), containing a single copy of the *nifH* gene. In this copy, we inserted an integrative plasmid harboring a modified 250bp fragment of the *nifH* gene, containing an I-SceI site. The resulting plasmid contains two 250 bp *nifH* repeats, flanking the integrative plasmid. Depending on the construction, I-SceI sites are located either in one of the *nifH* repeats (strain D1) or in both repeats (strain D2). DSBs were easily inflicted *in vivo* by conjugating a small, replicative plasmid that expresses the I-SceI nuclease into the appropriate strains.

For strain D2, since DSBs are generated on both *nifH* repeats, homologous recombination between these cannot occur. After analyzing 50 derivatives of strain D2 that contains the gene for SceI we found that: (i) in most cases (94%) DSBs were repaired by recombination between distant (50 kb away) repeated sequences, flanking the broken *nifH* repeats; (ii) no evidence for NHEJ was detected in this sample, and (iii) although theoretically possible, the symbiotic plasmid was not lost. For strain D1, since a DSB is generated on a single *nifH* repeat, homologous recombination between these repeats may readily occur. After analyzing 100 derivatives of strain D1 that contains the gene for SceI we found that repair by repeated sequences 50kb away from the DSB site was still the most common event (62%). Despite this, crossover and gene conversion events between the *nifH* repeats were found at a frequency of 15%; interestingly, crossover and gene conversion occurred at roughly equal frequencies, suggesting the operation of the double-strand break repair model for recombination. The remaining derivatives displayed inactivation of the SceI gene or possible NHEJ events, which are currently under characterization. With the aim of detecting NHEJ events without the hindrance of homologous recombination, we decided to use strain D2 but on a *recA* mutant background. Surprisingly, all the 50 derivatives of strain D2 *recA* containing SceI analyzed thus far had SceI inactivated by different mutational events. These data suggest that the NHEJ is relatively inefficient in Rhizobium.

P O S T E R S

Low levels of polar flagellin expression in mature biofilms from *Azospirillum brasilense*.

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Azospirillum brasilense is one the most important Plant Growth-Promoting Rhizobacteria (PGPR). Biofilm formation on root surface is essential for successful colonization and to observe a beneficial effect on plant–*Azospirillum* association (1). The biofilm matrix is primarily composed of exopolysaccharides, extracellular DNA, and proteins, which are required for cell-cell and cell-surface interactions, and development of mature *Azospirilla* biofilms (3, 4). Two cell-surface structures, the single polar flagellum, and putative TAD pili, have shown to be critical for initial attachment and biofilm formation (4). The aims of this work were to show the presence of polar flagella during *Azospirilla* development in biofilm production, as an approach to define the role of flagella in biofilm matrix construction. We used two different methods: Quantitative western blot analysis and confocal laser scanning microscopy. Western blot analysis of planktonic and biofilm grown cells indicated a decrease in polar flagellin expression profiles in biofilm grown cells after three or four days grown in NFB medium. We did not find changes in polar flagellin expression in planktonic cells under the same conditions. Mature biofilm were analyzed by confocal microscopy with anti-flagellin antibody FITC tagged and DAPI stain. Under these conditions it was observed that, the flagella were localized at basal layers. However, once biofilm formation has been started, flagellin expression decreases as a biofilm matures. This result could be attributed in the first place to the high energetic cost involved in the synthesis of flagella in a state where it does not fulfill its main function, on the other hand *Azospirillum* is a pleomorphic bacterium that during the maturation of the biofilm undergoes a high differentiation where we can find a large number of cysts (5).

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Limited diffusion of OmpA2 in Caulobacter crescentus is determined by its interaction with cell wall and outer membrane.

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The cell envelope of Gram-negative bacteria is formed by an outer membrane (OM), a peptidoglycan cell wall (CW) and the cytoplasmic membrane. In enterobacteria, the integrity of the OM is conserved mainly by the Braun´s lipoprotein (Lpp) and in a minor proportion by the Tol-Pal system and the OmpA protein. *Caulobacter crescentus* lacks Lpp and its cell envelope integrity is maintained by the Tol-Pal system and by its two OmpA homologues. OmpA2 is a very abundant outer membrane protein that forms a concentration gradient from the old to the new cell pole. This protein is constituted by an integral OM β-barrel at its N-terminus and a C-terminal domain that interacts with the CW. In a previous work, we demonstrated that the direction of the OmpA2 gradient depends on the position of the gene in the chromosome. This observation indicates a restricted diffusion of the mature protein probably caused by a fast and stable interaction with the OM, the CW or both. The diffusion of integral OM proteins in *Escherichia coli* has been shown to be restricted, suggesting that only the integral OM β-barrel could be sufficient to support the formation of the gradient.

To test the relevance of the different protein domains in the formation of the OmpA2 gradient, we generated fluorescent protein fusions lacking the N- or C-terminal domains. These protein versions were stable and localized evenly through the cell body, however OmpA2 lacking the β-barrel showed a lower concentration at the cell division site and caused the formation of OM vesicles (OMVs). Additionally, we evaluated if the function or the presence of the C-terminal domain was important for the formation the OmpA2 gradient. For this, we generated point mutations on conserved residues in OmpA2 that are important for the interaction between OmpA and the CW in *E. coli*. These mutations caused OMV formation and a slight instability and degradation of OmpA2. The instability was abolished by over-expression of SurA, DegP or Skp periplasmic chaperones, however the OmpA2 point mutants were still distributed evenly.

Taken together, our results show that the localization pattern of OmpA2 is determined by both, its interaction with the outer membrane and the cell wall. Is possible that early after its translocation to the periplasmic space, the C-terminal domain interacts with the CW stabilizing the position of the protein while the b-barrel is integrated into the OM. We are currently evaluating this hypothesis by testing the diffusion of the independent domains.

The effect of *Thymus vulgaris* on the formation of biofilm from uropathogenic *Escherichia coli* in a needleless system

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Introduction. The urinary tract infections are the most recurrent and produced by uropathogenic *E. coli* and Gram-negative bacteria. *E. coli* has the capacity to produce virulence factors such as the production of toxins, adhesins and biofilm. The biofilms have been of special interest in recent years due to the increase of nosocomial infections. It has been demonstrated that the formation of biofilm allows the bacteria to resist to withstand environment stress and increased resistance to antibiotics. It has been a particular interest the study of uropathogenic *E. coli* forming biofilm in hospital medical devices (for example: bladder probes or intravenous catheters), as well as to search the strategies that inhibit the formation of biofilm.

Objective. To determine the effect of *T. vulgaris* on the formation of biofilms from uropathogenic *E. coli* in a needleless system.

Material and methods. In this study the commercial essential oil of *T. vulgaris* was used. Uropathogenic *E. coli* was grown at 37^{o}C for 7 to 10 days in Petri dishes containing tryptic soy broth and sterile fragments of needleless system. On the other hand, the fragmentsof needleless system were exposed to essential oil during 1 to 20 minutes. Then, the fragments of needleless system were washed and biofilms using 0.1% crystal violet and 0.2% calcoflour white staining were determined.The biofilm was quantified by measuring absorbance at 595 nm. The effect of *T. vulgaris* on the growth of uropathogenic *E. coli* using a plate diffusion assay was measured.

Results. The results obtained in this study indicated that the essential oil of *T. vulgaris* had a strong inhibitory effect on the formation of biofilm from uropathogenic *E. coli*in the medical device.

Conclusion. The essential oil of *T. vulgaris* affected the growth and the formation of biofilm from uropathogenic *E. coli* in systemtested.

The introduction of a gene of a pyruvate kinase of *Vibrio cholerae* is toxic for *Escherichia coli*. Zoe Alba-Martinez, Carlos Guerrero-Mendiola, Leticia Ramírez-Silva and Gloria Hernández-Alcántara. Departamento de Bioquímica, Facultad de Medicina, UNAM., 04510. (52)5556232133. galcantara72@yahoo.com.mx

In contrast to other bacteria, *Vibrio cholerae* (a gamma-proteobacterium) has three pyruvate kinase: VCIPK, VCIIPK and VCIIIPK. Its genome consists of two circular chromosomes; VCIPK and VCIIPK are located in chromosome 1 and VCIIIPK in chromosome 2 (megaplasmid). In general, this megaplasmid contains duplication of genes. The individual roles of VCIPK and VCIIPK were previously studied (Guerrero-Mendiola C. *et al*. (2017) PlosOne). VCIPK and VCIIPK were K^+-dependent and K^+-independent enzymes, respectively. This result is coincidental with a previous phylogenetic study of the family of piruvate kinase, where it stands that those sequences that have Glu117 are K^+-dependent, whereas those with Lys117 are K^+-independent enzymes (Oria-Hernández, J. *et al.* (2006) *J. Biol. Chem.* 281, 30717-30724). In this respect, VCIIIPK should be a K^+-independent enzyme. In this work we studied VCIIIPK. The VCIPK and VCIIPK proteins were transformer and expressed without any difficulties in *E. coli* BL21(DE3)-Codon Plus strain. However, VCIIIPK, which exhibited near to 36% and 50% identities to VCIPK and VCIIPK, respectively; resulted in low efficient of transformation in *E. coli* BL2(DE3) expression system. Eight BL21(DE3) competent cells with different characteristics were studied, in six of these cells the transformation failed; only in BL21-AI and origami (DE3) cells the gene was transformed. A possible explanation for the failure could be the instability of the plasmid or that the gene is toxic for the strains. Supported by PAPIIT-RA204816.

Characterization of chaperonine (HpGroEL) of *Helicobacter pylori* which binds iron

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*Helicobacter pylori (H. pylori)*is a human pathogen that causes gastritis. This bacterium is a bacillus Gram negative that invades preferentially the human gastric epithelium. In some cases the infection caused by *H. pylori* can generate peptic ulcer and develop stomach cancer. This pathogen can support its cellular growth using for instance Hb (human haemoglobin), hem (hemin) or Tf (transferrin) as only iron source. To obtain iron from those sources, this bacterium expresses proteins such as FrpB1, FrpB2 or FrpB3. These proteins were characterized as Hb or hem-bindproteins. Another protein that also binds hem is the chaperonin HpGroEL, this protein bound the iron from hem. *In silico* analysis revealed the identity of this protein as chaperonine. The characteristic of iron-binding protein was compared with the chaperonine of *Escherichia coli* (EcGroEL), interestingly this protein did not bind iron. Another characteristic observed was that *H. pylori* secreted the chaperonine while *E. coli* not. These results allowed us to suggest that *H. pylori*has a special mechanism, which consists on secreting the chaperonine to bind iron from hem, maybe this mechanism allows to maintain the structure of other proteins such as urease that is necessary tomaintain the alkaline microenvironment to support the hostile conditions of the stomach.

Structural stability of the glucose-6-phosphate dehydrogenase from *Pseudomonas aeruginosa*, provided by its substrate.

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P. aeruginosa is the causative agent of frequent intrahospital infections. Its enzyme glucose-6-phosphate dehydrogenase (G6PDH) participates in the Entner-Doudoroff (ED) pathway, transforming glucose-6-P (G6P) to 6-P-glucono-delta-lactone and simultaneously reducing the coenzymes $NAD(P)^+$ to NAD(P)H.Several studies highlight the importance of this enzyme in the adaptation of the microorganism to the oxidative stress that is frequently found in the sites that invade.Considering this, and the difficulty in combating infections caused by *P. aeruginosa*, we are interested in studying its G6PDH to know if it could be considered a potential target of antipseudomonal compounds. To this end, we recently cloned the gene encoding it and purified the recombinant enzyme to homogeneity from *E. coli* (33 mg/L of the culture of the transforming bacterium).In the present work, we evaluate the stability of the recombinant enzyme against increases in temperature and the concentration of the chaotropic agent urea, as well as the proteolytic activity of trypsin. We also determine the degree of protection that could provide saturating concentrations of coenzyme $NADP^+$ and substrate G6P, against the inactivating factors mentioned. The general methodology involved monitoring the residual enzyme activity by spectrophotometry at a wavelength of 340 nm, and structural changes by fluorometry, following the emission of the ANS fluorescent probe between 400 and 600 nm and the intrinsic emission of their tryptophans between 300 and 400 nm. The results show that the G6P, but not $NADP^+$, significantly increases the stability of the recombinant G6PDH and protects it from heat and urea inactivation and trypsin proteolysis. Fluorescence studies suggest that this increased stability is due to conformational changes in the protein caused by the binding of the substrate to the enzyme. The increase in structural stability had also been reported for the G6PDH of the bacterium *Leuconostoc mesenteroides* but conferred by the binding of the coenzyme $NADP^+$. This microorganism lacks the Entner-Doudoroff pathway, so its enzyme participates exclusively in the reductive pathway of the pentose phosphate pathway (PPP).

The above difference, together with the fact that the *P. aeruginosa* G6PDH and the enzymes of at least five other bacteria that also possess the ED pathway, cooperatively bind the G6P substrate, could reflect a different kinetic mechanism than the enzymes involved in the PPP.

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The non-conserved C terminus of the flagellar muramidase (SltF) is crucial for its localization during flagellar rod formation in *Rhodobactersphaeroides.*

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Motility in bacteria derives mainly from an organelle named the flagellum. Structurally, it can be divided into three substructures: basal body, hook and filament. Upon basal body formation, the rod needs to penetrate the peptidoglycan layer (PG), since its diameter (4 nm) is much smaller than the rod structure (11 nm), the opening of a space in the cell wall is crucial for rod formation.

In *Salmonella enterica* the protein FlgJ consists of two functional domains: the N-terminus acting as a scaffold for rod assembly and the C-terminus acting as a β-Nacetylglucosaminidase that facilitates rod penetration through the peptidoglycan layer. In the photosynthetic bacterium *R. sphaeroides*FlgJ works only as a scaffold for rod assembly. In our laboratory we identified the lytic transglycosylase responsible for breaking the cell wall: SltF. Previous work has established that the non-conserved C-terminus is relevant for its interaction with the single domain FlgJ. We are currently exploring the role of the C terminus in the interaction of SltF with the five structural proteins that comprise the rod.

The details of the interaction SltF with the flagellar rod are being explored by dissecting the non-conserved amino acids of SltF. We studied the interaction between SltF and rod proteins on *in vitro* assays by performing coimmunoprecipitation and far western blot assays.

Our results show that the non-conserved C terminus of SltF is essential for the interaction with several flagellar components and therefore we propose that this domain is crucial for the localization of SltF during flagellar rod formation.

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A new player in flagellar system 2 of *Rhodobacter sphaeroides**

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Bacteria possess different mechanisms to move onsolid surfaces or liquid media. One of the most used is swimming in liquid media by means of the flagellum. The bacterial flagellum is composed of several proteins which together form a propeller. This structure is a reversible rotatory motor powered by the H^+ or Na^+electrochemical potential to propel the cell through the media.

Rhodobacter sphaeroides is a purple non-sulfur alpha proteobacterium and it has two sets of flagellar systems (*fla1* and *fla2*). Both encode for flagella for swimming in liquid media. It has been shown that Fla1 is constitutively expressed under laboratory growth conditions, while Fla2 can be expressed when a FleQ mutant contains a gain-of-function mutation in the histidine kinase CckA.

The flagellar locus of *fla2* has some Open Reading Frames of unknown function, one of them is RSWS8N_12065, which is located in a putative operon with *fliL2*(motor associated component), *motA2* (stator component) and two more ORFs. The objective of this work was to study the role of this ORF. The phenotype of a mutant in this ORF is Mot^- so the flagellum is assembled but it doesn't rotate. The protein encoded by this ORF has a Sec-type signal sequence, so possibly it interacts with other periplasmic proteins. So, a coimmunoprecipitation assay was performed between 12065 and MotB2, a main component of flagellar stator, and we found positive interaction between them. Also, the location of 12065 was determined by means of sfGFP fusion. A single fluorescent focus per cell at the pole of the cell was observed. In addition, the localization of 12065-sfGFP in different genetic backgrounds was determinated, it seems that its localization is dependent on FliL2 but not on MotA2. The results suggest that 12065 is a new associated component of Fla2 stator.

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STRUCTURAL AND MOLECULAR CHARACTERIZATION OF PROTEINS GroEL WITH INSECTICIDAL ACTIVITY DERIVED FROM SYMBIOTIC BACTERIA

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Molecular chaperones or heat shock proteins are an large family of proteins without relationship. This proteins have been characterized for your vital function into cell as is the folding protein newly sinthetized as well as those damaged for some kind stress, degrading and prevetion of the proteins agregattion and proteostasis. The subfamily best characterized have been Hsp60 or chaperonins 60 (GroEL in the case of bacteria belong to this group). These proteins form a complex in the form of double ring that is in charge of assisting the folding of poorly folded proteins or that have not reached their native state, these chaperones also require the assistance of a co-chaperonin Hsp10 (GroES) for its proper functioning. Genes of these proteins have been identified in all bacterial genomes sequenced to date, with differences in number of copies between different groups, as well as between members of the same group.

An interesting finding several decades ago was the discovery of alternative functions for these proteins, nowadays considered as moonlighting proteins. Although several alternative functions have been characterized for these proteins for example as activators of the immune response in mammals (activation of monocytes and macrophages), participation in the formation of biofilm, function as adhesin, function as tyrosine kinase among others. Perhaps one of the most surprising and recently discovered activities of the members of this family has been to exert toxic activity against eukaryotes. As is the case of a protein from *Enterobacter aerogenes*, which is a bacterium showing an endosymbiotic association with insects of the genus *Myrmeleon* that showed toxic activity on cockroaches of the genus *Blatella* and that when characterizing the molecule responsible for activity was identified as a protein GroEL. Even more recent studies demonstrate the insecticidal activity of these proteins identified as GroEL from symbiotic bacteria of the genus *Xenorhabdus* specifically associated to entomopathogenic nematodes of the genus *Sterneinema*, which when evaluated were shown to be toxic against larvae of *Galleria mellonella*. What is interesting is the fact that all these proteins come from microorganisms with some kind of symbiotic association. Bacteria from entomopathogenic nematodes have been isolated from our laboratory. The study and characterization of these proteins through bioinformatic, biochemical and molecular techniques is of great interest as potential agents with insecticidal activity for the management of insect pests.

Characterization of the C-terminal of MotF in *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 MotA and MotB complexes.

Rhodobacter sphaeroides is an alphaproteobacterium with two full sets of flagellar genes. One of these sets (Fla1) is constitutively expressed under laboratory growth conditions. This flagellar set was acquired through an event of horizontal transfer. The second flagellar set is expressed only in mutant strains that have been selected to swim in the absence of Fla1. The proteins encoded by the flagellar genes that belong to Fla1 assemble a single subpolar flagellum.

In the genomic sequence of *R. sphaeroides*, within the flagellar locus 1 (Fla1) there is a putative bicistronic operon composed of RSP_0067and 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). The Mot- phenotype resulting from the deletion of *motF* can be suppressed by secondary mutations in *motB*. So it can be inferred that *motF* plays a role in the functioning of the MotAB complexes. Further evidence suggests that *motF* is capable of interacting with another flagellar protein, FlgT, since in its absence MotF is unable to be located at the base of the flagellum.

motF encodes a 239 amino acid polypeptide. The analysis of the primary sequence of MotF predicts a transmembrane segment, extending from residues 54 to 74, which is flanked by zones that predict motifs with potential to form coiled-coil structures, and a long unstructured periplasmic region in the C-terminal rich in lysines, arginines, alanines and proline from residues 213 to 239. Recent studies have shown that the deletion of the last codons of *motF* produces a Mot- phenotype, revealing the importance of this region as a possible interaction site with other flagellar proteins.

Using MotF as a query sequence in a BLAST search, other marine metagenome sequences from the rhodobacteraceae family could be retrieved, presenting some highly conservated residues in the C-terminal region. In this work we obtained mutant versions of MotF that specifically change these conserved residues. Replacing three different proline residues did not affect MotF function. However, we also observed that in the absence of tyrosine 238 and proline 239, the protein is unable to localize and generates a Mot- phenotype. We suggest that these residues are relevant for the MoF-FlgT interaction.

Advances in the structural determination of the membrane-bound metaloenzime Alcohol Dehydrogenase from *Gluconacetobacter diazotrophcus.*

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Nitrogen-fixing bacteria *Gluconacetobacter diazotrophicus* is highly relevant due to the oxidative fermentation of ethanol to acetic acid that releases to the medium high amounts of the product while provides electrons to the bacterial respiratory chain for energy production. This oxidation is mediated by the membrane bound PQQ-dependent type III alcohol dehydrogenase (ADH). To the date, the structure of any type III alcohol dehydrogenase remains unknown as well as its mechanism for the electron transfer.[1]

In our group have been identified, by UV-Vis and EPR spectroscopy, the cofactors that are present in the protein. One PQQ, one [2Fe-2S] cluster (being the only ADH that contains an iron-sulfur cluster) and four heme *c* groups. Additionally the redox potentials of the cofactors[2] have been determined as well as the bifunctionality of the protein to oxidize ethanol to acetic acid without releasing acetaldehyde to the medium.[3]

In this work we present the advances in the structure determination of the type III ADH from *Ga. diazotrophicus* with special emphasis in the structure and spectroscopic and magnetic properties of the cofactors. By an analysis of the amino acids sequence and by comparison with type II alcohol dehydrogenases, a model has been proposed for each of the cofactors highlighting the [2Fe-2S] cluster that presents a novel coordination mode to a couple of cysteines, one glutamate and one bidentate aspartate. In addition we have found an antiferromagnetic coupling between the PQQ and the [2Fe-2S] cluster.

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Probing the Heme Centers of Alcohol Dehydrogenase from N_2-Fixing *Gluconacetobacterdiazotrophicus*with NO

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Alcohol dehydrogenase (ADH) from N_2-fixing *Ga. diazotrophicus*Bacterium which is an obligatory aerobe that fixes dinitrogen and participates in the oxidative fermentation of alcohols, aldehydes and sugars.Thisis a membrane-bound heterodimeric enzyme (SUI ≈ 72 kDa, SUII ≈ 44 kDa) and constitutes an important component of this organism (1). It carries one pyrroloquinoline quinone (PQQ), four *c*-type cytochromes, and one ferredoxin-type [2Fe-2S] cluster as cofactors. Surprisingly, the heme centers tend to be in the reduced Fe(II) state in the enzyme as isolated (even when purified in the presence of dioxygen), as documented by UV/Vis and EPR spectroscopy. The X-band EPR spectrum (10 – 80 K) of ADH as isolated reveals two major sets of resonances which have been assigned to the PQQ semiquinone radical (*g* 2.0034) and the FeS cluster in the $[2Fe\text{-}2S]^{+1}$ state, with *g*-values of 2.007, 1.941, 1.920, g_{av} 1.956 (2).

In our effort to access the four *c*-type heme centers of ADH in the oxidized Fe(III) state, we reacted ADH with several oxidants including nitrite (NO_2^-), ferricyanide ($Fe(CN)_6^{3-}$), and peroxydisulfate ($S_2O_8^{2-}$). In addition, we probed the interaction of ADH with several NONO-ates, such as the Spermine derivative, applying both UV/Vis and EPR spectroscopy under various conditions. We present the interaction of NO with the Fe(II) heme center leading to the formation of EPR-active Fe(II)-NO species. Additionally, we found that NO acts as an oxidant of the ADH showing the unexpected oxidationof the Fe(III) heme centers.

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Inhibitionby Pyridoxal Phosphate of the enzyme glucose-6-phosphate dehydrogenase from *Pseudomonas aeruginosa.*

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P. aeruginosa is a pathogen of clinical relevance because it is the causal agent of numerous intrahospital infections, especially in patients immunocompromised by burns or diseases such as cystic fibrosis. Also, this bacterium has an intrinsic ability to resist a wide variety of antimicrobial agents, making it difficult to combat it. Because of this, the search for new therapeutic targets in the microorganism is of vital importance.An essential molecule for *P. aeruginosa*, and thus a potential target for antipseudomonal agents, is the enzyme glucose-6-phosphate dehydrogenase (G6PDH), which participates in the alternative pathway to glycolysis, the Entner-Doudoroff route, responsible for catabolizing glucose to pyruvate. Specifically, G6PDH oxidizes glucose-6-P (G6P) to 6-P-glucono- δ-lactone, with the concomitant production of NADPH; this coenzyme is used for the synthesis of various biomolecules and in the adaptation of the bacterium to oxidative stress. During the search for substances that inhibit *P.aeruginosa* G6PDH, in the laboratory where this work was carried out, we find that pyridoxal phosphate (PLP), the main bioactive form of vitamin B6 and which acts as a cofactor for a large number of enzymes, inhibits the activity of the aforementioned recombinant enzyme.Here we present results indicating that PLP is a slow-binding reversible inhibitor, of a single-step, with an IC_{50} of 7.34 μM and a *K*i of 170 nM (the $K_{0.5}$ for the G6P is of the order of 150 μM).It is shown that PLP inhibits the enzyme competing for the site to which G6P binds, so preincubation of the enzyme with saturating concentrations of this substrate (> 2.0 mM), but not the $NADP^+$ coenzyme, will protect it from this inhibition. Fluorescence studies, which evaluated the emission of the hydrophobic probe ANS (1-anilino-8-naphthalene sulfonate), which binds to hydrophobic surfaces of partially folded proteins, reaffirm the competitive inhibition of PLP by showing that this compound causes conformational changes similar to which causes the binding of the substrate to the enzyme.However, binding of PLP to the protein does not provide the structural stability conferred by G6P against thermal inactivation (see "Structural stability of the enzyme glucose-6-phosphate dehydrogenase *of Pseudomonas aeruginosa* ..." in this Abstract book). We are currently investigating whether PLP binds to the enzyme through a Schiff base, such as when acting as a cofactor, and if so, attempt to meet the lysine residue modified during the interaction.

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Study of the p6, p7, and p8 reverse and p2, p5 and p4 forward promoters of the *leuO* gene in *Salmonella typhi*

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The LeuO regulon contains a group of genes that have been involved in the survival under stress and virulence, in *Salmonella enterica* serovar Typhi and other pathogens. LeuO is a quiescent transcriptional regulator in Typhi and many aspects concerning the regulation of its gene are still unknown.

The 5' *leuO* regulatory region is comprised by ca. 900 bp. It contains numerous promoters, 5 forward and 3 reverse. They are repressed by H-NS and Lrp, two regulators that appear to work cooperatively. In particular, the p6, p7 and p8 reverse promoters and the p2 and p5 forward promoters are derepressed in a double *hns lrp* mutant.

The p6, p7, and p8 reverse promoters seem to code for sRNAs with a putative regulatory role. In addition, the nature of the H-NS/Lrp complexes is being investigated regarding their role in the regulation of *leuO*.

A highly complex regulatory scheme appears to have evolved for this quiescent gene that allows for its regulation under a wide variety of environmental cues.

*Both authors contributed equally to this work.

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Protein dosage of the *lldPRD* operon depends on processing of the primary transcript.

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The ability of *Escherichia coli* to grow on L-lactate as a sole carbon source depends on the expression of *lldP*, whose product, LldP, transports L-lactate across the membrane, and *lldD*, whose product, LldD, oxidizes L-lactate to the central metabolite pyruvate. These genes are on the lldPRD operon, which in addition contains the *lldR* gen, a dual transcriptional regulator.

An interesting feature of this operon is that the stop codon of the upstream gene overlaps the start codon of the downstream gene in such a way that the three coding regions are arranged in three different reading frames. Also, it is curious that the cistron encoding a transcriptional regulator (*R*) is located between the permease (*P*) and the dehydrogenase (*D*) encoding genes. This genetic organization portrays a paradox as the regulator protein is expected to be required in relatively small amounts whereas the two enzyme-encoding genes are required in much higher amounts. In this study we report that protein dosage of the *lldPRD* operon is not modulated on the transcriptional or translational level. Instead, modulation of protein dosage is attributed to RNAse E-dependent mRNA processing events that take place within the *lldR* mRNA, leading to the immediate inactivation of *lldR*, and to differential segmental stabilities of the resulting cleavage products. Thus, RNAse E-dependent mRNA processing ensure the fine-tuning of the expression of the individual genes of the *lldPRD* operon, and thereby provide a notable variation of the previously documented examples of gene expression regulation.

A Two-Component System Involved in *Streptomyces coelicolor* Morphogenesis.

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Streptomyces is a genus of filamentous gram positive bacteria within the phylum *Actinobacteria*, whose members are adapted to growing in soils. They deploy a complex developmental cycle which begins with spore germination and growth of a vegetative mycelium inside the substrate.Unlike unicellular bacteria, this morphology presents a challenge for *Streptomyces* growth in liquid media, since hyphae tend to aggregate and form pellets. In some species, like the model *Streptomyces coelicolor*, pellets can become so tight that nutrient flow towards the center is reduced and growth stops. Thickening agents are added to the medium to increase viscosity and reduce hyphal aggregation, making the pellets more loose. This is necessary both for routine laboratory manipulation of *Streptomyces* and large scale industrial fermentations.

Much work has been done to study *Streptomyces* morphology and development, but most of it has focused on the transition between vegetative and aerial mycelium, and on the sporulation process. In order to study the genetic traits controlling the morphology on liquid media, in particular pellet formation, we designed an experiment to select mutants that sediment slowly, in contrast to the wild type strain, which forms dense pellets that sediment quickly. With this strategy we obtained strains that form loose pellets on liquid media, even in the absence of thickening agents.

One of these strains, 2L12, showed remarkably loose, dispersed pellets, with no other detectable difference regarding differentiation and secondary metabolism. Genome sequencing of this mutant strain revealed that its phenotype was caused by a single nucleotide change that caused an amino acid substitution in a sensor histidine kinase of a putative two-component system which has not been yet characterized. A sensor kinase null mutant, and a double null mutant lacking both kinase and response regulator showed the same phenotype as the wild type strain, indicating that the mutation identified was not a loss-of-function mutation. Complementation with the mutant allele restored dispersed growth to the kinase null mutant, but not to the double mutant, showing that the effect is requires specific activation of response regulator.

Phosphorylation activity assays comparing wild type and mutant kinases are underway, as well as further physiologic and genomic characterization of the mechanism underlying the observeddispersed growth phenotype.

HilD and PhoP independently regulate the expression of *grhD1*, a novel gene required for *Salmonella enterica*serovar Typhimurium invasion of host cells

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When *Salmonella* is grown in the nutrient-rich lysogeny broth (LB), the AraC-like transcriptional regulator HilD induces the expression of genes required for *Salmonella* invasion of host cells, such as the *Salmonella* pathogenicity island 1 (SPI-1) genes. On the other hand, when *Salmonella* is grown in minimal media, the two-component system PhoP/Q activates the expression of genes necessary for *Salmonella* replication inside host cells, such as the SPI-2 genes. Recently, we showed that the regulator PhoP induces the expression of the *ecgA* virulence gene located in a *S*. Typhimurium genomic island, which also contains the *SL1344_1872* hypothetical gene. Additionally, we found that *SL1344_1872*, but not *ecgA,* is co-expressed with the SPI-1 genes. Therefore, in this study we investigated whether HilD and PhoP are involved in the expression of *SL1344_1872*, and if this gene plays a role in *Salmonella* virulence.

By using transcriptional fusions to the *cat* reporter gene and Western blot assays, we demonstrate that HilD induces the expression of *SL1344_1872* when *S*. Typhimurium is grown in LB. Therefore, we named *SL1344_1872* as *grhD1* for gene regulated by HilD. Furthermore, we found that PhoP induces the expression of *grhD1*, independently of HilD, when *S*. Typhimurium is grown in LB or N-minimal medium. Moreover, we demonstrate that the *grhD1* gene mediates invasion of *S*. Typhimurium into HeLa epithelial cells, RAW264.7 mouse macrophages and NRK-49F rat fibroblasts. Thus, our results reveal a novel factor required for the *Salmonella* invasion of host cells, whose expression is independently controlled by the HilD and PhoP regulators.

Study of expression of *pvdS* and *csbC* genesin *Azotobacter vinelandii*

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Siderophores are low molecular weight compounds capable of solubilizing and chelate iron and they are classified in order to functional group that interacts with iron, in three types: carboxylates, hidroxamates and catecholates, also there are mixed siderophores which have different type of functional groups. In *Pseudomonas aeruginosa,* transcription of genes involved in pyoverdine (mixed siderophore) production is activated by a sigma factor encoded by the *pvdS* gene, and regulation of *pvdS* is related to Gac-Rsm regulation pathway, which includes the two-componentGacS/GacA system and a post-transcriptional control system comprised of Rsm small regulatory RNAs and the RsmA repressor protein. *Azotobacter vinelandii* belongs to *Pseudomonadaceae* family and produces pyoverdine at concentrations ≤ 3 µM of iron. Also, this microorganism has homologous genes to those of *Pseudomonas aeruginosa* that are involved in pyoverdine production, including *pvdS* and *pvdI,* which encode for the transcriptional regulator and a non-ribosomal peptide synthetase, respectively. In addition to pyoverdine, *A. vinelandii* produces four catecholate siderophores (azotochelin, aminochelin, protochelin and DHBA or dihydroxybenzoic acid) that are synthetized at concentrations ≤ 10 µM of iron. About the genes involved in catechols synthesis, is known that the gene encoding for isochorismate synthase enzyme, *csbC,* is the key in catechol production.

In *A. vinelandii*, GacS/GacA-Rsm regulatory system is involved in the regulation of pyoverdine production;GacA mutants do not produce pyoverdine and increase catechol production compared to wild type. The main objective of this work is to study the expression of *pvdS* and *csbC* genes by using transcripcional and translational fusions with the reporter gene *gusA* to study the effect of the mutation in *rsmA* on the expression of pyoverdine and catechol biosynthetic genes. Therefore,the integrative vectors pUMATcgusAT and pUMATcgusAPT were used to clone the*csbC* regulatory region (217 bp) as well as the regulatory regions of *pvdS* and *pvdI* (435 bp). The regulatory regions were amplified with primers containing artificial restriction sites *Sac*I to subsequently clone it in the aforementioned vectors. Once obtained the transcriptional and translational fusions *pvdS-gusA, pvdI-gusA,csbC-gusA* and *csbX-gusA*were used to transform *A. vinelandii* wild typestrain and its derivate *rsmA* mutant and subsequently to measure enzymatic activity of β-glucoronidase to determinate the regulatory relation between the Gac-Rsm pathway with the siderophore synthesis in *A. vinelandii*.

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Identification of two novel genes of phage ϕPs56 that exclude heterologous superinfection of *Pseudomonas aeruginosa***.**

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ϕPs56 is a temperate phage that was isolated from a clinical strain of *Pseudomonas aeruginosa* in Mexico City. In its prophage state, it prevents infection of the bacteria by a variety of superinfecting temperate and virulent phages. To identify the ORFs responsible for exclusion, the ϕPs56 genome was sequenced, annotated and compared to genomes in databases. The comparisons showed that ϕPs56 is highly homologous to B3-like phages, such as JBD18 and other phages from our lab. However, clusters of non-identical variable (accessory) ORFs were identified on the left arm of the genomes. Since ϕPs56 and JBD18 coincidentally share the same seven ORFs in the variable cluster and (almost) the same profile of superinfecting phage exclusion, we proposed that both phages should carry the genes for exclusion in the same cluster. Likewise, we left out phages whose genomes differed in the ORF composition of the cluster and did not exclude superinfecting phages. This strategy led to identify the most likely genes for exclusion. After cloning in the expression vector pHERD30T and testing each candidate ORF for exclusion of 12 virulent phages, the results showed that ORFs 7 and 8 mediated exclusion. We are studying at which step the superinfecting phages are excluded by these novel genes.

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Transcriptional response in *Salmonellaenterica* serovar Typhimurium strains with different susceptibility to peroxide stress

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Eighteen *Salmonella enterica* serovar Typhimurium strains of clinical relevance to Mexico were screened for their susceptibility to stress by hydrogen peroxide. Thus, a resistant, an intermediate, and a sensitive strain were chosen for further study together with a reference strain.

Analysis of the transcriptome of the four strains revealed that 1,314 genes were expressed differently in the absence or presence of H_2O_2. Twenty-six genes were differentially down-regulated more in the most resistant strain as compared to the most sensitive one. They included several carbon metabolism genes and that for the OmpD porin, the latter being previously shown to be down-regulated in H_2O_2. All these genes responded to a variety of different genetic regulators. Likewise, twenty-five genes were differentially up-regulated more in the most resistant strain as compared to the most sensitive one. They included many of those of the SoxRS and OxyR regulons, previously shown to be involved in the response to peroxide stress. Moreover, there were five genes that have been involved in the response to stress that were regulated in a different manner.

Conceivably, there are genetic networks that coordinate the expression of various regulons that determine a more or less effective response to peroxide stress.

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PerA, the activator of the bundle-forming pilusoperon of enteropathogenic *Escherichia coli*, interacts with the transcriptional machinery

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Enteropathogenic *E. coli* (EPEC) is an important human pathogen that causes childhood diarrhea in developing countries. EPEC infections are characterized by two distinctive phenotypes: localized adherence (LA) and formation of attaching-and-effacing lesions (A/E). The genes required for these phenotypes are encoded mainly within the EAF plasmid (EPEC adherence factor)and the pathogenicity island LEE (locus of enterocyte effacement). EPEC virulence genes are under the control of various regulators, one of which is the PerA protein, a regulator belonging to the AraC/XylS family. PerA directly promotes its own expression and that of the *bfp* operon encoding the genes involved in the biogenesis of the bundle-forming pilus (BFP) that mediates the LA phenotype; it also activates PerC expression, which in turn stimulates LEE activation through the LEE-encoded regulator Ler.

Monomeric PerA directly binds to the *per* and *bfp*regulatory regions;however, it is not known whether interactions between PerA and the RNA polymerase (RNAP)are needed to activate gene transcription as has been observed for several AraC/XylS-like regulators. Here we aimed to test these potential interactions by means of pull-downs and proteomics. Protein extracts were obtained of EPEC WT and Δ*perA*grown in DME medium at 37°C. Pull-downs with these extracts were performed using MBP-PerA or MBP as a control. The candidate proteins were analyzed by MALDI-TOF.Ourresults suggest that PerA interacts with RNAP subunits, including the α, β and σ subunits. These results support the idea that PerA aids recruiting the RNAP to activate its own expression, as well as that of the *bfp* operonseemingly interacting with the transcriptional machinery.

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AT-rich region in *argK* promoter is required to expression of *argK* gene, encoding the phaseolotoxin resistant ornithine carbamoyltransferasein *Pseudomonas syringae* pv. phaseolicola

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Pseudomonas syringae pv. phaseolicola NPS3121 producesphaseolotoxin[N^{δ}(N´-sulfodiaminophosphinyl)-ornithyl-alanyl-homoarginine], an antimetabolite toxin that functions as a reversible inhibitor of the enzyme ornithine carbamoyltransferase (OCTase), which catalyzes the formation of citrulline from ornithine and carbamoylphosphate in the sixth step of the arginine pathway. The production of phaseolotoxin is temperature dependent, being optimally produced between 18 °C to 20 °C, while no detectable amounts of phaseolotoxin are present above 28 °C. *P. syringae* pv. phaseolicola is insensitive to the effect of its own toxin and the reason for this immunity was attributed to the presence of a phaseolotoxin-resistant OCTase (ROCT), product of the *argK* gene, which is expressed under conditions leading to phaseolotoxin synthesis. The promoter for *argK*gene has been previously determined and shown to be a Pribnow-type (σ^{70}).The *argK* promoteris divergent to the promoter of genes *phtABC* and the interpromoter region contains AT-rich regions.

The purpose of the present study was to analyze the participation of the AT-rich regions on the *argK* transcription. For this, we constructed plasmids containingthree deletions: D0 deletes 136 bp affecting almost completely the AT-rich region,D1 and D2 in which was eliminated 114 bp and 71 bp of the *argK* promoter leaving 35 bp and 78 bp of the AT-rich region, respectively.This constructions were electroporated into the strain CLY233, which is a *P. syringae* pv. phaseolicola that does not produces phaseolotoxin since it lacks the Pht cluster. Our work group has used strain CLY233 to perform heterologous expression assays of genes from the Pht cluster in a genetic background similar to strain NPS3121. RNA was isolated from cultures of *P. syringae* pv. phaseolicola grown in M9 medium at 18 °C or 28 °C and Northern blot analysis were made.

The hybridization results showed that in strain CLY233,*argK*containing the complete promoter region was expressed at 18°C and 28°C, in agreement with previous reports. Respect to deletions, our results revealed that in deletion D0,*argK* expression is notably decreased but not completely abolished when compared with the strain containing the complete promoter region,in which we observed*argK*expression both 18 °C and 28 °C. On the other hand, in the deletions D1 and D2 we observed expression of *argK* at 18°C, in a similar way to NPS3121 and unlike the expression observed with the strain containing the complete promoter region.It is been observed that in some promoters, AT-rich regions known as UP elements, are recognized by the sigma subunit of the RNA polymerase.According to this, we suggest that RNA polymerase may use these AT-regions to achieve an optimal orientation regard the canonical -10 and -35 elements and in consequence,could be increasing the activity of the *argK* promoter, interacting with the RNA polymerase sigma subunit.

The CtrA regulon in *Rhodobacter sphaeroides*.

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CtrA is a response regulator widely distributed amongthe alpha-proteobacteria. In*Caulobacter crescentus* this protein is essential for viability, acting as the master regulator of the cell cycle, controlling the transcription of a broad group of genes involved not only in cell cycle progression, but also in several cellular processes like motility and chemotaxis. In other alpha-proteobacteria like *Rhodobacter capsulatus*, CtrA has shown not being essential for viability;however, itis required for proper expression of genes involved in gas vesicle formation, RcGTA (gene transfer agent), pili, flagellum, chemotaxis and other signaling pathways.

CtrA is phosphorylated by the histidine kinase CckA through the phosphotransferase protein, ChpT.Phospho-CtrA is able to bind to specific DNA motifs, the complete motif (TTAA-N7-TTAAC) and the half motif (TTAA), to activate or repress the transcription of its target genes.

Rhodobacter sphaeroides is an alpha-proteobacterium which possesses two functional flagellar systems, Fla1 (acquired by horizontal transfer from a gamma proteobacteria) and Fla2 (its endogenous flagella). It has been previously shown that CtrA is not essential for *R. sphaeroides* viability, but it is required to transcribe the *fla2* genes and the major chemotaxis operon that controls Fla2. However, the remaining genes that conform the CtrA regulon in *Rhodobacter sphaeroides*are unknown.

To identify the CtrA regulon in this bacteria, we determined the transcriptional profiles of the strains AM1 (a strain with an active CckA/ChpT/CtrA system) and AM1 *ΔctrA* (a derivative strain lacking the response regulator CtrA) by RNA-seq. The genes differentially expressed were identified. We also performed a bioinformatic search to detectthe putative binding sites for CtrA. In addition, we explored the role of this transcriptional factor on some cellular processes.

We identified at least 216 genes that depend on CtrA, including those involved in motility (flagellum and chemotaxis), stress response, gene transfer agent (GTA), gas vesicles, signaling pathways and photosynthesis. Being the photosynthesis genes a new target for CtrA, that had not been reported so far. Additional results support the idea that CtrA modulates the expression of the photosynthetic genes. We also show that gas vesicles (regulated by CtrA) are needed for adequatebuoyancy in this bacteria.

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InvF acts as a classical regulator of *sopB* in *Salmonellaenterica* serovar Typhimurium

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The epithelial cell invasion ability of *Salmonellaenterica*is encoded mainly in the *Salmonella* Pathogenicity Island I (SPI-I), a chromosomal locus containing 39 genes, which code for a type three secretion system (T3SS-1), several cognate effector proteins and their chaperones, as well as for transcriptional regulators that control the expression of the genes within this island.Expression of the SPI-I genesis tightly regulated by a complex regulatory cascade that includes global and *Salmonella*-specific transcriptional regulators. InvF is an AraC-like transcriptional regulator encoded in SPI-1 that induces expression of genes for effector proteins and chaperones of the T3SS-1, including genes located in other SPIs, such as *sopB*, which is located in SPI-5 and codes for the SopB effector. Here we aimed to further characterize the role of InvF in the expression of its cognate genes. For this, we analyzed the expression of a *sopB-cat* transcriptional fusion in *Escherichia coli*K-12, which lacks the SPIs and thus InvF and *sopB*. Several *Salmonella* regulators induce the expression of virulence genes by antagonizing repression mediated by H-NS; thus, in the absence of H-NS these regulators are no longer required for the expression of target genes. Activity of a *sopB-cat* fusion was not detected inwild type and Δ*hnsE. coli* K-12 strains, indicating that H-NS is not regulating *sopB* and that InvF is required for its expression.Therefore InvF seems to be necessary for recruiting the transcriptional machinery on *sopB*.As expected, expression of *sopB* in *Salmonella enterica* serovar Typhimurium, measured by qRT-PCR,also needed InvF. Currently, we are analyzing the interaction of a purified MBP-InvF fusion protein with the *sopB* regulatory region.

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The*Citrobacter rodentium ecp* fimbrial operon is positively regulated by the putative phosphodiesterase CreR

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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). EPEC is one of the major causes of infantile diarrhea and EHEC causes hemorrhagic colitis and the hemolytic uremic syndrome (HUS), whichcould lead to kidney failure and be fatal. *C. rodentium,* EPEC and EHEC produce the A/E (attaching and effacing) lesion on the surface of intestinal epithelial cells, which ismediated by the gene products encoded within the locus of enterocyte effacement (LEE). In addition, adherence to host epithelial cells is often mediated by multimeric filamentous structures known as fimbriae or pili. The *E. coli* common pilus(ECP), present in commensal and pathogenic *E. coli*, and also found in *C. rodentium*, has been shown to play a role in pathogenic *E. coli* interactions with environmental reservoirs or host epithelial cells.

As for *E. coli,* the *C. rodentiumecp* operon is a cluster of five genes (*ecpABCDE*) encoding proteins involved in the assembly of the fimbria,which expression is favored during stationary phase and in static DMEM cultures at 26°C. However, the *ecpR* gene, encoding the positive regulator of *ecp* in EPEC, is not at the start of the operon but downstream in the opposite direction and separated by the *creR* gene that is not present in *E. coli.* Interestingly, while EcpR does not seem to be expressed nor have a role in *ecp* regulation in *C. rodentium,* we found that *creR*, encoding a putative phosphodiesterase, is essential for *ecp* activation.

Using transcriptional fusions to the *cat* reporter gene and the respective mutant strains we found that the global regulators IHF and H-NS regulate, as in EPEC and EHEC, *C.rodentium ecp* expression in a positive and negative manner, respectively, and that CreR and IHF were still needed even in absence of H-NS. Moreover, we have identified a regulatory sequence, named Distal Regulatory Element (DRE) that is essential for CreR-mediated activation of *ecp* expression and perhaps the binding site of a positive regulatory protein responding to cyclic di-GMP levels.CreR does not act through c-di-GMP binding proteins containing the PilZ domain, as *C. rodentium* mutants in the genes encoding the PilZ-like proteins YcgR and BcsA still expressed *ecp* as the wild type strain. Moreover,CreR is not a key regulator for biofilm formation but its overexpressionexerts a negative effect.

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The c-di-GMP signaling protein MucR is necessary for cyst formation but not for alginate synthesis in *Azotobacter vinelandii*

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Azotobacter vinelandii is a gamma Proteobacteria of the *Pseudomonadaceae* family. During its life cycle it undergoes a differentiation process leading to the formation of cyst resistant to desiccation (Segura et al 2014). One of the main components of the mature cyst is the exo-polyssacharide alginate, which conforms the two layers surroundingthe differentiated cell. Alginate is also involved in the formation of biofilms.The second messenger c-di-GMP has emerged as a central regulator in bacteria for a variety of cellular processes. The intracellular levels of c-di-GMP are regulated by the opposing activities of diguanilate-cyclases (DGC) and phosphodiesterases (PDE). In *Pseudomonas aeruginosa* c-di-GMP has been shown to be essential for alginate production (Hay et al 2009); MucR, an inner membrane protein possessing both DGC and PDE active domains, provides the c-di-GMP necessary for activating the alginate co-polymerase Alg8-44 complex. In the present work we evaluated the role of MucRof *A. vinelandii* in alginate production, biofilm formation and during encystment. We found that, contrary to that observed for *P. aeruginosa*, MucR was not required for alginate synthesis but it was necessary for the initial adhesion and dispersion during biofilm formation.Interestingly, we found that MucR was essential for the formation of desiccationresistantcyst in an alginate-independent manner. Electron micrographs of the formed cysts revealed that MucR was necessary for structuring the alginate envelope of the differentiated cells as the *mucR* cysts lacked these outer alginate layers. Western blot analysis confirmed that MucR was required for the expression of several enzymes essential for the formation of the cyst envelope. qRT-PCR assay demonstrated that expression of *mucR* was under the positive control of the response regulator AlgR, which has been described to control the c-di-GMP pool in *P. aeruginosa*. Collectively, our results indicate a central role for MucR in the differentiation process of *A. vinelandii* and strongly suggest that this process is controlled by the intracellular levels of c-di-GMP.

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Phenotypic evaluation and construction of *abrB* overexpression strains of *Bacillus thuringiensis*

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The transition state is considered a key step in which cells start to express adaptative functions that are needed to survive in a nutrients exhausted environment and high cell density. One of the most important transition state regulators, identified in *Bacillus* spp. and specifically studied in *Bacillus subtilis* is the AbrB protein. It has been reported that AbrB is involved in preventing inappropriate gene expression in actively growing cells, and during the transition phase it reorganizes the expression of more than 100 post-exponential-phase genes with different biological functions including biofilm formation, antibiotic production, motility, development of competence for DNA uptake, synthesis of extracellular enzymes and sporulation (Chumsakul et al., 2011). On the other hand, *Bacillus thuringiensis* (Bt) is an entomopathogenic bacteria widely used for the production of biopesticides due to its capability for producing δ-endotoxins named Cry proteins during sporulation phase. However, most of the research about Bt has been focused only on the optimization of Cry proteins production and the construction of recombinant strains in order to broaden its effectiveness. Then, there is scarce information about the gene expression regulation carried out during the bacterial growth cycle.

Therefore, the goal of the present work is focused on the phenotypic effect of *abrB* overexpression in *Bacillus thuringiensis* ser. *kurstaki* HD73. For this purpose, it has been carried out the construction of a strain that overexpresses *abrB* using a pHT-01 expression vector inducible by IPTG. Recombinant strains have been verified by restriction and sequencing analysis. Posteriorly, it will be examined by changes in phenotype and morphology, profile of growth kinetics in bioreactor and that will be compared to those of wild type strain.

Expression of the small RNAs CrcZ and CrcY determine the degree ofCarbon Catabolite Repressionin the nitrogen fixing bacteriumAzotobacter vinelandii

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Introduction.*Azotobacter vinelandii* is a nitrogen-fixing soil bacterium that prefers the use of organic acids than carbohydrates, this preferential use isregulated byCarbon Catabolite Repression (CCR) that prevents transcriptional expression of genes required for degradation of the less prefer substrates (Sonnleitner *et al.,* 2009). In *Pseudomonas spp*. Crc and the RNA chaperone Hfq represses the translation of mRNA involved in the uptake of non-preferred compounds. The CbrA/CbrBTwo Component System (TCS)activates the sRNAs transcription to sequester the Crc/Hfq complex(Valentini *et al.,* 2014).

Background. In a previous study we demonstrated that the CbrA/CbrB-Crc-Hfq system functions in a similar fashion as that observed in *Pseudomonasspp*. The Crc-Hfq protein complex binds to the *gluP* mRNA (encoding the glucose transporter), inhibiting translation, whereas the sRNAs CrcY and CrcZ sequester the Crc-Hfq protein complex antagonizing its repressor activity (Quiroz-Rocha *et al.,*2017). We hypothesized that the amount of the Crc-Hfq protein complex remains constant under different types of carbon sources (i.e. preferred vs non-preferred) whilst the expression levels of the sRNAs CrcZ and CrcY determine the strength of CCR during diazotrophic growth of *A. vinelandii*. Based on previous reports about the lack of CCR in the presence of fixed N_2, we also hypothesized that in the presence of NH_4 the expression of the sRNAs is high allowing the simultaneous assimilation of different types of carbon sources.

Results. We identified a σ^{54} promoter driving expression of the*crcZ*gene. This is in agreement with the fact this promoter is activated by the enhancer binding protein CbrB.The role of CrcZ and CrcY during CCR was evaluated using a mixture of acetate and glucose as carbon sources (preferred and a non-preferred substrate, respectively). We found that CrcZ and CrcYpromoter activity increasedin low CCR condition,that is, during glucose uptake. Real timeqPCR performed with RNA from cells growing exponentially in the mixture of acetate-glucose, confirmed that the number of transcriptsduring low CCR (glucose) was more abundant than those during high CCR (acetate),whereas Crc levelswere constant under bothconditions. These results confirm that theCCR is modulated by theexpression levels of the sRNAs CrcZ and CrcY. In addition,our resultsclearly indicate the existence of CCR process even in the presenceof a fixed nitrogen source, like NH_4, and that sRNAs expression is necessary for the assimilation of glucose under this condition.

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LC-MS/MS proteomic analysis of the BarA/SirA and Csr regulons in *Salmonella enterica* serovar Typhimurium

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In many bacteria, the BarA/SirA (UvrY) and Csr regulatory systems control the expression of genes encoding a wide variety of functions, such as carbon metabolism, motility, biofilm formation, stress responses and virulence. BarA and SirA form a two-component system, where BarA is the sensor kinase protein and SirA its cognate response regulator. In response to the presence of short-chain fatty acids, such as acetate, the BarA/SirA system induces the expression of CsrB and CsrC, two small noncoding small RNAs that contain several motifs recognized by the RNA binding protein CsrA. CsrA binds to sequences overlapping the Shine-Dalgarno (SD) sequence and the start codon, thus blocking translation and promoting degradation of target mRNAs. Furthermore, CsrA also positively regulates the expression of some genes, by preventing the formation of RNA structures that block translation or favor RNA degradation. Therefore, the BarA/SirA system induces gene expression through CsrB and CsrC, which bind to and sequester CsrA, thus antagonizing its activity.

Previous studies have shown that BarA/SirA and Csr play an important role in the pathogenesis of *Salmonella*. Specifically, these systems are at the top of a complex regulatory cascade controlling the expression of the genes located in the *Salmonella* Pathogenicity Island 1 (SPI-1), which are required for the *Salmonella* invasion of host cells. To further investigate the role of BarA/SirA and Csr on the virulence and physiology of *Salmonella*, we performed a LC-MS/MS proteomic analysis using the wild-type (WT) *S. enterica* serovar Typhimurium strain and its isogenic Δ*sirA* and Δ*csrB* Δ*csrC* mutants grown in the nutrient-rich medium LB at early stationary phase, at 37^{o}C, conditions that favor expression of *Salmonella* virulence genes. The expression of 100 proteins was affected in the Δ*sirA* and Δ*csrB* Δ*csrC* mutants, compared with the WT strain. Several of these proteins are encoded in genes known to be targets of BarA/SirA and Csr, like SPI-1 and flagellar genes. Interestingly, our study revealed 32 new targets in *Salmonella* for these systems, including some hypothetical genes. Notably, the expression of 30 proteins was differentially affected in the Δ*sirA* and Δ*csrB* Δ*csrC* mutants, suggesting that BarA/SirA and Csr could regulate gene expression independently of each other. Thus, our results further expand the BarA/SirA and Csr regulons in *Salmonella* and reveal a possible additional layer in the mechanism by which these regulatory systems control gene expression.

Functional analysis of MAP3373c recombinant of *Mycobacterium avium* subsp. *paratuberculosis* in MAP Zinc regulon.

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ABSTRACT

Introduction:*Mycobacterium avium* ssp. *paratuberculosis* (MAP) is the cause of paratuberculosis in ruminates that is characterized as a progressive chronic disease. MAP possesses a specific region of the lineage named LSP14 and LSP15, implicated in its pathogenicity and in metal homeostasis. The zinc metabolism could be dependent on its pathogenicity, this research was exclusively in the genes containing a Zur box, of which the gene *map3773c* is also part. **Objetive:**This study was designed to analyze the function of the Zur MAP3773 protein in the regulatory Zur box.
Materials and methods: We performed the purification of the protein induced with IPTG and the IMAC column with Zinc, and determined the interaction de MAP3773c with its possible Zur box and with the one of 12genes that contain a box Zur in agreement with the one of other mycobacteria, by the technique of EMSA.
Results: Protein purification revealed monomer form.The following genes and promotor region sequences: *map3778 -236, map3772c-30, map3770 -88, map3770 -33,map3765-139, map 3764c-307, map 3747c-88, map3747c-33, map3740-118, map3739c-21, map3737-197, map3737-248, map3736c-65d map3736c-440d, map3736c-491d, map0489c-66*of which interaction was positive. We believe that MAP3773c may participate in its regulation. Otherwise for *map3769c rpmG-283, map3769c rpmG* -338, *map3765 -270*, in which there was no interaction of MAP3773c in these regions of the promoter.**Conclusions:**We concluded that MAP3773c is a functional protein that interaction with the MAP zinc regulon, and the region CGTTAATGATAATCATTTTCA, is a box Zur localized in -49 site start translation of*map3773c*.

Keywords: Zur box, regulation, metabolism.

Characterization of the*Arabidopsis thaliana*-PGPR interactionunderconditions of salinity stress.

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SUMMARY

Theaim of thisstudywastoevaluatetheeffect of volatileorganiccompounds (VOCs) emittedbyplantgrowthpromotingrhizobacteria (PGPR) onbiomassproduction, salt stress mitigation, biochemicalchanges and gene expressionof *Arabidopsis thaliana.*The PGPR LBEndo1 and KBEcto4 wereisolatedfromtherhizosphere of thehalophilegrass*Distichlis spicata*, thegrowthpromotingeffecton*Arabidopsis thaliana*wasdetermined in ansystem*in vitro*of Petri dishdividedwith Murashige and Skoog (MS) mediumtoevaluatethe VOCs in conditions of 0, 50 and 100 mM of NaCl, seedswere placed ononeside and thebacterialinoculationwas placed ontheother at a concentration of $1x10^9$CFU, after 12 daystheparameters of rootlength, lateral roots and freshweightfromtheplantsweremeasured,observingthattherhizobacteriaincreasedsignificantlythebiomass of *Arabidopsis thaliana*seedlings at allsalinityconcentrations, theamount of proline, NaCl and chlorophyll in aerialpartwassubsequentlydetermined. Once the VOCs producedby PGPRs wereshowntodecreasethenegativeeffect of saline stress onseedlings, assayswereconductedonadult*Arabidopsis thaliana*plantsto test thelong-termeffect of VOC protectionagainstsaline stress. In thisway, theeffect of theprotectioncontinuesafterbeing in contactwiththe VOCs, determiningthepercentage of senescentleaves per treatment, stemlength, number of siliques and freshweight of *Arabidopsis thaliana*plants. Expression of the*AtHKT1*, *AtSOS1, AP2, PDF 1.2, AtYUCCA1, AtNIT1 y AtYUCCA1* genes of *Arabidopsis thaliana*werealsoanalyzed, which are relatedtothesalinity stress response, throughqRT-PCR.

Key words: PGPR, VOCs, proline, qRT-PCR

Genetic tools for study of wheat colonization by strains of *Azospirillum brasilense* tagged with mCherry fluorescent protein.

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Among plant-beneficial microorganisms, plant growth-promoting rhizobacteria (PGPR) are able to colonize plant root systems and to enhance plant growth and nutrition through a variety of mechanisms, including direct effects on nutrient uptake (e.g., N2-fixation and P-mobilization, iron chelation), and enhancement of root growth through the production of phytohormones. *Azospirillum brasilense* is one of many plants PGPB and it is known to have a broad host plant range. It has been isolated from the rhizospheres of wheat and other crops and several different agronomic plants.

This study reports the introduction of *mCherry* marker in *Azospirillum brasilense* Sp245, Sp7, M40 wild-type strains as well as a bacterial mutant strain to monitor the colonization in wheat (*Tritucum aestivum*). To this end, it was constructed one suicide plasmid derived from pSUP202 harboring the gene *mCherry* expressed under kanamycin resistance promoter and recombination was done in locus *hisC1.* The broad ranged plasmid pMP2444 was also constructed harboring the gene mCherry under lactose promoter. Stability of the plasmid encoding *mCherry* was confirmed *in vitro* for at least seven days of bacterial growth and after the colonization to root wheat, each of them under non-selective conditions. The utility of the labeled strains was proven by adherence and colonization studies by observed using fluorescence microscopy and confocal laser scanner microscopy (CLSM) in wheat plants inoculated with labeled strains. The method was suitable to the *in situ* formation of mini-colonies, enables visualization of bacterial colonization sites on large root fragments, and an outstanding root colonization was seen with most strains genotypes by direct microscopic examination of roots, and we are able to quantify structure as biofilm formation by strains. We concluded that it is a fitting method for analysis *in situ* of phenotyping showed by mutants affected in colonization.

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Cloning and purification of BfpI and BfpJ minor pilins of the enteropathogenic *Escherichia coli* BFP pilus in expression vector pET-3a

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The objective of this study was to expressed and purified two minor pilins called BfpI and BfpJ of type IV pili or BFP Bundle Forming Pilus, this pilus is expressed for enteropathogenic *Escherichia coli* (EPEC).

Materials and Methods. The sequences of the gene *bfpI* and *bfpJ* were obtained to the genbank. The primers were design for amplified through PCR the region of the gene *bfpI* and *bfpJ* deleted hydrophobic N-terminal segment, this segment not affect the final conformation this proteins. Next the product of PCR was digested with *NdeI* and *BamHI* restriction enzymes for clonated into pET-3a vector (previously digested with the same restriction enzymes). The products digested were ligated and transformed in HB101 *E. coli* electrocompetent cells. The cells that grown in LB broth with ampicillin were submitted to plasmid extraction. The cells that integrated the BfpI-pET3a and BfpJ-pET3a constructions were screening through electrophoresis in agarose gels. The constructions were verificated using restriction (digestion with *NdeI* and *BamHI* restriction enzymes), PCR, and sequentiation.

Results and discussion. BfpI and BfpJ proteins were expressed in strain BL21 (DE3) *E. coli* and Rosseta-gamiB (DE3) *E. coli*, and through electrophoresis in polyacrylamide gel was verified the expression of BfpI and BfpJ proteins and next purified this proteins by urea in denaturing conditions.

Regulatory effect of the histidine kinase RetS over the expression of the Rsm-sRNAs in *Azotobacter vinelandii*

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Two-component regulatory systems (TCS)are type of signaling pathway that is widely distributed in prokaryotes, which are typically constitutedby a histidine kinase and its effector protein called response regulator. The TCS generate adaptive responses based on the detection of an environmental signal, subsequently activating a phosphorylation cascade that culminates in the transcriptional regulation of target genes of the system.

In *Azotobacter vinelandii* the ability to produce metabolites of industrial interest, as the intracellular polyester poly-β-hydroxybutyrate and the extracellular polysaccharide alginate, is controlled by the TCS GacS/A, as well as the production of other secundary metabolites likealkylresorcinols and the siderophore pioverdine. The TCS GacS/A have as an intermediary the RsmA/Z/Y system, a post-transcriptional regulation system which in *A. vinelandii* has a largenumber of regulatory RNAs (sRNAs), seven belong to the RsmZ family and one to RsmY family.

In *Pseudomonas aeruginosa*has been proposedthat additionally to GaS thehistidine kinases RetS and LadS controlsthe activation of GacA so that alsoregulates the expression of*rsmZ*. RetS control negatively and LadS positively the *rsmZ* expression. Homologuesto LadS and RetS were foundin *A. vinelandii*, and were constructed mutants of thesekinases. Interestingly, the mutants exhibitdifferent phenotypes of alginate, PHB, alkylresorcinols and pioverdines, the aim of this study was to quantify the differential production of these metabolites.Contrary to expected the data suggest that RetS acts as positive regulator while LadS do it as negative regulator.

Since in *Pseudomonas* spp. GacS/A control the expression of the *rsm*-sRNAs, wemonitor the expression of *rsm*-sRNAs in *retS* mutants using transcriptional *gusA*fusions.We transfer the *retS*mutation to each one of the strains to carried the *rsm-gusA*fusions and the results obtainedcorroborate the previous finding, RetS acts as a positive regulator.

Characterization of the Avin 08930 gene involved in polyhydroxybutyrate and alkylresorcinol synthesis in *Azotobacter vinelandii*

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Azotobacter vinelandii is a gram negativebacterium. Under unfavorable conditions undergoes a process of differentiation through morphophysiological changes that results in the formation of cysts resistant to desiccation, mechanical disintegration and ultraviolet radiation. The cyst morphology consistsof a cell called central body, which is surrounded by a capsule composed of an inner layer, called intine and outer layer, called exine. The three most important compounds due to their abundance and structural role in the cyst are the exopolysaccharide alginate, the polyester polyhydroxybutyrate (PHB) and the phenolic lipids alkylresorcinols (AR) and alkylpyrones, being the first two, products of industrial interest. (Segura *et al.,* 2014)

The GacA/GacS global regulatory system through interaction with the Rsm system and the phosphotransferase system (PTS^{Ntr}) are involved in AR and PHB production and therefore in the encysting process. In order to search for alternative PHB and ARs regulationpathwaysby GacA, an algorithm in the Perl languagewas designed to search for possible GacA targets, considering the binding site of the response regulator of GacA to its target genes known as GacA box. Resulting in 8 possible targets, which corresponded to the rsmZ1-7 genes. The eighth target corresponded to an intergenic sequence upstream of the Avin 08930. (Hernández-Eligio, 2012).

Based on the above, we propose that Avin 08930 gene participates in a regulatory pathway headedby GacA and controls PHBand ARsynthesis in *A. vinelandii*. Thus the aim of the present work is to investigate the role of Avin08930 gene product in the control of the PHB and AR synthesis. We found that the inactivation of the Avin08930 gene reduced the synthesis of PHB and ARsResults on the effect of the Avin08930 on the transcription and translation of the AR and PHB biosynthetic and regulatory genes*arpR*, *phbR*, *arsA* and *phbB* will be presented.

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Functional analysis of the *Rhizobium etli* OmpR/PhoB regulators.

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The two-component systems (TCS) are one of the most prevalent signals schemes in bacteria for coordinating responses to environmental changes. The prototypical TCS consist of a receptor histidine kinase (HK) and a response regulator (RR) who elicits a response when it is phosphorylated by the HK. The OmpR/PhoB subfamily is the largest subfamily of RRs and includes well characterized transcriptional regulators such as *E. coli* OmpR and PhoB (important for osmoregulation and phosphate assimilation respectively). In *R. etli* CE3, a soil bacteria that can establish an effective symbiosis with the common bean *Phaseolus vulgaris*, 17 of the 68 putative response regulators belong to the OmpR/PhoB subfamily. Despite its importance for survival under harsh conditions in multiple organisms, OmpR/PhoB-like regulators remain poorly characterized in *R. etli*. The main objective of this work is to understand how these regulators are implicated in the response of *R. etli* to different environmental conditions. By RT-PCR experiments we demonstrate that these genes and their putative HKs are expressed under aerobic and microaerobic conditions. Since biofilm formation and motility are key traits that allow bacteria to persist or escape from different environments, we analyzed the ability of mutant derivatives in *ompR*/*phoB* homologues to form biofilms or migrate in soft agar. We also analyzed the ability of mutant derivatives to fix nitrogen. We found that the absence of FxkR, the response regulator that allows the transduction of the microaerobic signal for the activation of the FixKf regulon, and the *RHE_PC00057* gene affected motility in opposite ways. In contrast, we did not observe any significant difference in biofilm formation capacity of the mutant derivatives tested. From the mutants analyzed, only the strain lacking *RHE_PC00057* showed a defect in nodulation, although this did not translate into defective nitrogenase activity. Our results unveiled a previously unnoticed role for FxkR as a motility regulator and identified an OmpR/PhoB homologue in plasmid C that modulates motility and nodulation.

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The response regulator DctR is involved in the repression of *cckA* mediated by C4-dicarboxylic acids in *Rhodobacter sphaeroides*.

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In many alpha-proteobacteria the two component system CckA-ChpT-CtrA, controls the expression of the flagellar genes. The alpha-proteobacterium *Rhodobacter sphaeroides*, has two complete sets of flagellar genes. Under the growth conditions commonly used in the laboratory the vertically inherited *fla2* genes are not expressed whereas the horizontally acquired *fla1* genes are highly expressed. The presence of the Fla2 flagella was detected for first time in mutant strains that were selected to swim in the absence of the *fla1* genes (Δ*fla1*). In these strains a gain of function mutation in CckA accounts for the expression of the *fla2* genes and the assemble of the Fla2 flagella. Analysis of the expression of *cckA* showed that the presence of C4-dicarboxylic acids in the culture medium represses the expression of *cckA*. In *R. sphaeroides* these organic acids are mainly transported by the DctPQM transport system. The expression *dctPQM* is under control of the two components system DctSR. DctS is a membrane histidine kinase and DctR is the response regulator (RR) that activates the expression of *dctPQM* genes in the presence of a high concentration of C4-dicarboxylic acids. It has been reported that several RR related with the control of specific transport systems, also control the expression of genes related with the survival and adaptation to environmental changes. To test if DctR could be implicated in repressing the expression of *cckA* in the presence of C4-dicarboxylic acids, we isolated a *ΔdctR* mutant strain and the expression of *cckA* was determined. We observed that *cckA* is up-regulated in the absence of DctR. Given that in the absence of DctR the C4-dicarboxylic acids are not efficiently transported, the growth of the *ΔdctR* strain was compromised when succinic acid was used as a carbon source. The growth defect was corrected when *dctR* or the *dctPQM* operon were expressed from a plasmid. In the *ΔdctR*/p*dctPQM* strain, we still observed an increase in the expression of *cckA* as compared with the parental strain. In addition, the swimming ability of the *ΔdctR* and *ΔdctR*/pdctPQM strains exceeded the proficiency of the parental strain. These results suggest that DctR is implied in the transcriptional repression of *cckA*.

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Study of the effect of (p)ppGpp, H-NS and LRP on the *leuO* gene promotors in *Salmonellaenterica* serovar Typhi

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Salmonella enterica serovar Typhi is the etiological agent of typhoid fever. LeuO is a dual LysR-type transcriptional regulator that has beeninvolved in the regulation of genes implicated in the survival of the bacterium under stress and in virulence, in several pathogens. The precise signals that trigger the expression of *leuO* are unknown, given that it is a quiescent gene in the wild type under standard laboratory growth conditions.

In this study we found that *leuO* expression is repressed by the H-NS and Lrp nucleoid proteins; and that its regulatory region contains eight promotors: five forward and three reverse. These findings were obtained through the study of the expression of various fragments of the 5' regulatory region fused to*lacZ*, in the wild type and isogenic*hns*, *lrp*, and*hns lrp* strains. Moreover, by electrophoretic mobility assays it was observedthat both H-NS and Lrp bind to the 5´regulatory region. Recently, we have observed that the (p)ppGpp alarmone negatively regulates at least three of thepromotors, through the study of the expression in *relA* and*relA spoT* strains.

Our current model for regulation will be presented, which reveals a high degree of complexity that apparently allows the bacterium to respond to a diverse series of environmental signals in the host.

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Quorum sensing inhibition using antisense RNAs in *Pseudomonas aeruginosa*

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*Pseudomonas aeruginosa*is a Gram negative opportunistic pathogen of plants and animals, itsintrinsicresistance 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, alkaline protease, rhamnolipids, pyocianin, among others,the synthesis of these factorsis regulated 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; twoof them based on the detection of N-acyl-homoserine lactones (*las* and *rhl*systems), and onebased 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.However, strains deficient in *las* system, such as the environmental strain ID4365 still produce virulence factors. In this work, antisense RNAs are designed and used to inhibit expression of *lasR*and *rhlR,* genes encoding the regulator proteins of the *las* and *rhl* systems respectively, in order to determine which of these two systems can be the best target to inhibit the QSS and reduce the virulence factors production. Additionally giving an antecedent of the use and scope of this technology in this species for further applications even in the medical treatment area.

Inducible plasmidsto expressantisense RNAsagainst*lasR* and *rhlR*were constructed. They were first tested indirectly using transcriptional fusions of a target gene of each regulator protein on reference strain PAO1, the RNA that showed the best inhibition of each one was selected for further analysis. The LRL-RNA was the selected one against *lasR*, it showed 38% inhibition detected by the transcriptional fusion, and showed a similar rate in the apparently decrease of LasR detected by western blot;howeverthis decrease has no impact in the production of elastase or quinolones which are regulated by LasR. The RR1-RNA which was the selected one against*rhlR* showed 23% inhibition on the transcriptional fusion, but this was not enough to impact in the production of pyocianin or elastase which are regulated byRhlR. Additionally, synthesis of pyocianin and elastase were analyzed on PAO1*lasR* and *rhlR*single mutants and a double mutant strain, and on the ID4365 strain which is natural *lasR*mutantand its isogenic *rhlR*mutant strain.This analysis showed that even though *lasR* has a role on the synthesis of both metabolites, this effect was smaller compared with *rhlR* since its mutation impairs the synthesis of elastase and abolishes pyocianin production. In conclusion, *rhlR*is a better target than *lasR*to reduce the production of at least two virulence factors, thus the perspectives of this project is to get a better antisense RNA against *rhlR*in order to inhibit the virulence of *P. aeruginosa*.

Expression of the *phoH*ancestral gene was adapted to be controlled by the HilD virulence regulator in *Salmonella enterica* serovar Typhimurium

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Introduction.*Salmonella enterica* serovar Typhimurium (*S.* Typhimurium) causes severe enteritis in humans and various animals, as well as systemic infection in laboratory's mice, chickens and immunocompromised humans. Most of the genes required for the *S*. Typhimurium invasion of intestinal epithelial cells are in the*Salmonella*Pathogenicity Island 1 (SPI-1), a chromosomal region consisting of about 39 genes. The AraC-like transcriptional regulator HilD, encoded in SPI-1, positively controls the expression of the SPI-1 genes and several other genes located outside SPI-1. In a recent study, we determined that HilD directly regulates the expression of the *phoH* gene, when *S.* Typhimuriumis grown in the nutrient-rich LB medium. *phoH* is an ancestral gene present in many bacteria, including *Escherichia coli*, where its expression is induced by the PhoR/PhoB two-component system, in response to phosphate starvation. The PhoH protein has ATP binding activity; however, its cellular function remains unknown.Sincethe regulation by HilD represents a novel mechanism for the control of *phoH* expression, in this study, we investigated whether in *S*. Typhimurium the expression of *phoH* was adapted to be regulated by HilD, or by both HilD and PhoR/PhoB.

Results. We analyzed the expression of *phoH* in *S*. Typhimurium and *E*. *coli* through transcriptional fusions to the *cat* reporter gene. Interestingly, *S*. Typhiumrium *phoH*, but not *E. coli phoH,* was expressed in LB, in a HilD-dependent way,even when the *E. coliphoH* was assessed in *S*. Typhimurium. Surprisingly, the expression of *E. coli phoH*, but not that of *S*. Typhimurium *phoH,* was induced by the PhoR/PhoB system, in response to phosphate starvation. Consistently with these results, we found that the sequence upstream of *phoH* is different between*S.* Typhimurium and *E. coli*. Together, our results indicate that the expression of the *S.* Typhimurium *phoH* gene was adapted to be controlled by the HilD virulence regulator, but not by PhoR/PhoB, through the gain and lost of *cis*-regulatory motifs in its upstream region.

RNA-seq profiling reveals new elements involved in palladium reduction in *Geobacter sulfurreducens*

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Geobacter sulfurreducens is an anaerobic soil bacterium able to carry out dissimilatory reduction of Fe(III), Mn(IV), and other metals coupled to organic matter degradation. This relevant feature confers to these bacteria the capability to be utilized in bioremediation of soils contaminated with heavy metals and other pollutants. Besides their potential role in cleaning up the environment, *G. sulfurreducens* has also been employed in the production of electricity through the extracellular electron transfer to electrodes in bioelectrochemical systems.
G. sulfurreducens is also capable to reduce Pd(II) to Pd(0); however, the biochemical mechanism of this process remain unknown. In this work, we used transcriptome profiling analysis to identify genes involved in the reduction of Pd(II) to Pd(0). Our results showed that 254 genes were up-regulated and 142 genes were downregulated after Pd(II) exposure/reduction. Both RNA-seq and qRT-PCR data showed also that PilA encoding genes (the structural protein of pili), and several c-type cytochromes as OmcH, OmcM, PpcA and PpcD were upregulated during Pd(II) reduction; while, OmcB, OmcC, OmcZ and OmcS involved in the reduction of Fe(III) and Mn(VI) were downregulated. Also some regulatory and central metabolism genes were diferentially expressed. With those results we will propose a putative model of the mechanism of Pd(II) reduction in *G. sulfurreducens*

The extracellular electron transfer is controlled by the GSU1771 repressor a member of the SARP family in *Geobacter sulfurreducens*

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Geobactersulfurreducens 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 generated by directed evolution, which has several mutations along the genome, reduces more efficiently insoluble Fe(III) oxides. Among the mutations that this strain presents 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 was evaluated. We first constructed the mutant ΔGSU1771 in *G. sulfurreducens* by deleting 100-pb of the coding region using the pK18mobsacB system. The mutant strain ΔGSU1771 exhibits a growth delayin fumarate as electron acceptor (NBAF medium).However, it can reduce Fe(III) soluble to Fe(II) faster than the wild-type strain. Interestingly, mutant strain ΔGSU1771 produces more *c*-type cytochromes than the wild-type 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 ΔGSU1771 produces more PilA protein (structural protein of the Pili) than the wild-type strain as was observed by immundetection. These results point outthat GSU1771 represses the transcription of the *pilA* gene and *c*-type cytochrome involved in soluble Fe(III) reduction, positioning this strain as potential to be used in bioremediation and bioelectricity generation processes.

Ihfα-2 is part of the IHF complex that controls the transcription of relevant genes involved in electron transfer in *Geobacter sulfurreducens*

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In *G. sulfurreducens,* metal reduction and bioelectricity production require the participationof several elements, including multiple *c*-type cytochromes and electrically conductive pili. The pilus is composed of PilA monomers (*pilA* gene) and their expression depends mainly of the σ^{54} factor andPilRan **E**nhancer **B**inding **P**rotein regulator[1].In general, EBP regulators require ofIHF complex (α-β heterodimer) to completely activate the transcription of their target gene.*G. sulfurreducens* has 2 genes coding for the αsubunit (*ihfα*-1and*ihfα*-2) and 2 for the β subunit(*ihfβ*-1and*ihfβ*-2). Experimental evidence demonstrates that mutant strains in the subunits of the IHF complex in *G. sulfurreducens, ihfα*-1, *ihfβ*-1 and *ihfβ*-2 presented a deficient phenotype in growth and Fe(III) reduction, as well as alterations in the expression of the *pilA* and some *c*-type cytochromes importantin metal reduction[2]. In this work, we study the role of Ihfα-2 in controls the expression of the genes involved in electron transfer. We construct the *Δihfα-2* mutant strain of *G. sulfurreducens*using the pK18mobsacB system. The strain *Δihfα*-2 showeda drastic deficiency in growth andreduction of soluble Fe(III). Furthermore,this mutant did not produce PilAand it is deficient in productionof some *c*-type cytochromes, mainly those located in the outer membrane. Recently, a transcriptome analysis by RNA-seqof the *ihf* mutant strains, resulting in several differentially expressed genes in each mutant, suggesting that although *G. sulfurreducens* contains 2 copies for each subunit, each heterodimercan regulate specific genes and processes. Also, these results demonstrate that the IhfA-2 protein is part of the IHF complex, which positively regulates the transcription of the *pilA* gene, several genes coding for *c*-type cytochromes, and possibly several important enzymes for bacterial growth.

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Regulation of arginase expression in *Sinorhizobium meliloti* 1021

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In bacteria, the proteinogenic amino acid L-arginine is a precursor of antibiotics, polyamines, ornithine lipids and peptidoglycans and can also serve as a source of carbon and nitrogen. Five metabolic routes for arginine degradation are known. The arginase catabolic route is widely distributed in nature but has been little studied in most bacteria. Arginase (EC 3.5.3.11) transforms L-arginine into L-ornithine and urea. Ornithine auxotrophs of the *Medicago* nitrogen-fixing microsymbiont *Sinorhizobium meliloti* are unable to form an effective symbiosis [1]. *S. meliloti* 1021 encodes two possible arginases, annotated *argI1* and *argI2*, but lacks an *argR* transcriptional regulator of arginine metabolism. The objective of this work was to genetically and biochemically characterize the *S. meliloti* 1021 arginases and determine their physiological importance in free life and symbiosis. We independently overexpressed ArgI1 and ArgI2 in *E. coli* BL21 (DE3) (which lacks arginase activity) and found that only ArgI1 was active. A 1021 *argI1*::*loxSp* mutant lacked detectable arginase activity and its specific growth rate in minimal medium-succinate (MMS) containing arginine as sole nitrogen source of was 0.086 generations h^{-1} versus 0.119 generations h^{-1} for the wild type. Normal growth of the mutant was restored by complementing with *argI1 in trans* or by adding 1 mM ornithine to the medium. In strain 1021 containing a transcriptional ß-glucoronidase (*gusA*) reporter gene fusion to *argI1*, we found that *argI1* expression was induced 4.2 fold in MMS-NH_4 cultures containing 1 mM exogenous arginine, very similar to the increase in arginase activity observed in strain 1021 grown under the same conditions. A putative gene encoding a leucine responsive protein (Lrp) is oriented divergently from *argI1*. A 1021 *lrp*::*loxSp* mutant showed no increase in arginase activity in response to arginine, but arginine induction in the mutant was restored by expressing the *lrp* gene *in trans*. Arginine induction of *argI1* transcription did not occur when the *argI1*::*gusA* fusion construct was introduced into the *lrp* mutant. The *lrp* mutant grew similarly to the wild type in MMS with arginine as nitrogen source, indicating that the basal expression of *lrp* and *argI1* were sufficient to sustain growth. The ArgI1 is currently being purified for biochemical characterization and the symbiotic phenotype of the *argI1* mutant is being determined.

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The c-di-GMP turnover protein MucG affects the alginate process in *Azotobacter vinelandii*

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Azotobacter vinelandii is a nitrogen-fixing soil bacterium that produces the linear exo-polysaccharide alginate during both vegetative growth and encysting conditions. Alg44 is an inner-membrane protein that together with Alg8, constitute the alginate-polymerase complex. Alg44 has a domain for binding the second messenger c-di-GMP; upon binding c-di-GMP Alg44 triggers the polymerase activity of Alg8 for the synthesis of alginate. It has been postulated that the final molecular mass (MM) of the alginate is determined by the enzymatic activity of the Alg8-Alg44 polymerase complex and by the activity of alginate lyases which degrade this polymer. The Tn5 mutant strain GG9 exhibited increased levels of alginate of higher MM when compared to its parental AEIV strain. The gene affected in this mutant (Avin07910), named *mucG*, encodes a protein with putative domains for the synthesis (GGDEF) and degradation (EAL) of the second messenger c-di-GMP; it also contains a PAS domain for the detection of oxygen levels. Therefore, we hypothesized that the MucG protein negatively affects the Alg8-Alg44 polymerase complex activity by reducing the intracellular pool of c-di-GMP through its EAL domain, which degrades this second messenger. We have constructed a mutant expressing the MucG protein lacking phosphodiesterase activity ($MucG^{AAA}$) by alanine substitutions (EAL→AAA), a second mutant in which the EAL domain of MucG was deleted ($MucG^{\Delta EAL}$) and also a mutant $mucG^{-}$ by deleting the gene *mucG* (ΔMucG). These mutants showed a similar alginate phenotype observed in the GG9 mutant, indicating that the EAL domain of MucG affects negatively the production of alginate and its MM. In order to investigate a possible mechanism related to c-di-GMP, we determined the levels of this second messenger by LC/MS-MS. We found 4-fold increased levels of c-di-GMP in the ΔMucG mutant when compared to the WT strain, while in the $MucG^{AAA}$ mutant the the levels of this second messenger were 6-fold higher. Additionally, the overexpression of the gene *Avin_00420*, which encodes a diguanylate cyclase, resulted in an increment of c-di-GMP of about 19 times relative to the WT strain. Interestingly, all these mutants phenocopied the alginate effect of mutant GG9. These results indicated that high levels of c-di-GMP triggers an overproduction of alginate of higher MM. Moreover, MucG emerges as the main regulator controlling the c-di-GMP pools at the membrane levels, thus modulating both the amount and the chain length of this polymer.

Analysis of PHB production in an heterologous model.

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Poly-β-hidroxybutyrate (PHB) is a polymer produced by several species of microorganisms in which is used as storage of carbon and energy. PHB is synthesized and accumulated in cytoplasm when the cell is exposed to stress occasioned by the lack of an essential nutrient but an excess of carbon source (Anderson *et al*. 1990). PHB is a polyester of industrial interest because of its biodegradability and similar characteristics to polypropylene so, it can be used as an alternative source of plastics derivate from petroleum (Byrom *et al*. 1987).

Three enzymes are needed to catalyse the PHB production: β-ketothiolase (*phbA*), wich condense two acetyl-CoA molecules; acetoacetyl-CoA reductase (*phbB*), that reduces the molecule formed in first reaction; and PHB synthase (*phbC*) that polymerizes the β-hidroxybutyrate (3HB) monomers to form PHB wich is stored as intracellular granules.

This work evaluates the PHB production in the non-natural polymer producer: *Escherichia coli.* As *E. coli* do not have the three essential genes for de the polymer production, these were introduced: two different constructs using the pMMB206 vector containing the genes *phbA, phbB* and *phbC*in different positions were transformed into competent*E. coli*cells.

The PHB production was monitored and compared with a third *E. coli* strain containing the pMMB206 constructs and an extra plasmid with a translational fusion of GFP and a granule associated protein named Phasin; the polymer production seems to be enhanced in the presence of the GFP-Phasin fusion.

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Polyamines are required for normal growth, motility and exopolysaccharide production in *Sinorhizobium meliloti* Rm8530

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Polyamines are ubiquitous polycations derived frombasic L-amino acids thathaveimportantbut generally ill-defined roles in growth, stress resistance, pathogenicity and translation. Their importance in symbiotic nitrogen-fixing rhizobia has not been systematically investigated. An analysis of the genome seqeuence of the *Medicago* microsymbiont *Sinorhizobium meliloti* revealedthat *smc02983* and *sma0680* encode two putativeornithine decarboxylases (Odc) for synthesizing putrescine (Put)from L-ornithine. Activity assays with purifiedSMc02983 and SMa0680 showed them to be abifunctional lysine/ornithine decarboxylase (L/Odc) and a ornithine-specific Odc, respectively.In comparison to the *S. meliloti*wild type strain Rm8530, a*smc02983* mutant had 45 % as much Odc activity and drastically reducedintracellular levels of Put, spermidine (Spd) and homospermidine (HSpd).The growth rate of the mutant was reduced over 40% in minimal medium and it had only 60 % theswimming motility of the wild type.Semiquantitative assays showed that the mutant produced little calcoflour-binding exopolyasaccharide relative to the parent strain. Wild type phenotypes were restored when the mutant was grown in the presence of exogenous (1 mM) Put or Spd or when the *smc02983* gene was introduced *in trans*. In contrast, a*sma0680* mutant showed no significant alterations in Odc activity, polyamine production or other phenotypes in comparison to the wild type.These results indicate that the L/Odc SMc02983 is the major basic amino acid decarboxylase for the synthesis of Put and the polyamines derived from it (Spd and HSpd), and that one or more of these polyamines are important for a variety of physiological processes. We are currently determining the symbiotic phenotype of the *smc02983* mutant in combination with alfalfa and characterizing other phenotypes that are altered in the mutant.

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Regulation of Polyamine Biosynthesis in *Sinorhizobium meliloti*

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Polyamines are biologically active polycations with two or more amine groups.In *Sinorhizobium meliloti*, the ability to synthesize thepolyamine precursorsL-arginine (Arg), L-ornithine (Orn) and l-lysine (Lys)is importantin both free life and symbiosis [1-3].We have shown that polyamines are required for normal growth and stress resistance in *S. meliloti* and one of our current aims is to understand how polyamine synthesis is regulated in this organism.In *S. meliloti* 1021 and Rm8530, the putrescine (Put) precursor Orn is produced by the deacetylation of *N*-acetylornithine (NAO)catalyzed not only byglutamate *N*-acetyltransferase (ArgJ)but also by NAO deacetylase (ArgE) and 3 hippurate hydrolase orthologs [3]. Orn is also produced from Arg by arginase (ArgI1), whose transcriptional induction by Arg is regulated by a leucine responsive protein (Lrp). The redundancy of NAO deacetylating enzymes and the highly active ArgI1 appear to ensure the regulatedproduction of Orn for polyamine synthesis [2,3]. We have biochemically confirmed the production of Put, homospermidine (HSpd), 1,3-diaminopropane (DAP), spermidine (Spd)and norspermidine (NSpd), all of which exist free forms except for NSpd, which is ligated to yet-unidentified macromolecules. The Lys/Orndecarboxylase (L/Odc) SMc02983 is the major route for producingPut (by decarboxylating Orn), from which HSpd and Spd are produced. The purified L/Odc uses Orn and Lys as substrates with high and low specific activities, respectively, but we have not detected cadaverine (from Lys decarboxylation) in *S. meliloti*.DAP and NSpdare produced by a pathway starting with L-aspartate ß-semialdehyde [2]. A ß-glucuronidasetransciptional fusion to the *l/odc* showed that its expression is many fold higher than those of fusions to thebiochemically confirmed Orn decarboxylase (Odc) *sma0680* or the predicted Arg decarboxylase (Adc) *sma0682.* This, and the near absence of Put, Spd and HSpd in a *l/odc* mutantindicate a major role of the L/Odc in Put synthesis, while the polyamine/basic amino acid antiporters encoded near *odc* and *adc*suggestthat they function in acid resistance. We are now determining the transcriptional characteristics of the 3 decarboxylase genesand of HSpd synthase (*hss*). We are alsostudying the effects of inactivatingthe*hss*and genes encoding enzymes for making DAP, Spd and NSpd on polyamine production, physiology, and global gene expression.

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Large antagonistic activity of *Burkholderia cenocepacia*TAtl-371

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The *Burkholderia cepacia* complex (Bcc) is comprised by a group of 22 species known as human opportunistic pathogens. Most of the antimicrobial compounds characterized so far in the Bcc are produced by clinical isolates and mostly have antifungal activity. However, there is a lack of information about antibacterial compounds produced by environmental strains of Bcc. The aim of this work was to characterize the antimicrobial spectrum of *B. cenocepacia* TAtl-371, a strain isolated from tomato rhizosphere. To carry out this study, the antagonistic spectrum was determined using the double-layer agar technique, showing a large activity spectrum, including several Gram negative bacteria, highlighting the multidrug resistant strain *Acinetobacter baumannii* BAA-007*,* Bcc species from cystic fibrosis patients such as *B. multivorans* LMG 13010^{T}and *B. dolosa* LMG 18943^{T},*Salmonella* Typhimurium LT2, rice pathogen *B. glumae* LMG 2196^{T} and soil isolate *Tatumella terrea* SHS 2008^{T}. Additionally, antifungal activity was observed inhibiting the growth of fluconazole resistant yeasts *Candida albicans* and *C. glabrata* and the phytopathogens fungi*Phytophthora* sp. PTCA-14, *Pythium* sp. PYFR-14 and *Rhizoctonia* sp. RHCH-14.Afterwards, TAtl-371 strain was grown on a batch bioreactor containingpotato dextrose broth. The cell-free supernatant wasfreeze-dried and tested on FPLC, obtaining a peak with antimicrobial activity. The activity was stable atdifferent thermal treatments demonstrating that it is thermo-resistant. It was active over a wide pH range, from 1 to 11, and the activity was also stable at pH changes, keeping the activity after overnight exposition to pH values between 1 and 13. No antimicrobial activity was detected after a treatment with a peptidase enzyme. The genome of strain TAtl-371 was gathered through a project with JGI-DOE-USA. The genome contains genes related to antimicrobial activity, such as a non-ribosomal peptide, a poliketide, two bacteriocins, and a chitinase. We are studying which of these genes are responsible for the large antimicrobial spectrum of *B. cenocepacia* TAtl-371.

Nowadays, there is a concern on multidrug resistant microorganisms, and it is encouraged researchers to look for alternatives that tackle these problems. Studying new soil bacteria for antimicrobial production might be a source of novel compounds. The special features of strain TAtl-371 antagonism show us the potential use in biomedical and biotechnological applications.

Distinct distribution of lipids between outer and inner membrane of *Sinorhizobium meliloti*

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The soil bacterium *Sinorhizobium meliloti* can form a nitrogen-fixing root nodule symbiosis with legume plants such as alfalfa. Upon growth of *S. meliloti* in complex media, the bacterium produces phosphatidylethanolamine (PE), phosphatidylglycerol, cardiolipin, and the methylated derivatives of PE, monomethylphosphatidylethanolamine, dimethylphosphatidylethanolamine, and phosphatidylcholine (PC) as major membrane lipids. In contrast, when *S. meliloti* is cultivated in minimal media under phosphate-limiting conditions of growth, membrane phospholipids are largely replaced by lipids that lack phosphorus in their structure, such as diacylglyceryltrimethylhomoserine (DGTS), ornithine lipids (OL), and sulphoquinovosyldiacylglycerol (SL). Gram-negative bacteria usually possess two membrane systems, an inner (cytoplasmic) membrane and an outer membrane. Whereas the inner membrane is thought to be composed of a typical phospholipid bilayer, the outer membrane is highly asymmetrical with phospholipids covering the inner leaflet and lipopolysaccharides (LPS) the outer leaflet of the outer membrane. In the course of this work, separation of inner and outer membranes of *S. meliloti* has been refined in order to permit a quantitative assignation how individual lipids are distributed. The distribution of individual lipids between the outer and the inner membrane has been studied for *S. meliloti* wild type, mutants that lack PE or PC or that overproduce phosphatidylserine. In addition, data will be presented how DGTS, OL, and SL are distributed between the two membrane fractions.This work will permit to decide whether, in addition to LPS, other membrane lipids might be enriched in the outer membrane fraction.

Biosynthesis and functions of zwitterionic membrane lipids and their hydroxylated derivatives in the opportunistic pathogen *Burkholderia cenocepacia* J2315

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The Gram-negative bacterium *Burkholderia cenocepacia* J2315 (*Bc*J2315) is a member of the Burkholderia cepacia complex (BCC), a subgroup of important virulent opportunistic pathogens, that infects humans with cystic fibrosis, chronic granulomatous disease, and immunosuppressed individuals, causing a high mortality rate. *Bc*J2315 is multi-resistant to various antibiotics. Zwitterionic lipids constitute the majority of lipids in this bacterium and they comprise ornithine lipids (OLs) and phosphatidylethanolamine (PE) as well as their hydroxylated derivatives, namely 2-OH-OLs (OLs hydroxylated at the C2 of the esterified acyl), NL1 (OLs hydroxylated at the C2 of the amidated acyl), NL2 (OLs hydroxylated at the C2 of both acyls) and 2-OH-PE (PE hydroxylated at the C2 of the acyl esterified at the *sn*-2 position). The presence of hydroxylated versions of membrane lipids has been associated with resistance to stress. In the last two decades most genes involved in standard and hydroxylated OLs biosynthesis have been identified. However, the functions and biosynthesis of hydroxylated OL versions have not been fully elucidated in *Bc*J2315. The genes *olsB* and *pssA* participate in the first step of the biosynthesis of the OLs and of PE, respectively. This research aims to generate a double mutant in *olsB* and *pssA* in *Bc*J2315 that would be unable to synthesize zwitterionic membrane lipids or their hydroxylated derivatives, in order to determine whether these lipids are essential or if they contribute to tolerate stress. Previously a mutant deficient in the *olsB* gene was generated and it does not synthesize any form of OLs. This mutant will be used as genetic background to delete the *pssA* gene (*bcal2355*) and thus generate the double mutant. So far we have made the genetic construct to delete the *bcal2355* gene and the same gene was cloned into an expression vector. The results of this project will clarify the roles of zwitterionic membrane lipids in *Burkholderia* species.

Genetic analysis of the synthesis of phosphatidylinositol mannosides in *Streptomyces coelicolor*

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Phosphatidyl-inositol mannosides (PIMs) are unique glycolipids that are based on a phosphatidyl-*myo*-inositol lipid anchor carrying one to six mannose residues and up to four acyl chains. They are abundant in the inner and outer membranes of the cell envelope of all *Mycobacterium* species and have a fundamental role in pathogenesis by this bacterium, since they are the precursors of lipomannan and lipoarabinomannan, two major lipoglycans implicated in host-pathogen interactions.

The early steps of PIM biosynthesis have been elucidated, with the identification of the integral membrane phosphatidyl-*myo*-inositol phosphate synthase (PIP synthase), and that of three enzymes working at the protein membrane interface, the phosphatidyl-*myo*-inositol mannosyltransferases responsible for the synthesis of PIM1 (PimA), PIM2 (Pim B'), acyl-PIM1 and acyl-PIM2 (AcylT) and acyl-PIM5 (PimE). PimA has been shown to be essential in *M. tuberculosis* (Rv2610c) and *M. smegmatis* (MSMEG_2935). PimE (Rv1159, MSMEG_5149) is the only PPM-dependent mannosyltransferase so far identified in PIM biosynthesis, this enzyme has been shown to be also involved in the *O*-mannosylation of proteins in *Mycobacterium,* however, this does not occur in *Streptomyces.*

Homologues of PimA and AcylT from *M. tuberculosis* (SCO1525 and SCO1526, respectively) are found in a putative operon with the gene encoding PIP synthase in most *Streptomyces* genomes. *S. coelicolor* also has a PimE homologue (SCO2335). The presence of these genes in *S. coelicolor* predicts that this species is at least able to produce PIM1, PIM2, acyl-PIM1 and acyl-PIM2. These four PIMs have been identified in lipid extracts from this species.

The aim of this work is to demonstrate that the products of the *S. coelicolor* genes SCO1525 (homologue to PimA) and SCO2335 (homologue to PimE) are responsible for the early and late steps in PIM biosynthesis. And to determine if any higher PIM species similar to those found in *Mycobacterium* are produced in *Streptomyces*, such as PIM3, PIM4, PIM5, PIM6, or their acylated forms.
S. coelicolor null mutants of SCO1525 and SCO2335 were obtained by PCR-targeting, this strains did not show severe morphological abnormalities in their development, suggesting that these genes are not essential in *S. coelicolor.*

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Isolation and Analysis of Membrane Microdomains in *Escherichia coli*

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A common feature to all living cells is the presence of a lipid membrane that defines the boundary between the inside and the outside of the cell. Proteins that localize to the membrane serve a number of essential functions, for example mediation of signal transduction and protein secretion. In eukaryotic cells these proteins are often localized in membrane microdomains, commonly referred to as ‘‘lipid rafts’’ or ‘‘membrane rafts’’, enriched in certain lipids, such as sterols and sphingolipids. Interestingly, a group of proteins with SPFH domains (Stomatin, Prohibitin, Flotillin and HflC/K), having structural and recruitment functions, have been shown to be associated to the eukaryotic lipid-rafts and are therefore used as lipid raft markers. The fact that the *Escherichia coli* chromosome encodes for four SPFH domain containing proteins prompted us to propose that membrane rafts also exist in the E. coli. Here, we present the development of a simple method to obtain Detergent Resistant Membranes (DRMs) from *E. coli* inner membranes, and the results of a proteomic analysis of these isolated DRMs are discussed.

V Congreso de Bioquímica y Biología Molecular de Bacterias

Gene Expression of the PEP-Pyr-Oxalo node enzymes in *Streptomyces coelicolor* M145

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INTRODUCTION

In aerobic bacteria, the phosphoenolpyruvate-pyruvate-oxaloacetate node (PEP-Pyr-Oxalo node) constitutes a distribution point for carbon flux between carbon metabolic pathways like glycolysis, gluconeogenesis and tricarboxylic acid cycle. The PEP-Pyr-Oxalo enzymes vary among different organisms (Sauer y Eikmanns, 2005). Commonly, the node or some of the enzymes, has been studied with the aim to identifying targets for metabolic engineering that allow to improve production of secondary metabolites of commercial interest (Sauer & Eikmanns, 2005; Papagianni, 2012).

In wild-type *Escherichia coli* phosphoenolpyruvate carboxylation is the sole anaplerotic reaction observed when grown on glucose (Yang et al., 2002). On the other hand in *Corynebacteruim glutamicum* grown on glucose, parallel and bidirectional fluxes of carbon has been observed between phosphoenolpyruvate and malate, and pyruvate and malate (Petersen et al., 2000).

In *Streptomyces,* the available information about the PEP-Pyr-Oxalo node is scarce, limited to particular enzymes. So far, in *S. coelicolor* has been reported phosphoenolpyruvate carboxylase (PEP Cx) activity only during stationary phase of growth in cultures supplemented with glucose (Bramwell et al., 1993) and two malic enzymes has been characterized (Rodriguez et al., 2012).

In the present study, we aim to determine the conditions where genes of the PEP-Pyr-Oxalo node are expressed in *S. coelicolor* and the enzymes show activity by growing it in minimal medium using different carbon (glycolytic and gluconeogenic) sources. As well as determine the impact of malate dehydrogenase knock-out in the expression patterns and the activities of this enzymes.

METHOD

We used two *S. coelicolor* strains: wild-type M145 and a Δ-*mdh* mutant. Both strains were grown on minimal medium using casamino acids as carbon and nitrogen sources. The measurement of growth was determined using the Lowry method for protein. To quantify gene expression, we used real-time RT-PCR, using absolute quantification method. To measured enzymes antivities cell-free extracts were used.

RESULTS

In the WT, we observed a relation between gene expression levels and the enzymatic activities of the enzymes that code during the firsth 24 hours of incubation. With the lack of malate dehydrogenase, we observed an increment in the activitie of one of the malic enzymes (protein-coding gene SCO5261).

CONCLUSION

The PEP-Pyr-Oxalo enzymes are expressed in both strains of *S. coelicolor* during the different growth phases in minimal medium supplemented with casamino acids. However, the lack of malate dehydrogenase modifies the gene expression patterns of the enzymes of interest.

Role of *Bacillus cereus* A1 ChrA protein in the interaction with hexavalent chromium

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Background:

Bacillus cereus A1 isolated from chromium-contaminated wastewater of a tannery factory displayed high Cr(VI) resistance. This strain reduces 0.57 mm Cr(VI) under aerobic and anaerobic conditions without a decrease in growth rate.

Introduction:

The presence of high concentrations of chromate (hexavalent chromium) in the environment has selected microorganisms possessing mechanisms that allow them to tolerate the toxic oxyanion[1]. Bacterial resistance to chromate has been documented widely and may be conferred by chromosomal or plasmid genes[2]. The best-studied bacterial chromate resistance system is that of*Pseudomonas aeruginosa* ChrA membrane protein, which functions as a chemiosmotic pump that expels chromate from cell cytoplasm using the proton motive force[3]. ChrA belongs to the chromate ion transporter (CHR) superfamily[4]that includes hundreds of homologues from all three domains of life[5]. Nevertheless, other CHR family homologs have not been functionally characterized.Therefore, our main aim was to investigate if *B. cereus* A1 strain presents the chromate resistance *chrA* gene, and determine the role of its product in the interaction with Cr(VI).

Results and Conclusions:

We found that strain A1 displays only a*chrA* gene, which was cloned and sequenced; this gene was termed *Bc*A1-*chrA2*.Moreover,it was established that *chrA2* predicted product corresponds to a bidomain protein LCHR2 that showed high identity with putative chromate transporters of *B. cereus* and *B. thuringiensis*. On the other hand, by using the pMUTIN4cat-*chrA2*construct, which contains an internal fragment of the *chrA2* gene and the chloramphenicol resistance marker, a *B.cereus* A1 derivative disrupted in the *chrA2* gene (*Bc*A1Δ*chrA2*::Cm^r)was obtained.The determination of the survival rate at different Cr(VI) concentrations indicated that *Bc*A1Δ*chrA2*::Cm^r mutant has a higher sensitivity to Cr(VI) as compared to the wild–type strain and that this sensitivity in the mutant is more pronounced at high concentrations of chromate. To obtain additional evidence on the role of the BcChrA2 protein in Cr(VI) resistance, the *Bc*A1-*chrA2* gene was inserted into the IPTG modulable expression vector pDG148 and the construct pDG148-*Bc*A1-*chrA2*was introduced by transformation into *Bacillus subtilis* 168. The chromate sensitivity analysis of the *B. subtilis* 168/pDG148- *Bc*A1-*chrA2* transformant showed that when incubated in the presence of IPTG it exhibited higher chromate resistance, as compared to the control without IPTG and the untransformed *B. subtilis* 168 strain.Taken together, these results indicate that the BcChrA2 protein confers Cr(VI) resistance, possibly acting as a chromate expelling pump inside the cells.

Acknowledgments:-

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Qualitative expression of the TCA genes and four regulation genes involved in carbon metabolism in *Streptomyces coelicolor* M145

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INTRODUCTION

Since 2002, with the publication of the complete genome of *S. coelicolor* A3 (2) it's clear that the genome in general have large number of genes involved in carbon uptake and utilization; also, have multiple gene copies encoding similar or homologous gene products of the TCA enzymes (Van Keulen*et al*., 2011). In bacteria is common that primary metabolic pathway genes are usually clustered in operons, enabling simple coordinated regulation of gene expression, but in *S. coelicolor* the multiple copies of central carbon metabolic genes are scattered over the core and arm regions of the chromosome (Siebring*et al*,. 2010).On the other hand, prokaryotes can adapt and respond to environmental cues by either modifying their gene expression or by post-translational modifications that affect the activity or stability of existing proteins. In bacteria, transcription is mostly controlled at the stage of RNA synthesis by regulatory proteins that interactwith a specific regulatory DNA element. Regulation at transcriptional initiation is probably the most common example of metabolic adaptation in bacteria. Exist proteins capable of binding DNA known as transcriptions factors (TFs) that repress or activate transcription of certain genes (Rodríguez *et al*., 2015).

We have studied previously some of the TCA enzymes in *S. coelicolor*, but in this work, we studythe qualitative expression of all the genes encoding the TCA enzymes and the genes codifying for the regulatory proteins (CRP, DasR, GlnR and TamR)in *S. coelicolor*cultured in glucose 1% at 24 h and 72 h of growth.

METHOD

We used RNA from *S. coelicolor* wild-type M145grown for 24 h and 72 h using glucose 1% as carbon source. We realized a two-steps RT-PCR and the amplicons were compared with the qualitative expression of the RNAr 16S in the same conditions. Results were a ratio of band intensity.

RESULTS

We observed differences in the expression of the multiple genes of the same enzyme, citrate synthase for example, at 24 h the SCO2736 was expressed 2.84 times more than the control, while at 72h SCO4388 was mainly expressed. In the case of enzyme complexes there were also differences between the subunits and a single subunit can be expressed up to 4.17 times more than the control. It was also observed that the genes that are located consecutively, like an operon, showed different level of expressionand that some were not expressed in any of the two sampling times and in the conditions used.

CONCLUSION

The TCA enzymes are regulated in very different ways and the genes are not clustered in operons even if the genes are together.Also not all the genes were expressed in the same proportions at 24 h and 72 h, suggesting that some SCOs are activated in other conditions or another growth phase.

Effect of co-inoculation of PGPR on *Arabidopsis thaliana* under conditions of abiotic stress.

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The plants are exposed to biotic and abiotic stress, this affects the development and growth of plants, there are alternatives that help to mitígate these negative effects. A viable alternative is the use of plant growth promoting rhizobacteria (PGPR), not only because they stimulate growth, but also because they actívate resistance to different types of stress. Bacterialconsortia have emerged as a viable option to enhance the positive effects on plant growth and development. The present work will determine the effects of co-inoculation of the rhizobacteria LBEndo1 and KBecto4 (isolated in previous study) on *Arabidopsis thaliana* under stress conditions due to drought and salinity. An experiment was performed under stress conditions to observe the *in vitro* interaction of *A. thaliana* in Petri dishes with Murashige and Skoogmedium (MS) for both diffusible and volatile compounds, making a simple inoculation with the bacteria LBEndo1 and KBEcto4, and the co-inoculation of both (LBEndo1 and KBEcto4), in addition to an uninoculated control. Thus, when positive effects are obtained over growth of Arabidopsis, it will proceed to analyze under conditions of stress by salinity and drought with the same treatments; In salinity stress, concentrations of 0, 50 and 100 mMNaCl will be added to plates with solid medium and the morphological parameters (rootlength, number of lateral roots and freshweight) will be measured. The expected results are that the rhizobacteria being in synergy in co-inoculation could protect the plants under stress bysalinity. As for drought, a positive effect was observed in total weight and lateral roots in co-inoculation.

Co-inoculation, rhizobacterias, *Arabidopsis thaliana*,droughtstress, salinity stress

Isolation and characterization ofa crude oil-degrading *Pseudomonas aeruginosa* from the Gulf of Mexico.

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Marine environments have been severely affected by oil spills due to its high recalcitrance. Alkanes represent an important fraction of total hydrocarbons that conform the crude oil and its biodegradation is a challenge for different microorganisms due to their low solubility and reactivity. Never the less, some bacteria have the metabolic capacity to degrade alkanes. The aim of this study was to characterize the hydrocarbon-degrading capacity of a strain isolated from Gulf of Mexico.

Initially, a bacterial consortium from water column (55 meters of depth) was isolated from the Gulf of Mexico in mineral medium with a mix of crude oil and kerosene as carbon source. From this consortium, a strain named B9MF-1was isolated and phylogenetic analysis of 16S rRNA sequence showed that this strain is closely related to *Pseudomonas aeruginosa* species.

B9MF-1 strain was able to grow in mineral medium using different crude oils (light, medium or heavy) as sole carbon source. Growth kinetics with alkanes from C8 to C28 showed that B9MF-1 strain had preference for medium and long-chain alkanes (C12-C28). Cells of B9MF-1 strain previously grown in medium with light crude oil showed higher adherenceto aliphatic hydrocarbons (80%) than those grown with glucose (25%).

Alkane hydroxylase coding genes *alkB1*, *alkB2* and *almA* showed a differential expression dependent of alkane chain length.The B9MF-1 strain exhibited higher growth than the human pathogen *P. aeruginosa* strain PAO1 in mineral medium added with light crude oil, suggesting a high adaptation of B9MF-1 strain to use hydrocarbons as carbon source. Collectively, these results show that the marine *P. aeruginosa* strain B9MF-1 has the metabolic capacity to degrade different aliphatic hydrocarbons.

Sphingolipid biosynthesis and function in bacteria

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Sphingolipids exist in all eukaryotic cells, playing essential roles as structural components of cellular membranes and as signaling molecules that participate in a wide range of physiological and pathological processes. In contrast, sphingolipids occur only in few bacteria and their presence is so exotic that many of them have "*Sphingo*" as prefix in their genus name. However, in some bacteria, sphingolipids might be formed only under certain physiological conditions to resist different types of stress such as acidity or high temperatures. Although the eukaryotic genes and enzymes involved in the sphingolipid biosynthesis are reported, little knowledge exists for bacteria. An exception is the first step, catalyzed by serine palmitoyltransferase (SPT) thatcondensates serine and a fatty acyl-CoA to form the first intermediate 3-oxo-sphinganine. Phylogenetic analysis suggests that operons for sphingolipid biosynthesis exist in many more bacteria. Upstream of the *spt* gene, some of these operons contain a putative acyl carrier protein (*acp*) gene. This finding suggests specialized ACPs, instead of coenzyme A (CoA), are used in some cases during the initial step of sphingolipid biosynthesis in bacteria. Ourbioinformatic analysis of the *Caulobacter crescentus*genome shows that it contains a cluster of 8 genes, probably organized in two operons,that seem to be involved in sphingolipid biosynthesis. Specifically, in one operon, a gene encoding for a putative SPT (CC_1162) is preceeded by a gene encoding a putative ACP (CC_1163). Also, theother operon harbors a gene predicted to encode an acyl-CoA synthetase (ACS;CC_1165). In our simplest model, the putative ACS CC_1165 would be in fact an acyl-ACP synthetase (Aas), which converts the ACP CC_1163 to its acylated form. Then, SPT CC_1162 would use L-serine and acyl-ACP CC_1163 to form 3-oxo-sphinganine,which would then be further modified to produce diverse sphingolipids.Studying the biochemical function of specific genes we could show that the predicted ACP CC_1163 is an ACP, since it carries the 4'-phosphopantetheine prosthetic group. Also, the enzymatic activity of SPT from both *C. crescentus* and *Escherichia coli* BL21 (DE3) was studied, using SPT from *Sphingomonas wittichii* as a positive control. After expression of *spt* candidate genes in *E. coli*, *in vitro* formation of 3-oxo-esfinganine was observed, in addition to other lipid compounds that are possibly sphingolipids. Additionally, the predicted ACS CC_1165 was able to acylate CoA. However, acylation of the special ACP CC_1163 by CC_1165 could not be demonstrated so far. Using the Acyl-ACP synthetase (AasS) from the bioluminescent *Vibrio harveyi* strain B392,it was possible to synthetize the acylated forms of the constitutive ACP (AcpP CC_1677) from *C. crescentus*, with fatty acids from C6 to C18. However, it was not possible to observe the acylated forms of the special ACP CC_1163 by urea-PAGE. Acylation assays using [^{3}H]-labeled fatty acids will be performed to resolve this. Enzymatic assays using both acyl-ACPs and acyl-CoA will be done to determine which is a better thioester substrate for the different SPTs.

Evaluation of the antimicrobial effect of gallium maltolate in clinical isolates of *Pseudomonas aeruginosa*

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This year the WHO, published al ist of the most dangerous bacterial species for public health, *Pseudomonas aeruginosa*,a notorious multi-drug resistant opportunistic pathogen occupies the second place of that list.in Mexico itcauses 10 % of nosocomial diseases, some of them with high mortality rates. Hence, it is very important to discover novel forms to combat their infections.

In our laboratory, we are interested in elucidating the mechanisms used by*P. aeruginosa*to resist different types of stress including that derived from novel antimicrobials. Gallium is a metal with clinical applications as radiotracer as well as for the treatment of hypercalcemia. Gallium (Ga 3+) also has remarkable antibacterial properties against *P. aeruginosa* and other recalcitrant bacteria, those properties are related with its physicochemical similarities with iron 3^+ with the important difference that it cannot be oxido-reduced, a crucial activity of the iron functions in key metabolic processes such as the electron transfer in the respiratory chain, the DNA replication and the stress response (e.g. it mediates the activities of catalase and superoxide dismutase).

Interestingly Gallium is being used in clinical trials with cystic fibrosis patients infected with *P. aeruginosa* with the goal to reduce bacterial loads and improve lung function. Previously our group described that the laboratory strain PA14 can acquire gallium resistance by reducing their entrance to the cell, via the functional loss of the HitAB iron transporters or by the overproduction of pyocyanin a phenazine that can reduce iron 3+ to iron 2+ for which Ga 3+ is not a good competitor (1). But little is known about the mechanisms of gallium tolerance and resistance in clinical strains, hence we screen a collection of clinical *P. aeruginosa* isolates from cystic fibrosis and pneumonia patients with the objective to identify those that presented high tolerance against gallium formulated as gallium nitrate and as gallium maltolate, the screening revealed that in general all tested isolates presented similar tolerance levels against gallium nitrate that the reference strains PA14 and PAO1 (MIC50~ 20 uM), in contrast, some isolates tolerated higher concentrations of gallium maltolate and even grew better at 100 uM than at 10 uM, currently we are exploring if this behavior is related to the activity of efflux pumps by using respiratory uncouplers, efflux pumps inhibitors and a set of mutants defective in different efflux pumps.

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Role of *Pseudomonas aeruginosa* alkaline protease during nutritional stress

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Quorum sensing in *Pseudomonas aeruginosa* controls the production of exoproteases such as alkaline protease encoded by the *aprA* gene and elastaseencoded by*lasB*and *lasA* genes. These are costly public goods susceptible to be exploited by selfish non producer individuals (social cheaters) (1).

Here we show using the reference strains PAO1 and PA14 that the alkaline protease activity is high during several conditions of nutritional stress such as prolonged starvation, the presence of social cheaters, and the addition of the respiratory uncoupler CCCP. The elastase activity seems to have no important modification over these conditions. In addition we evaluated some of these phenotypes in clinical strains from burned patients.

We hypothesize that since the energy cost of producing alkaline protease is high, its production during starvation should confer and advantage to the bacteria that produce it. The possible physiological relevance of this behavior is currently being explored using PAO1 mutant strains (*aprA*, *lasB*) by the evaluation of survival during long time incubation (in conditions which favor cell lysis) (2), biofilm formation, biofilm dispersal, bacterial competitions, etc.

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Removal kinetics of dicophol in liquid culture by soil microorganisms from uncontaminated agricultural soil

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ABSTRACT

In the present work we demonstrate that soil microorganisms are capable of degrading organic contaminants like organochlorine pesticides. The aim of this study was to evaluate the removal Dicophol in liquid culture by microorganisms obtained from an uncontaminated agricultural soil at laboratory conditions. Sample with microorganisms was dissolved in mineral medium previously sterilized. Aliquots were taken with 3 ml of inoculum was added to 30 ml of mineral medium and 50 ppm of Dicophol was performed in liquid culture at 28ºC, 150 rpm during 286 h (12 days). Cell growth was evaluated by measuring the turbidity spectrophotometer at 600 nm, and total protein by the Bradford method. A kinetic of microbial growth and removal of Dicophol at 50 ppm and supplemented with 25 ppm of glucose, was performed. Dicophol residual concentration was determined by gas chromatography. Results showed that Dicophol was removed in a high percentage (90%) for twelve days. That is, uncontaminated soil microorganisms were able to use the Dicophol as sole carbon and energy source in a liquid medium contaminated with Dicophol.

Keywords: organochlorine pesticides, soil microorganisms, water pollution.

Characterization of Plant Growth Promoting Rhizobacteria isolated of *Prosopis spp.*

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Abstract

The plant growth promoting rhizobacteria has abilities for to ameliorate salinity stress in plant. RizoNFB, LBrizos3, LBecto3 and Cryrizos1rhizobacteria were identified as *Achromobacter sp*., *Bacillus mojavensis*, *B. subtilis* and *B. aryabhattai,* respectively, which were isolated from*Prosopisspp*., andevaluated for growth in medium with NaCl and plant growth promoting mechanism. Evaluated for, phosphate solubilization; indol-3-acetic acid, 1-aminocyclopropane-1-carboxilate (ACC) deaminase and siderophores production. Rhizobacteria were tested for amplification of nifH and acdS genes by PCR method.The PGPR isolated were tested*in vitro* on*Arabidopsis thaliana* (col-0), with 0, 100 and 150 mMNaCl, and different methods of inoculation (diffusible to distance and contact or volatile compounds). For tolerance to growth in saline medium, all bacteria growth at 10 %NaCl. The results indicated that only RizoNFB have the ability for siderophore production and phosphate solubilization. In indole-3-acetic acid production,Cryrizos1 showed production of 19.3 µg mL^{-1}. In ACC deaminase activity, RizoNFBand Cryrizos1revealed their ability to produce 1409.088 and 669.9 nmol α-ketobutiric$mg^{-1}h^{-1}$, respectively. In determination of amplification of nifH and acdS genes, one strain RizoNFB amplify to 800 bp for acdS gene. The *in vitro* assay with seedlings of*A. thaliana*, we evaluated growth parameters (primary root length, lateral root, fresh leaf weight and fresh root weight). In parameters of diffusibles in distance inoculation method were higher than uninoculated controlLBrizos3 and RizoNFb. In diffusibles in contact, RizoNFB was higher than uninoculated control in all parameters. In volatile compounds, was higher than uninoculated control,LBecto3 and Cryrizos1. Thisresults indicate that depending on inoculation method under salinity stress *in vitro*, the rhizobacteria mechanism are activated.

Study of the role of the proteins Avin34710 and Avin34720 in the metabolism of polyhydroxybutyrate (PHB) in the bacterium *Azotobacter vinelandii*

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Polyhydroxybutyrate (PHB) is a natural polyester synthesized by various bacteria, including *A. vinelandii*, as a reserve of carbon and energy. Bacteria accumulate PHB when there is abundance of the carbon source, and degrade it (mobilize it) when it is exhausted. This mobilization of PHB is carried out by intracellular PHB depolymerase enzymes (iPHB depolymerases), which are attached to the PHB granules, as well as the enzymes involved in its synthesis. Other PHB granule associated proteins called Phasins constitute the major protein component of the granules, although they have no catalytic activity in PHB metabolism.

In the genome of *A. vinelandii* we identified seven genes that could encode PHB depolymerases. Among them, the product of gene *Avin34710*, presents a 33% identity with well characterized PHB depolymerases.

Our analysis of the proteins associated to the PHB granules of *A. vinelandii* by mass spectrometry, revealed the presence of a new protein, which not found in any other bacterium and whose gene (*Avin34720*) is located next to the *Avin34710* PHB depolymerase gene. Because in some bacteria phasin proteins have been found to affect PHB depolymerase or PHB synthase enzymatic activities, we studied the role of the Avin34710 and Avin34720 proteins in the metabolism of PHB by generating single and double mutants inactivating these genes, and also by heterologous expression in *E. coli* for their biochemical characterization *in vitro*.

The Δ34710::Km^R mutant presented a whitish and opaque phenotype, compared to that of the wild-type strain, suggesting that the mutant could have a higher PHB content than the wild-type strain. The quantification showed that the mutation did not affect the growth and increased the specific production of PHB in *A. vinelandii* at 72 h of culture by 74%. We also characterized the enzymatic activity of these proteins and the molecular weight of the polymer they produce. The results suggest that Avin347109 is a PHB depolymerase that, together with the previously characterized enzyme Avin03910, participate in the degradation of PHB in this bacterium.

Single-step anaerobic downstream fluidized bed bioreactor for wastewater treatment

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Wastewater is a key indicator of anthropogenic impact on the environment. Different treatment strategies has been proposed to tackle this problem, prevailing the industrial wastewater treatment facility, composed by filters, flocculators, decanters/sedimentation tanks, and aerobic-anaerobic bioreactors where interlinked processes are carried out, consisting in ammonification, conversion of organic nitrogen compounds to ammonia; nitrification, reduction of organic nitrogen into oxidized inorganic nitrogen compounds such as nitrate $(NO_3)^-$; and denitrification, the last step in the nitrogen cycle where the nitrate is transformed through several intermediary products (NO^{*-}, NO, N_2O) unto N^2. Despite the existence of diverse types of bioreactors, the most used are multi-step sequenced reactors, which needs separated vessels to perform nitrification and denitrification. On the other hand, in single-step reactors both processes are carried out in the same vessel, each one in different depth levels according to the O2 bacterial requirements. Being thus a compact option in wastewater treatment with a comparable efficiency; nevertheless, its energetic cost is higher due their upstream nature. In this work, we propose a single-stage anaerobic downstream fluidized bed bioreactor, conducting a comparative study of its nitrogen remotion performance against similar types of reactors used in wastewater treatment.

Molecular analysis of the tyrosine phosphatase, BCAL2200 of *Burkholderia cenocepacia*

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Burkholderia cenocepacia, member of the *B. cepacia* complex (Bcc), is an opportunistic pathogen causing chronic lung infections in cystic fibrosis patients and other immunocompromised individuals. *B. cenocepacia* is distinguished by their extraordinary plasticity to adapt and persist in different ecological niches. Several virulence factors including, fimbriae, metalloproteases, phospholipases, and membrane polysaccharides, have been described in the Bcc. However, recent studies suggest that the establishment of a chronic infection by these bacteria is closely related with its capability to adapt and persist intracellularly. Tyrosine phosphorylation has emerged as an important posttranslational modification modulating the physiology and pathogenicity of Bcc bacteria. We have recently performed a genetic and biochemical analysisof the enzymes achieving tyrosine phosphorylation in *B. cenocepacia*. Interestingly, we found that the three *B. cenocepacia* LMW-PTP (low-molecular-weight tyrosine phosphatases) BCAM0208, BceD, and BCAL2200are not functionally homologous enzymes. While, BceD and BCAM0208 contribute to biofilm formation;BCAL2200 is the only LMW-PTP required to support the growth of *B. cenocepacia* under nutritional limiting conditions. Furthermore, we showed that, unlike to BceD andBCAM208, BCAL2200 was unable to dephosphorylate the tyrosine kinase, BceF.In order to understand the molecular basis dictating the specific role of the LMW-PTP encoded by *B. cenocepacia*, here we achieved a protein sequence analysis of LMW-PTP from several organisms. First, we found that LMW-PTP can be separated into two different groups,(*i*) the "*classical"* phosphatases, including BceD and BCAM208, commonly encoded within a cluster related to capsular polysaccharides biosynthesis, and (*ii*) the "*atypical"* phosphatases, including BCAL2200,encoded next to the *isc* operon, and with higher similarity to eukaryotic LMW-PTP. Through multiple sequence alignments we were able to identify eightamino acids common to the group of a typical phosphatases which are not conserved in the classical LMW-PTP. Conserved amino acids were mapped into a structural model of BCAL2200. To investigate the relevance of these conserved amino acids a set of point mutations were performed in *BCAL2200* by PCR site-directed mutagenesis. Plasmids encoding the different BCAL2200 point mutants were incorporated into *B. cenocepacia ΔBCAL2200*and we are currently evaluating their ability to complement the growth in minimal media. Also, phosphatase activity of eachBCAL2200 point mutant will be confirmed by *in vitro* activity assays. We consider that the results obtained by this work will contribute to understand the mechanisms of protein substrate recognition by the LMW-PTP.

Transcriptional responses to riboflavin biosynthesis and uptake in the riboflavin opportunistic bacteria *Vibrio cholerae*.

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Riboflavin (vitamin B2) is an ancient redox cofactor essential for metabolism in all organisms. Riboflavin-derived molecules, mainly flavin mononucleotide and flavin adenine dinucleotide, facilitate electrons transfer reactions required in a myriad of core biological processes such as oxidative phosphorylation, carbohydrates metabolism and oxidative stress response. Bacteria may obtain riboflavin by endogenous biosynthesis through the riboflavin biosynthetic pathway (RBP) comprised by the RibBADHE proteins. In addition, bacteria may also use riboflavin uptake systems. Although either riboflavin biosynthesis or transport are sufficient to sustain life in a variety of bacterial species, both functions coexist in many others. The role of riboflavin uptake in such riboflavin prototrophic species has not been studied. The diarrheagenic pathogen *Vibrio cholerae*, causing the cholera pandemic, has a full RBP plus a riboflavin importer of the RibN family. We performed atranscriptomic study of the response to exogenous riboflavin in *V. cholerae*by RNA-Seq on which constitutes the first determination of the riboflavin regulon in any bacteria. In addition, this work also compared the transcriptomic responses of a strain lacking endogenous biosynthesis (Δ*ribD*) and a strain impaired in riboflavin uptake (Δ*ribN*). Transcriptomics results were validated by real time PCR analysis. Most of the genes identified are affected by the lack of either function. Notwithstanding, our analysis pinpointed sets of genes whose expression is specifically affected by riboflavin biosynthesis or uptake. Notably, many of the genes responding to riboflavin availability were previously reported to be regulated by iron, suggesting that riboflavin and iron regulons highly overlay in this species. Overall, our results suggest that although biosynthesized and internalized riboflavin may have a core of common uses, specific functions for each ability exist. This is in contrast with the extended hypothesis that riboflavin importers simply substitute for the RBP in riboflavin opportunistic species when the vitamin becomes environmentally available.

Mutation in a diguanylate cyclase of PGPR *Azospirillum brasilense* Sp245 causes an alteration in biofilm formation

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Azospirillum brasilense is a PGPR capable of increase growth and yield of crops. To enable a beneficial bacteria-plant association, the bacterium forms biofilms, communities of aggregated bacteria embedded in an extracellular polymeric matrix containing eDNA, proteins, exopolysaccharides and water. We study diguanylates cyclase (DGC), enzymes synthesizing the 3',5'-cyclic diguanosine monophosphate (di-GMPc), involved in motility, biofilm formation and dispersion. By bioinformatics, we found a gene that codes for a DGC (DGC-C) in *A. brasilense* Sp245. To evaluate its function we performed a mutation *cdgC::*Sm^R. This mutant showed an alteration in biofilm formation, as well as a thick pellicle formation. To confirm *cdgC* activity we carried out a genetic complementation of this gene using pJB3Tc20 vector. Biofilm formation assays were performed using crystal violet staining. To visualize biofilm formation, we tagged all strains with the enhanced green fluorescent protein. Additionally we employed calcofluor, a fluorochrome that binds to exopolysaccharides. Biofilms were visualized employing a Nikon CLSM. Under biofilm formation conditions,this mutant did not form a thick and irregular pellicle in comparison with wild type strain. This pellicle was composed mostly by cyst-like structures. Crystal violet assays showed that mutation in cd*gC* decreased biofilm formation compared with wild type strain and staining with calcofluor confirmed this data. This result was accord with literature, because mutations in DGCs lead to a decrease in di-GMPc intracellular level and a poor biofilm formation. We suggest that with insertion of streptomycin cassette a polar mutation was generated. It would be suitable to analyze gene downstream of *cdgC*::Sm^R, which could be responsible of this phenotype.

Resonant acoustic mixing technology for the production of recombinant proteins in *Escherichia coli*

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Shaken flasks are widely applied in bioprocesses due to their low cost and ease operation. Resonant Acoustic Mixing (RAM) enables noncontact mixing by the application of low frequency acoustic energy, and is proposed as an alternative to solve oxygen limitations in shake flasks orbital mixing (OM). Recently, we demonstrated that RAM mixing and aeration mechanisms differ with those from OM and empirical correlations were validated (from experimental data) for the volumetric mass transfer coefficient (K_La) as a function of shaking frequency and superficial area/filling volume ratio. In the present study, we use a recombinant *E. coli* BL21(DE3) producing phospholipase A2 as a model protein, in order to investigate how recombinant protein productivity can be affected if high RAM agitation are used and comparing those results with those from OM. Cultures were conducted at different agitation rates in conventional shake flasks using either OM at 200 rpm and 350 rpm, and three different agitation rates in RAM (7.5 x g, 12.5 x g and 20 x g). The resonant acoustic mixer works on the use of low frequency, and a high intensity acoustic field to induce oscillation at resonance. On the other hand, a traditional orbital shaker was operated with a shaking diameter of 25 mm. Growth of *E. coli*was followed by optical density (OD). Organic acids were quantified by HPLC. Glucose was measured by a Biochemistry Analyzer YSI2900. Dissolved Oxygen Tension (DOT) and pH were follow online and offline, respectively. We observed an improved oxygen transfer and higher biomass production under RAM agitation than OM at the same initial K_La (200 rpm vs 7.5 g). Increasing RAM at 12.5 g, up to five times the maximum biomass concentration was reached, as also an improve of the specific growth rate, glucose consumption, oxygen transfer rate and acetate production was observed. However, the increase up to the maximum RAM agitation level (20 g), diminished the specific growth rate around 20%, explained by the formation of vesicles and deformation of *E. coli* membranes (observed by transmission electron microscopy). No significant changes were observed in the specific production of the recombinant protein (by SDSPAGE). However, an increase in five times in biomass production reflects the same overall production of the heterologous protein.

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Characterization of cyanobacteria from microbialites along a depth gradient in the Alchichica crater lake.

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Microbialites are microbial induced organo-sedimentary structures formed either by mineral accretion, precipitation or binding. Previous works have shown that cyanobacterial photosynthesis is an important factor for mineral precipitation and microbialite formation. Since photosynthesis elevates local pH by bicarbonate assimilation, favoring thus carbonate precipitation. It's presumed that cyanobacterial external polymeric substances (EPS) act as a template for mineral nucleation. Microbialite biotechnological applications have been also suggested. Nonetheless, microbialite formation it's not fully understood. Hence, to fully comprehend its complex formation and function, it is crucial to describe the cyanobacterial component.

We described and characterized the cyanobacterial diversity in spongy microbialites from lake Alchichica (Puebla, Mexico), along a depth gradient in the water column (z=3 m, 10 m, 20 m, and 30 m) during the two hydrodinamic periods, (mixing and stratification) using culture methods and non-culture methods. For non-culture methods, dried microbialites samples were examined by optical microscopy, and the cyanobacterial taxa were then characterized by standard morphological identification. While for molecular analysis, microbialites were analyzed through 16S rRNA gene amplification with specific primers for cyanobacteria (27F and 23S). Sequencing analysis confirmed the previous characterizations by microscopy identification. For culture methods, fresh microbialites fragments were inoculated in solid medium and isolated during 6 months. We obtained 10 different cyanobacterial axenic cultures which are being characterized by microscopy with morphological identification and through 16S rRNA gene sequencing. Interestingly, we found a diverse cyanobacterial component adapted to different light wavelengths along the vertical gradient such as *Chroococcopsis*, *Acaryochloris* and *Leptolyngbya* (among others), each cyanobacteria plays a key role in primary production and carbonate precipitation.

A metagenomic approach to survey nitrogen transformations in a shallow aquifer within an agricultural zone.

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Nitrogen is an important element for life, as it is a basic component of biomolecules such as nucleic acids and proteins. However, it is often a limiting factor for agricultural production. To overcome this situation, N-rich fertilizers and wastewater irrigation(which contain organic N-forms and N-NH_4^+ overloads)are used to increase N-inputs. These constant nitrogen overload are nitrified by the local soil microbiota and result in nitrate leachates reaching the aquifer. The Mezquital Valley is a perfect place to study the groundwater nitrogen transformations due to the constant N overloads by both, wastewater irrigation and fertilizers.

Previous 16S rRNA gene surveys suggest that anaerobic ammonium oxidizing bacteria (or anammox bacteria) are a key component of groundwater microbiota, and remove these N overcharges in the aquifer. The Brocadiales is a specific taxon that comprises 5 genera capable of carrying out anammox. This pathway was firstly discovered in reactors treating wastewater, and then, the process was confirmed in the deep ocean where it removes 30-50% of the fixed nitrogen. Thus far, anammox bacteria have been also detected in fertilized rice paddy soils, and more recently, in groundwater, where the information is still scarce.

In this work, we studied the bacterial diversity and specially the anammox bacteria in a shallow aquifer mainly formed by wastewater irrigation practices. Metagenomic DNA was extracted from waste and groundwater samples taken in different monitoring points. 16S rRNA analysis were done to survey the bacterial component. The potential anammox bacteria were screened using *hzo* gene encoding the hydrazine oxidoreductase involved in the anammox process. *hzo* gene surveys were carried out by cloning and sequencing. Our results suggests that anammox bacteria correspond to *Brocadia* spp. and *Scalindua* spp, and play an important role in removing N overloads in the aquifer.

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Comparative genomic analysis of pathogenic *Staphylococcus epidermidis* isolated from nosocomial infections.

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Pathogenic *Staphylococcus epidermidis* colonizes medical devices such as catheters, and it can reach the human bloodstream causing severe bacteremia and potential mortality. This is especially true in perinatal hospitals were children are very prone to contact with *S. epidermidis*. Therefore, to tailor adequate treatments, it is important to characterize *S. epidermidis* strains populating clinical settings. Here, we characterize several isolates of*S. epidermidis*by next generation sequencing (NGS) from catheters and blood from patients at the Instituto Nacional de Perinatología (México City).

We obtained whole-genome sequences by NGS using Illumina´s platform, NextSeq in 2 x 150bp paired-end reads with a depth coverage (50x – 100x)in18 strains. Genome assemblies were performed using Spades generating about 94 to 1,727 contigs with N50 around 36,000bp. Gene predictions were performed with Glimmer and annotations were performed utilizing the Patric server. The average number of predicted genes was 2292, which agreed with the overall gene content determined for other *S. epidermidis* strains.

Whole genome alignmentsshowed that all *S. epidermidis* genomes analyzed reached high levels of similarity (Average Nucleotide Identity, ANI = 99%), except for strain S10 which presented an ANI ~97%. In addition, 71common genes related to virulence were identified and only a few were strain-specific (19). For example, we observed, biofilm forming genes, *icaA, icaB, icaC*, in several strains. Genome analysis confirmed the presence of specificresistancegenes for several antibiotics including, penicillin, methicillin, oxacillin, quinolones, gentamicin, erythromycin, and clindamycin, distributed in different strains. New Multilocus Sequence Types (MLST) were identified among the *S. epidermidis* isolates, together with MLSTs of global distribution. Phylogenetic trees of *S. epidermidis* isolates were constructed using genomic information from several housekeeping genes, but were not conclusive. Inconsistencescould be due to the high rates of recombination measuredfor*S. epidermidis*and compared to *S. aureus*. In support, identification of prophages and CRISP-Cas elements in the *S. epidermidis* genomes suggest that the analyzedpopulation evolvesby recombination and gene exchange.

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Is it just a question of time? *Acinetobacter haemolyticus*, an emerging pathogen

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Introduction: *Acinetobacter haemolyticus* is an emerging opportunistic bacterial pathogen that generally affects immunosuppressed patients and can cause pneumonia, urinary tract infections, bacteremia, etc. Currently, most isolates are susceptible to antimicrobial agents routinely used, in contrast to the *Acinetobacter calcoaceticus-baummannii complex*, a group of species that are either multi-drug resistant or pan-drug resistant. However, there are some *A. haemolyticus* strains which harbor antibiotic resistance genes of great concern for public health, such as NDM-1 metallo-beta-lactamases that are able to inactivate most betalactams, including carbapenems –one of the last resources for antimicrobial therapy-.

Objective: We want to determine if *A. haemolyticus* may become soon a public health threat such as the *A. calcoaceticus-baummannii complex.*

Materials and methods: We sequenced 31 complete genomes of various Mexican isolates from hospitals in Oaxaca, Puebla and Mexico City. Those isolates were obtained by the hospital staff in 1998 and from 2008 to 2016. We included the 8 available genomes at the Genbank NCBI; those strains are from approximately 1960-1970 and most don't have clinical data. All of the Mexican strains have different plasmid profiles and clinical data. We analyzed the 39 genomes with various comparative genomics tools and performed an exploratory population genomics analysis.

Results: Most of the strains have few inactivating antibiotic resistance genes (aminoglycoside acetil-transferases and betalactamases) but one Mexican isolate has a NDM-1 betalactamase. The exploratory population analysis, at the *core* genome level, suggests that there is no clear distinction between isolates neither by geographic location nor by isolation year. Also, the observed groups are heterogeneous in their gene-content. This may be because *A. haemolyticus* has an open pan genome with around 8000 genes, and the *core* genome has about half of the genes found in each strain (~2000/~4000), but the chromosomes are not syntenic; they have lots of rearrangements. On the other hand, two plasmids are conserved between various strains even though some of the isolates are very old (1960-1970 and 1998).

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Functional annotation of the genome of virulent Avibacterium paragallinarum serotype A Strain AVPG5

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Avibacterium paragallinarum (AVPG) is the causative agent of infectious coryza (IC), an acute respiratory disease of poultry. This disease occurs worldwide and produces significant economic losses to the poultry industry due to a highnumber of sacrifices, poor growth, drop in egg production and mortality of infected birds. This bacterium expresses several virulence factors that must be considered to develop effective vaccines to control prevalent strains. Here, we present a comparative genomic study of AVPG strain 5 focused on the search for virulence factors.

The genomic DNA from Avpg5 was sequenced by 454-technologyand assembled with Mira4 software program. The genome's leng this approximately 2.4 Mband was compared with other five AVPG genome sequences (JF4211, 72, 221, CL and AVPG 2015) retrieved from the GenBank database,in order to know differences in virulence factors among AVPG strains. A comparative analysis among genomic sequence of AVPG5 andgenomes of different animal pathogenic bacteria from the Pasteurellaceaefamily, including *Gallibacterium, Pasteurella, Actinobacillus, Haemophilus, Histophilus and Mannheimia*, was made using RAST software program, to identify additional DNA sequences encoding putative virulence factors in AVPG5. Genetic sequences encoding putative hemagglutinins, Avx and CDT toxins, colonizing factors and other metabolic genes related to capsule biosynthesis were identified and are in process of assay to corroborate their function and participation in IC.

Bacterial communities associated to insect intestine.

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Intestinal bacteria plays very important roles within hosts: Food digestion, secondary metabolite production, xenobiotic degradation, among others. Insects could contain a diverse microbial communities inside intestines, depending on morphological stage or food habits. Beneficial insects like praying mantis or green lacewing are used as effective pest control, both use a very different predatory strategy and consequently, uses different digestive method.

We have been using metagenomics and enzyme activity detection as complementary approach to describe functional role of bacterial communities in praying mantis and *Chrysoperla carnea*, in order to characterize and identify interesting bacteria activities to enhance biotechnological processes (food science, integrated pest management, etc).

Through shotgun metagenomics of *Stagmomantis limbata,* we described bacterial community composition and enzymatic capabilities. This insect contains a minimalistic bacterial community dedicated to degrade insect prey tissues, secondary metabolite production (vitamin and bacteriocin), antibiotic and insecticide resistance. Most abundant bacteria belongs to *Lactobacillus* genus, a common insect symbiont.

Chrysoperla carnea produces an enzymatic cocktail to liquefy inner tissues of their preys. We have detected several proteases, lipases and nucleases responsible for tissue digestion. Microbiological approach demonstrated that intestinal associated bacteria contains lipase and cation-chelating activities. A metagenomics study are underway to describe bacterial community.

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The mutation in diguanylate cyclase D from *Azospirillum brasilense* Sp 245 decreased biofilm formation.

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*Azospirillum brasilense*is a plant growth promoting rhizobacteria capable of increase growth and yield of crops through several mechanisms. To enable a beneficial bacteria-plant association the bacterium forms biofilms. The product of diguanylates (DGCs), c-di-GMP, is a ubiquitous second messenger involved in regulating various aspects of bacterial physiology and behavior, from motility and biofilms, to cell cycle, differentiation. The cellular level of c-di-GMP is decreased by the enzymes phosphodiesterase (PDEs). In most bacteria where c-di-GMP-dependent signaling pathways are present, they are involved in regulating bacterial transitions from the planktonic to the sessile lifestyle via inhibition of motility and formation of adhesive surface proteins or appendages [1].It was described hybrid proteins that have both activities, these activities are regulated by sensory domains occurring in DGAC-PDEs proteins [1].By bioinformatics analysis, we found a gene which translate product of 946 amino acid residues, shows the CHASE-PAS and DGC-PDEdomains (named diguanylate cyclase D) in *A. brasilense*Sp245. To evaluate the hybrid putative protein function, we performed a mutation *cdgD::*Km^R. Crystal violet assays showed that mutation *cdgD::*Km^R decreased biofilm formation as compared with wild type strain in a minimal medium supplemented with NO_3K as nitrogen source, as well as enhanced motility; however when minimal medium was supplemented with NH4Cl, both strains produced similar biofilm formation. The *cdgD::*Km^R mutant was complemented with the plasmid pVKdgcDand biofilm formation restored.

Conclusions

The preliminary results obtained herein,indicate thatgene *dgcD* has DGCD activity, when minimal medium was supplemented with NO_3K. We previously described that nitric oxide (NO) is synthetize by the enzymes periplasmic nitrate reductase and nitrite reductase. NO is a signal for biofilm formation in *A. brasilense* Sp245 [2]. We suggested that CHASE domaincould sense NO, then as sensory activity of CHASE domain was disrupted, the DGC activity was interrupted.

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Assembly of the genome and functional analysis of the annotation of Klebsiella sp., to identify genes that confer anticoccidialactivity

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Introduction

Coccidiosis is a disease induced by intracellular protozoa of the genus Eimeria, this organism infects the chicken by the intestinal epithelial tissues. The typical methods used for anticoccidial control are drugs, vaccines and ionophores; however, these methodologies generate disadvantages such as resistant strains, high costs and bioaccumulation respectively.

Methods

The SPAdes 3.8.1 assembler and the RAST platform (http://rast.nmpdr.org/) were used to obtain assembly and annotation of the genome of strain *Klebsiella* sp., we used the concatenated sequences of the genes gyrA, Mdh, parC, rpoB and 16s to molecularly identify *Klebsiella* sp.A comparison was made using two additional genomes of the KbMx and KvMx strains belonging to the genus *Klebsiella*, different growth times were selected in the strains, and bioassays were carried out in each of them to determine in which state of development they present the highest activity. We compared the sequences shared between the three strains to perform the search of the genes involved in the activity based on the function given by the annotation.

Results

Klebsiella sp. genome assembly with a 5.8 kb length was obtained in 34 contigs, molecularly identified as Klebsiella oxytoca, and named Kox Ac.In the bioassays performed with cell and supernatant at 4, 6 and 9 hours of growth, greater activity was obtained at 6 hours of growth, in the 48 hours of the bioassays performed with cell, and greater activity was observed in the bioassays performed with the intracellular extract the 48 hours of the bioassay. From the genomic comparison, we obtained the group of sequences shared between the three strains, where the search for the possible molecules involved in the activity was carried out, resulting in 5 sequences with possible lipase activity and 15 with possible protease activity.

Conclusions

- Themolecule (s) involved in the anticoccidialactivity of strains of thegenus *Klebsiella* sp. are secreted outside
- The genes encoding the molecule (s) involved in this activity are not constitutive, in addition to being present in three different species of the genus *Klebsiella*

'Candidatus Mycoplasma haemobos' strain INIFAP01, the first hemotrophic Mycoplasma identified in Mexico: Genome assembly and comparative genomics analysis.

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Hemotrophic Mycoplasmas (Hemoplasmas) are a group of wall-less bacteria and animal pathogens of the Mollicutes class. Currently, ten genomes of Hemoplasmas have been sequenced and deposited in GenBank database. These organisms were subjected to a genome reduction which provided the ability to surviving often evading the immune responses and establishing chronic infection. Recently, CeNID-PaVet, INIFAP reported a bacterial strain of the genus *Mycoplasma*, an uncultivated Hemoplasma isolated from blood of sick cattle. In this work, we compare genomic features of *Ca.* Mycoplasma sp. INIFAP01 and the relationship with other Hemoplasma species. Also, identification of genes involved in pathogenicity, infection and the probable synergistic mechanisms with other bacteria are of our interest.

In this study, we report the draft genome of *Ca.* Mycoplasma sp. INIFAP01 which consists of 935,638 bp total length. In total, 18 contigs were produced using *de novo* assembly with SPAdes program with N_{50} contig size of 256,799 bp, G+C content of 30.46% and ~48X coverage. Phylogenetic analyses based on 16S rRNA gene showed that '*Ca.* Mycoplasma haemobos' strain INIFAP01 is grouped with other *M. haemobos* species reported. Here, we propose a new classification of Hemoplasmas based on two main groups: group A, including *M. haemobos*, *M. haemocanis* and *M. haemofelis* species; and group B, including *M. suis*, *M. parvum*, *M. haemolamae*, *M. haemominutum*, *M. wenyonii* and *M. ovis* species. This result was confirmed by mapping of 16S, 23S and 5S rRNA genes; two heatmaps of ANIb identity and coverage alignment; and rooted parsimony pangenome tree for 10,534 protein sequences of 11 Hemoplasma genomes. All contigs of '*Ca.* Mycoplasma haemobos' strain INIFAP01 were analyzed with RAST web server, identifying 1,216 open reading frames (ORFs), 1,184 coding sequences (CDS) and 850 hypothetical proteins. The CDS were used to identify clusters of orthologous groups (COGs) resulting 277 CDS with predicted function. According to COGs classification, defense mechanisms have the largest number of CDS with 33 results.

Comparative study of human versus *Caenorhabditis elegans* microRNAome.

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MicroRNAs are single-stranded RNAs of 19-23 ntds that participate in diverse biological processes such as cellular proliferation, animal development, apoptosis and metabolism. They are considered as master regulators of genetic expression. Changes in their expression profiles represent landmarks of several diseases such as obesity, Diabetes mellitus type II, cancer, and neurodegenerative conditions. Different animal models are used as an approach to study the involvement of microRNAs in such multifactorial diseases. This is the case of the nematode Caenorhabditis elegans, whose genome is about 100 million base pairs long, consisting of six chromosomes. Introns make up 26% and intergenic regions 47% of the genome. Many genes are arranged in clusters, resembling bacterial operons. There are 20,470 protein-coding genes, of which 35% have human homologs. It is estimated that C. elegans contain about 16,000 RNA genes, of which rougly 500 are microRNAs. The objective of this study is to identify the microRNAs of C. elegans that are conserved in humans. First, we identified all of the microRNAs of human and C. elegans in miRBase version 21. Then, we classified the microRNAs of C. elegans depending of the genomic context into which they are located (intergenic (70%), intronic (28%), or exonic (2%)). Next, we analyzed which microRNAs from C. elegans are conserved in other animals of the Phylum Nematoda. We also found that 50% of the mature microRNAs of C. elegans have human homologs. Taking into consideration the seed region of the microRNAs, nucleotides 2-7, we were able to cluster 1932 human microRNAs (of a total of 2813) into 624 families, while 161 C. elegans microRNAs (from a total of 447) were clustered into 55 families. Interestingly, 160 seed regions are present in C. elegans and human microRNAs. Finally, microRNA families let-7, lin-4/miR-125, miR-1, miR-9/miR-75, miR-34, miR-100/miR-51, and miR-124, were found to be conserved in all of bilateria animals. It is noteworthy that many of them are involved in different multifactorial human diseases.

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Analysis of multi-resistance to antibiotics of bacterium *Escherichia coli* BOq isolated from poultry farms

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INTRODUCTION: Antimicrobial resistance has been observed since the first antibiotics were discovered, however, their indiscriminate use has led to increasing levels of resistance not only in clinical field but also in agriculture and livestock.
This problem has triggered a global health crisis. It is estimated that by 2050, without appropriate actions to solve this issue, infections caused by resistant bacterias could generate up to 10 million deaths each year. This due it is known that pathogenic bacteria can acquire resistance to these compounds by mobile genetic elements, which can be acquired by horizontal transfer processes. To contain this crisis, is necessary a depth analysis and monitoring of resistant strains by traditional methods such as antibiograms and the genomic studios with bioinformatic tools, which allow suggest possible mechanisms resistance. BACKGROUND: A bacterium identified as Escherichia coli BOq, which has anticoccidial activity, was isolated in the CeIB/UAEM Laboratory of Ecogenomic Studies. As part of the characterization of this bacterium was also to determined multi-resistant antibiotic strain, as it presented resistance to 10 antibiotics. METHODS: The strain was monitored with conventional methods as qualitative tests to propose a possible resistance profile. Identification of possible genes related to resistance to antibiotics was carried out using the following servers: Plasmid finder, CARD, RGI. RESULTS: The analysis of the genome with the Plasmid finder server showed the presence of two plasmids, in contings 18 and 46, which could be involved in the resistance to different antibiotics. The presence of these plasmids was confirmed with extraction for plasmids. The CARD server resulted in the presence of 95 genes that could confer resistance, which are found in 25 contigs. The RGI server showed the possible mechanisms that could be involved in resistance, including flow pumps, modulation at the exit of different antibiotics, enzymes that inactivate these drugs and proteins that modulate the permeability of these compounds. CONCLUSION: This strain presents resistance to 10 antibiotics by means of qualitative tests, besides by means of different servers it was observed that it could have different mechanisms of resistance to a great variety of these compounds. Therefore, these possible mechanisms should be investigated with experimental tests.

Antibiotic Susceptibility Pattern of Stenotrophomonas Species Isolated from Soil and Sewage in Mexico

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Stenotrophomonas are known to be intrinsically resistant to many classes of antibiotics. This property makes them bacteria of special interest, causing opportunistic infections in sick patient. *Stenotrophomonas* can also acquire resistance from other bacteria in their environment. Many strains of *Stenotrophomonas maltophilia* have been isolated with acquired resistance behavior especially from clinical infections. Acquired resistance in *Stenotrophomonas maltophilia*in clinical settings has been widely discussed in literature with limited attention to strains from the environment. We isolated 43 strains of Three *Stenotrophomonas* species from soil and sewage and investigated their pattern of susceptibility to commonly used antibiotics (Tetracycline, Augmentin, Amoxycillin, Ceftriaxone, Nitrofurantoin, Gentamicin, Imipenem, Ofloxacin, Sulfamethoxazole-Trimethoprim (SXT), Ampicillin, Ceftazidime, Polymyxin, Doxycycline and Levofloxacin) using both disc diffusion and broth dilution methods. We observed high rate of resistance in the isolates to most of the antibiotics tested. We noted high rate of resistance to Sulfamethoxazole-Trimethoprim (SXT) (81.4%) which is the recommended drug for the treatment of *Stenotrophomonas* infections. We also observed that *Stenotrophomonas* susceptibility seems to follow a chronological pattern being resistant to all the older antibiotics and showing more susceptibility to the new generation antibiotics. This observation could suggest that resistance might have been developed because of the selective pressure from their environment.

Spa-typing of *Staphylococcus aureus* isolated from fresh cheeses

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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 tocarry out a molecular characterization of *S. aureus* strains isolated from cheese samples. Two ATTCC control strains (27543and 43300*mecA*+) and a total of 22 *S. aureus* isolates from fresh cheeses donated by the Laboratorio Estatal de Salud Pública de Michoacán, Mexico, 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* genewas 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) and the less predominant *spa*-types were t008, t349, t5695 and t701 each with 4.3% (n=1). 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*, *spa*-typing, cheese, *mecA*.

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Generation of the mutant of the *fur*$_{1398}$ gene from *Gluconacetobacter diazotrophicus PAL5*

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Introduction:The iron is a versatile biocatalyzator, because of is extremely width redox potential; as cofactor of several enzymes and regulatory proteins. The bacteria have developed several strategies for the iron uptake. The ferric uptake regulator (Fur) is a global transcriptional regulator, which regulate the expression of the acquisition and storage systems in response to the intracellular iron concentration. *Gluconacetobacter diazotrophicus* (GDI) Pal5 is a plant growth-promoting rhizobacteria (PGPR) and a promising alternative to the massive use of the industrial fertilizers in the agriculture. Bertalan and coworkers in 2009 sequenced the genome of *G. diazotrophicus*, revelated two *fur*-like genes, GDI_1248 and GDI_1398. Pérez-Rodríguez in 2015 performed pEXP5CT*fur* 1398 overexpressed in *E. coli* BL21(pLys), she shows that, when cells are challenged with H_2O_2, overexpression pEXP5CT*fur*$_{1398}$increase significantly the cell survival. This study will be to generate a *fur*1398 mutant in *G. diazotrophicus Pal5*, for deletion of the gene *fur*$_{1398}$ and insertion of a cassette for kanamycin resistance.

Materials and methods: Design of specific primers of the *fur*$_{1398}$ gene, with 1300 pb upstream and 1500 pb downstream with the restriction sites *MluI* and *NheI* for the insertion of the Km cassette and the sites *HindIII* and *BamHI*for the subcloning in the suicide vector pSUP202. The amplicon of 2.4 kb was cloned into the TOPO 2.1 vector, and transformed in *E. coli* TOP10, these clone candidate pTOP*fur*$_{1398}$were analyzed by PCR and restriction with *EcoRI*; the plasmid pTOP*fur*$_{1398}$ was sent to the IBT-UNAM for the sequencing. After performing inverse PCRof pTOP*fur*$_{1398}$, with a length of 6.3 kb, it was realized double digestions with *MluI* and *NheI*, for the site-directed ligation and the insertion of the cassette for kanamycin resistance. The pTOPGDIΔ*fur*$_{1398}$::Km was transformed into *E. coli*TOP10 chemically competent;once obtained, the transformants were analysed by PCR and restriction with endocnucleases; the plasmid extraction by miniprep was realized to this clones, and the pTOPGDIΔ*fur*$_{1398}$::Km was digested along with the pSUP202 vector by double digestion, with *BamHI* and *HindIII,* proceeding with the subclonation in this vector. The construction pSUP:GDIΔ*fur*$_{1398}$:Km, will be electroporated in *G. diazotrophicus* electrocompetent cells, and it will be realize PCR and the mutants will be evaluated by tetracycline sensibility.

Conclusions: The generation of the GDIΔ*fur*$_{1398}$::Km mutant of *G. diazotrophicus* Pal5 will allow us to continue studies of phenotypic characterization in the presence and absence of iron.

Comparative analysis nitrogen fixing bacteria associated with *Acacia sp* from the mining tailings of Huautla, Morelos

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Introduction. The biological fixation of N_2 is a process that is carried out by diazotrophic bacteria in symbiotic relation with legumes. This relationship may be affected by anthropogenic activities. An example of this is heavy metal contamination, which is the result of mining-metallurgical activity. This has caused the accumulation of residues of fine particles rich in heavy metals called mine tailings; that when are exposed to environmental conditions have induced negative consequences on the different structures, biogeochemical cycles and biological systems of the ecosystem. A number of studies have been carried out in two mine tailings in the region of Huautla, Morelos: physicochemical analyzes, accumulation of metals in organisms, ecotoxicological effects until theto the impact of these metals on the different trophic levels. However, there are no reports describing the effects of these metals on the relationships established by microorganisms with the plants that colonize these ecological niches.Therefore, carrying out the isolation and characterization of *Acacia sp* endosymbiotic bacteria present in the mining tailings can determine the diversity and strategies to colonize environments contaminated with heavy metals. **Methodology and Results.**They were isolated in selective medium PY a total of 111 bacterial strains associated with Acacia cochliacantha and A. farnesiana. It has been made southern blot hybridization of the nifH gene and plasmid visualization by means of the eckardt technique to select isolates. Three isolates designated as: AC3, AC17 and AF25 were selected because they presented a plasmid profile and the *nifH* gene. These isolates were identified by means of the 16S RNA molecular marker. Isolates AC3 and AC17 were identified as *Sinorhizobium sp*and the AF16 isolate as *Microvirga sp*. Subsequently, the minimum inhibitory concentration (MICs) to heavy metals (Zn, Cd, Mn and Pb) in the three isolates was determined. AC3 and AC17 showed a high level of resistance to Mn (at a concentration of 207 mM) and a bacteriostatic effect was observed in AC3, AC 17 and AF25 at a concentration of 180 mM Pb. Finally, the symbiotic effectiveness of the isolates with the two species of acacias was evaluated, demonstrating a positive and effective nodulation. **Conclusion.** The present study prove that the diversity of bacteria that establish symbiotic interactions with *A. cochliacantha* and *A. farnesiana* is related to exposure to heavy metals. This is the first study of isolations and characterization of nitrogen fixing bacteria in the mining tailings of Huautla, Morelos. This opens the way to know the interactions plant-bacteria and the effects that metals exert on it.

GENOMIC CHARACTERIZATION OF THE BACTERIAL STRAIN *Escherichia coli* BOq WITH ANTICOCCIDIAL ACTIVITY

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Introduction. Avian coccidiosis is responsible for huge economic losses in the poultry industry worldwide. This is defined as a parasitic disease that is caused by protozoa belonging of the genus *Eimeria*. Agents used for prevention and control of coccidiosis infections are called anticoccidial drugs. Some are synthetic drugs, which are mainly used in the poultry industry for disease control. However, most of these products have limitations that can be counterproductive. Thus, natural alternatives have been sought among which are the use of microorganisms that have anticoccidial activity. To obtain this kind of alternative, disciplines such as genetic engineering, comparative genomics and structural techniques have been used, which allow us to propose means of communication to the conventional ones to infer and create new candidates that may be the answer to the problem in question.**Methods**. The*E. coli* BOq genomewas sequenced using a Paried-End run (double or paired readings) with the Illumina Genome Analyzer IIx (GAIIx) sequencer from a 5 µg genomic DNA sample.Data obtained from genome sequencing were evaluated to determine the quality of the sequences using the FastQC program.Later they were edited using the program dynamictrim. The genome assembly was performed using the SPADes program (3.1.1) and was annotated using the RAST 2.0 server database (http://rast.nmpdr.org/). **Results**. Sequencing, assembling and annotation of the *E. coli* BOq genome showed acceptable values to perform this work. A candidate sequence list was obtained, resulting from the genomic comparative between the *E. coli* genomes BOq and DH5a, to be identified as responsible for the anticoccidial activity, this because the DH5a strain has not anticoccidial activity. **Conclusions**. The evidence that we obtained in the experiments indicate that the anticoccidial activity is not only given by a single molecule, but is probably result of an interaction of several molecules and can even intervene a complex or a system. We identified the sequence of two plasmids present in the BOq strain and they have some sequences that indicate be responsibles for the anticoccidial activity.

Comparative Genomics and Biochemical-Molecular Characterization of a New *Pseudomonas alcaligenes* from Gulf of Mexico Involved in the Hydrocarbons Degradation

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The characterization of new bio-tools for biotechnology applications has been increased in the last years, the principal activity for this is the seeking of novel microorganisms, these discoveries are couple with the description of new ecosystems. The strategies used represent a new interdisciplinary approach that improves the way to obtain a specific tool for a specific application. Our aim is discovery microorganisms that have a natural evolve in the degradation of hydrocarbons. The natural hydrocarbon emanation in the Gulf of Mexico and the oil industry activity produce a potential ecosystem for the development of microorganisms with skills to consume oil. A microbiology approach let us isolated a new *Pseudomonas alcaligenes* from water of 1000 meters of depth, able to growth in a mineral media with hydrocarbon as a unique source of carbon. The complete sequencing of its genome let us to know that this organism is new specie with close relatives as *Pseudomonas alcaligenes* species, and in add a wide comparative bioinformatics analysis describes a conserved set of tools for alkane oxidation and also aromatics hydrocarbons. The genes of this cluster were previously described in other members of *Pseudomonas* exposing a level of adaptation to the presence of aliphatic hydrocarbons. Growth experiments with hydrocarbons as the only source of carbon, plus genomic data, suggest a taste for short chain alkanes. Further essays demonstrate the production of surfactants of the rhamnolipids type. The results obtained allow us to have a potential hydrocarbon degrading bacteria.

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Structural genomics and pathogenicity of isolated bacteria associated with the entomopathogenic nematode *H. indica* MOR03

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The entomopathogenic nematode *Heterorhbditis* are obligate parasites of insects that are associated with enterobacteria from the genus *Photorhabdus* in a mutualist symbiosis, which is lethal to the host insect and essential for the development and reproduction of the nematode. However, other bacteria have been isolated from different species of entomopathogenic nematodes as *Pseudomonas, Alcaligenes, Enterobacter* and *Ochrobactrum* and their relationship is not clear.Therefore, this is interesting to understand the interactions between entomopathogenic nematodes and no canonical bacteria.

In this study, we isolate an entomopathogenic nematode from samples of agricultural soil and was identified as *Heterorhabditis indica*using the regionD2D3 of 28s and ITS. Interestingly, two bacteriawere isolated and identified with concatenated sequences (16s, 23s, *dnaN, gyrA* and *gyrB*) and (16s, 23s and *gyrB)* as *Photorhadus luminescens* HIM3 and *Pseudomonas aeruginosa* NA04, respectively. Our objective is to analyze the structure of the genome of HIM3 and NA04 and to evaluate the pathogenicity of the nematode and the bacteria against insects, as well as determine its location and persistencein the nematode.

We analyzed the pathogenicity of *H. indica*MOR03 against insects and obtained DL50 of 9.4, 150, 172 and 182 JIs per larvae of *G. mellonella, T. mollitor, Heliothis* sp. and *Diatraea* sp., respectively at 48 hours. The virulence of the bacteria was also evaluated by the injection of different doses (10-5000 CFU) in the homolymph of the insect, demonstrating that both HIM3 and NA04 are highly virulent against larvae of *G. mellonella, T. mollitor* and only HIM3 generates high mortality (95-97.7%) in *Diatrae* sp. with doses of 3,000 and 10,000 CFU at 36 hours.

Additionally, the genomic DNA was sequenced using the Illumina HiSeq platform (2 X 300 bp paired-end) and assembled with SPAdes version 3.0. The draft genome of *P. luminescens* HIM3consists of 5.4 Mb. In total, 91 contigs were produced assembly and GC content of 42.6%. On the other hand, *P. aeruginosa* NA04consists of 6.3 Mb with a total 45 contigs and GC content of 66.5%.

Metagenomic analysis of astable microbial consortium capable to grow in a polyether polyurethane varnish reveals adaptive capabilities to metabolize xenobiotic compounds

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Microbial consortia are ubiquitous entities in nature since they are involved in processes of great importance for humans, from environmental bioremediation, to the maintenance of human health. Our understanding of microbial communities is limited by our capacity to discern which species coexist in the consortia and how each of them contributes to the phenotype at community level. Metagenomics allows discovering new genes and proteins or even complete genomes incomplex microbial communities. The main inconvenience, when analyzing complex samples, is the loss of information of the sequences belonging to one species. In this work, by using Hi-C-based contact probability map, a novel techniquethat allows to regenerate contiguity signals between sequences belonging to the same species-a process named deconvolution-,we were able to reconstructindividual genomes froma microbial consortia (BP8),capable to attack polyether-polyurethane (PE-PU) (Gaytán*et al*. this meeting). BP8 consortium was selected and cultivated for several generations in a minimal medium with a PE-PU varnish (PolyLack®) as the sole carbon source.The varnish, besides polyurethane, contains other solvents such as N-methylpyrrolidone, isopropanol, butoxyethanol and polyethylene glycols. The *de novo* metagenome assembly had 2,153 contigs with a total length of 16.23 Mb and a minimun N50 contig length of 3.3 kb. The binning analysis suggested the presence of 5 distinct clusters with genome completeness from 52.3to 97.6%that closelymatch with the species: *Agrococcus lahaulensis*, *Chryseobacterium antarcticum*, *Sorangium cellulosum*, *Paracoccus* sp. and *Ochrobactrum intermedium*. Gene annotationby KEGG, identified genes belonging to 11 xenobiotic degradation pathways.Thesefindings suggest that the consortium's population, besides to be able to attack PU, could play a significant role in the degradation of other environmental contaminants, such as the ones present in the varnish.The possible link between the genetic potential of the BP8 consortium and the biodegradation of thePE-PU varnishwill be discussed.

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Genomic diversity and local adaptation of bacteriophage communities associated with bean-nodulating bacteria.

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Bacteriophages are the most abundant and most diverse biological entities on earth;theyplay a key role in shaping bacterial communitiesbyadjusting the frequency of genotypes and promotinggene transfer among bacterial hosts. The diversity and ecology of bacteriophages associated with soil symbiotic bacteria, such as rhizobia, remains largely unexplored.

In this work, we are interested in know the genomic diversity and the host ranges of rhizobiophages associated with common bean-nodulating rhizobia. We isolated rhizobiophages from soil using a protocol previously established (3), but implemented in a high trought put format. The phages come from soil of three common bean (*Phaseolus vulgaris*) fields in Tepoztlán, Yautepec and Xoxocotla, Morelos, using as host 94 *Rhizobium* bacteria from different geographical zones and native *Rhizobium* strains from the same field.

We obtained a total of 196 rhizobiophages that were characterized by their host range and the estimation of their genome size by electrophoresis. Different phage communities were isolated from the three fields: in the Tepoztlán field, lytic phages with small genomes (~6 kb, Microviridae) or big genomes (~200 kb) were prevalent, whereas in Yautepecand Xoxocotla were the medium size genomes (~60 kb). From the Xoxocotla field, two classes of phages with distinct specificity were isolated, one infect *R. etli* and *phaseoli*, while other infect only*Sinorhizobium americanum*. The complete genomic sequence of about 18 phages indicate that the Microviridae group is exclusive for *Rhizobium* without any resemblance to other phages of the family deposited in databases.

Our results indicatethat local *Rhizobium* populations are more sensible to be infected by the local community of phages. Indeed, there is a clear distinction among the phage communities. Currently, we are testing hypothesis to understand the underlying evolutionary process on the phage differentiation and their ecological consequences for the *Rhizobium* populations.

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SEARCH FOR GENES RELATED TO RESISTANCE TO DESICCATION IN SENSITIVE BACTERIA THROUG METAGENOMIC STUDIES.

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In the last years, population growth has increased dramatically and is expected to continue to grow. As a result of this increase, agriculture has faced the challenge of how to obtain greater yields from crops to feed the world's population. In the last decades, large amounts of chemical fertilizers, pesticides, new crop varieties and the increased of irrigation have been used as a strategy to generate an increase in agricultural productivity. Unfortunately, excessive use of chemical fertilizers has generated adverse effects on the environment, such as water eutrophication, soil acidification and contamination of groundwater with nitrates in agricultural areas. As an alternative to the application of chemical fertilizers, the use of plant growth promoting rhizobacteria (PGPR) has been proposed. In agriculture, the beneficial use of PGPR in conjunction with other beneficial microorganisms also generates the potential for increased plant protection against soil microorganisms that can cause disease and may also increase protection against environmental pressures frost, salinity and to counter stress due to the presence of xenobiotic contaminants;which is translate into high agricultural yields and preservation of the environment. Although the use of PGPR bacteria in agriculture is incipient, the direct application of its properties is done through inoculants based on PGPR bacteria and other microorganisms. However, the efficiency of the inoculants can be affected by the water content present in the soil and its fluctuations. This is a limiting factor for the activity and survival of the bacteria since not all beneficial bacteria (PGPR) are resistant to desiccation. In our laboratory a multi-species inoculant has been developed, which is already patented (MX / E / 2013/048220) and contains bacterial strains that are able to coexist without antagonizing each other. Because other authors have developed formulations containing desiccation tolerant bacteria to be more efficient in environments with low water availability, the working group has focused on finding out what sort of genes are involved in the resistance to desiccation. Focuses mainly on identifying genes that can give resistance to the desiccation to a sensitive bacterium like Pseudomonas putida for its agro-biotechnological use through metagenomics studies.

Evaluation of Antibiotic Resistance, BLEE Production and Biofilm formation in Two *E. coli* strain collections Isolated of patients with ITU in the State of Puebla

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Uropathogenic *E. coli* (UPEC) is the main etiologic agent of urinary tract infections (UTI), which affects men and women of different age. UPEC represents a public health problem due to the emergence of strains multiresistant to antibiotics, making UTI treatmentdifficult. In this study, we analyzed 116 *E. coli* strains from two different groups: the first oneformed by 51 strains isolated fromurine of women cursing with UTI since a third-level public hospital of the city of Puebla in the year 2016. The second group with 65 strains from patients with UTI in public hospitals, women with cervical dysplasia, and private laboratories, belonged to serotype O25:H4 and O25:H-. These last strains were the most frequent isolated during the period 2011-2015.

The susceptibility profile was determined to 24 antibiotics, including aminoglycosides, fluoroquinolones, betalactamics, trimethoprim/sulfamethoxazole, chloramphenicol, nitrofurantoin, polymyxins, and phosphonates by Kirby-Bauer method following CLSI criteria. Resistance results were evaluated using Magiorakos, 2012 classification. The production of beta-lactamase of extended spectrum (BLEE) was carried out by double disk diffusion. The biofilm formation was realized using microplate assays reported by Cremmet 2013 and Tabasi 2015. The detection of virulence genes of aerobactin (*iucD*), autotransporter toxin (*satA*) and adhesin toxin of pili type I (*fimH)*was performed by multiplex PCR.A comparison made using resistance profiles from strains isolated in 2016 and O25 strains showed an increase in the resistance of the strains from 2016. 2016 strains showed 63% resistance to nitrofurantoin, 96% to ciprofloxacin and 100% for the betalactamic cefuroxime, with regard to the strains O25 with 9%, 83% and 71%, respectively.

Regarding the classificationby resistance phenotype, the strains isolated in 2016 were MDR 90.1% and XDR 9.8%, compared to O25 strains that were MDR 100%. In addition, 23.5% of the 2016´s strains and a 17% of the strains O25 were BLEE producers. Concerning biofilm formation tests, 8.33% of the strains isolated in the 2016 and 16% of strains O25 were biofilm producers. The virulence genes in the strains O25 were present in 100%*fimH*, *12% sat and* 89%*iucD*, compared to 2016 strains with 64.7% *iucD, 23.5 % sat*and 19.6% *fimH*. These findings showed 2016 strains were significant increase in resistance to first-line antibiotics for low urinary tract infections compared to isolated O25 strains in the period 2011-2015. Whereas UPEC O25 strains were more virulent and biofilm producers than 2016strains.This provide new signals regarding the prevalence of resistance to antibiotics used in clinical practices, as well as virulence characteristics that must be taken into account to guide and improve strategies for a better treatment and control of the ITU.

PvdQ as Quorum quencher in *Pseudomonas aeruginosa* isolated from burned patients in a third level hospital.

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Burns are the most severe trauma manifestations and are associated to high morbility and mortality rates. In addition, the loss of skin make them more susceptible to infectious process, therefore increasing mortality rates. There are two nosocomial microorganisms more frequently associated to infectious in these kind of patients, *Pseudomonas aeruginosa* (Pae) and *Acinetobacter baumannii*, in the present work we used *Pae* as the model infection. Pae has a huge arsenal of virulence factors including exotoxins, rhamnolipids, elastase, pigments, proteases, moreover, it can adapt quickly to the selective pressure caused by the use of antibiotics, in agreement our clinical isolate collection include a high proportion of Pae multidrug resistant (MDR) strains. Since high rates of antibiotic resistance is a severe global health problem. we are looking for strategies to reduce infections in burned patients with infectious caused by MDR. We are currently focused in blocking virulence factors, which are controlled by a social phenomenon known as Quorum Sensing (QS), Pae has 3 QS systems, two of them are dependent on acyl homoserine lactone (Las and Rhl system) and one on quinolone like signals..

We had analyzed 100 Pae strains, 57% of them MDR (resistant to three or more antibiotics families) and 43% were non MDR (at least resistant to one family of antibiotcs). 44.5% were pyocianin producers, 65.6% produced elastase, 81.5% produced proteases and 91% were rhamnolipids producers. At this stage we are purifying PvdQ an enzyme that degrades long chain acyl homoserine lactones and that inhibit QS of laboratory Pae strains by expressing it in *E. coli* and then using FPLC with HiTrap sepharose HQ 5 mL, phenyl sepharose and superdex columns. After obtaining enough pure protein we will test its activity by bioassays and then its effect in attenuating the QS dependent virulence factors of our clinical strains.

Correlation between high bacterial load and the presence of the O122 pathogenicity island in enteropathogenic *Escherichia coli* isolated from children with diarrhea.

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Enteropathogenic *Escherichia coli* (EPEC) is an important causative agent of diarrhea in children from less developed regions of the world. EPEC colonizes the surface of the epithelial cells inducing a histopathological lesion known as "attaching and effacing lesion (A/E lesion)", regulated by the locus of enterocyte effacement (LEE). LEE encodes and regulates a type three-secretion system (T3SS), as well as some other effectors involved in the A/E lesion. In addition, the T3SS also translocates non-LEE encoded effectors (Nle) into mammalian cells cytoplasm that are involved in EPEC virulence and host damage, as in the case of O122 pathogenicity island (PAI O122) effectors. PAI 0122 Nle effectors are lifA, nleE, nleB, espL. LifA (lymphostatin) inhibits mitogen-activated cytokine expression and associated with adherence and colonization, while NleE disrupts Nf-KB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway through IkB stabilization, preventing its degradation. NleB mediates GAPDH (glyceraldehyde-3-phosphate dehydrogenase) O-GlcNAcylation, disrupting the tumor necrosis factor type 2-GAPDH interactions, suppressing TRAF2 polyubiquitination and NF-kB activation. EspL is a cysteine protease that can degrades the TRIF (TIR-domain-containing adapter-inducing interferon-β) protein during infection. Objective: To establish EPEC load in the stools of children with diarrhea and the prevalence of non-LEE encoding genes among EPEC strains isolated form these children. Material and Methods. EPEC was isolated from 23 patients <5 years old. Strains from these children were characterized for the presence of several non-LEE encoded genes (nleA, nleB, nleE, nleH1, nleH2, espL and lifA), toxins (cdt and astA) and proteases(espC and pet). Bacterial load was established by Real Time PCR using as reference gene encoding for intimin (*eae*A, a LEE encoded gene). Results. We tested EPEC bacterial load in the stools of 23 patients and classified in two groups:< $1X10^8$bacteria/mg of stool and ≥ $1X10^8$ bacteria/mg of stool. Seven (30%) of the 23 patients had a bacterial load of ≥ $1X10^8$bacteria/mg of stool, six patients (86 %) harbored genes of the PAI 0122, compared with only three (17.6%) of the 16 patients with a lower bacterial load (p=0.0049, OR= 26.344 CI=1.66-231.4). Out of the 6 patients with a higher EPEC load in feces, five(71%) carried all four PAI 0122 genes and one patient (14%) carried nleE and nleB genes. Conclusion. PAI O122 genes were more prevalent among EPEC strains from children with diarrhea with a higher EPEC load in their stool. This suggest that the properties of PAI O122 effectors on reducing host inflammation and cytokine production limits the host capacity to eliminate EPEC from the gut, therefore, EPEC will multiply, colonize and will induce diarrheal illness in the host.

GEIs AS GENOMIC MARKERS FOR MULTI-INFECTION EVENTS BY *Pseudomonas aeruginosa*.

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Pseudomonas aeruginosa is an opportunistic bacterial pathogen in immunocompromised humans. It is one of the main etiological agents of nosocomial infections such as pneumonia, urinary tract infections, bacteremia, surgical or cutaneous wound infections in patients with burns. *P. aeruginosa* pangenome consists of a core and accessory elements which are variable among the specie strains. Example of accessory genomic material are the genomic islands (GEIs) which play important roles in environmental adaptation; in a nosocomial environment GEIs participate in biofilm formation, antibiotic resistance, virulence, etc. Since GEIs are elements that contributes to genotypic and phenotypic variation, the proposal of this study is that GEIs could be used as genetic variability parameters that may reflect and identify multi infection events by *P. aeruginosa* variants in the same nosocomial patient. From a *P. aeruginosa* isolates library that has been collected from nosocomial patients of Ciudad Juárez Chihuahua, we selected the cases in which more than one bacterial isolates come from the same patient. The phenotypic characterization of the isolates was performed by colonial morphology, pigmentation, and presence of bacteriophages. We found six patients as the source of more than one *P. aeruginosa* isolates, 47 in total. The genotypification was done using 18 molecular markers of seven GEIs. So far, 22 GEIs profile patterns have been identified, there were few cases where the bacterial isolates displayed the same GEIs genotype, suggesting a common clonal origin. GEIs could be used as a relevant genetic tool with potential contribution to the understanding, diagnosis and treatment of multi infection by strains of the same bacterial specie.

Evaluation of the interaction between *Fusarium oxysporum* and *Pseudomonas aeruginosa* isolated in vanilla beans (Vanilla planifolia).

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Mexico is the fifth producer of vanilla worldwide and this orchid is considered among the main natural flavoring. However green or cured beans are susceptible to microbiological contamination (bacteria and phytopathogenic fungi), causing in the fruit diseases that involve the use of agrochemicals for their control, which requires more environmentally friendly alternatives. The aim of this study was to evaluate the microbial interaction between *Fusarium oxysporum* and *Pseudomonas aeruginosa* isolated from green and curedvanilla,applying two methods (M1 and M2).Spore-washing was used for M1 or cup method and 5 mm of fungal mycelium was occupied with M2, potato dextrose agar (PDA) and nutrient agar (AN) plates previously inoculated with the bacteria (CFU / mL), were used for both methods.The plates were incubated at 30 °C for 7 days and radial growth and % Inhibition (% IN) were calculated. The% IN presented significant differences ($p \leq 0.05$) and were higher in nutritive agar with values of 100 and 79.7% for M1 and M2, respectively.The PDA medium was observed inhibition on F. oxysporum too, however the% IN were lower with values of 38.32 and 53.5% for M1 and M2, respectively. The results obtained in the interactions can be useful to propose studies on the bacterial metabolites that are participating in the inhibition of *F. oxysporum* as well as to continue investigating the behavior of the microbial interactions on vanilla beans for obtaining alternatives of biocontrol in diseases fungi.

Construction of *tsh* mutant of avian pathogenic *Escherichia coli* and evaluation of their pathogenicity

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Avian colibacillosis is the major cause of morbility and mortality in poultry and is responsable for significant economic losses worldwide. In avian pathogenic *Escherichia coli* (APEC) has been identified several virulence genes associated with the virulence including those encoding for autotransportes as the temperatura-sensitive hemagglutinin (Tsh). Tsh was the first serine protease autotransporters of the *Enterobacteriaceae* to be described, and is synthetized as a 140-kDa precursor that undergoes cleavage of its 52-amino-acid signal sequence in the periplasm. As a next step, the 33-kDa C-terminal domain inserts into the outer membrane to mediate translocation of the passenger domain to the cell exterior. Once surface localized, the secreted 106 kDa domain remains temporarily bound to the cell envolpe. Tsh binds to red blood cells, and to the extracelular matrix proteins fibronectin and collagen, and have proteolytic activity against mucin. The aim of this study was construct a mutant of *tsh* gene for study in a future functional activities of Tsh that haven not been analyzed so far, in the virulence of APEC.

According to the methodology and results, one isolate designed APEC-RS4 was obtained from a lung from a clinic case of avian colisepticemia and the occurrence of the *tsh* gene was analyzed using PCR assay. To construct the *tsh* knockout, RS4Δtsh, in-frame deletion of a 2133 pb of *tsh* was performed by overlap extension PCR as follows: the first overlap PCR was performed with one primer par F1/R1, the second overlap with primer par F2/R2, and the fusion PCR was performed with F1/R2. The PCR products were inserted into the suicide plasmid pRE112, resulting in pRE112Tsh. SM10 λ pir was transformed with pRE112Tsh, and the transformants were conjugated with RS4. The transconjugated were selected with sacarose and chloramphenicol. This strain was named RS4Δtsh.

Relationship between lipid rafts and the type III secretion system of EPEC.

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Abstract

Bacterial cell membranes, like their eukaryotic counterparts, have been shown to have membrane microdomains, also known as lipid rafts. It has been proposed that one of the most important properties of lipid rafts is that they can compartmentalize and spatially organize cellular processes by serving as organizing centers for the assembly of specific proteins or protein complexes. Associated to these nanoscale raft-based membrane regions are found the SPFH (stomatin/prohibitin/flotillin/HflK/C) domain-containing proteins, and are therefore used as markers for lipid rafts.

In this study, we present results indicating that the type III secretion system (T3SS) of EPEC, a multiproteic complex used by Gram-negative bacteria to secret proteins directly into the cytoplasm of eukaryotic cells, is associated to lipid rafts. Also, we demonstrate that deletion of the HflK, a SPFH containg-protein, affect T3SS dependent secretion. Thus, membrane microdomains and the associated SPFH domain-containing protein may play an important role in T3SS biogenesis/function.

Expanding the protein interaction network of EscQ, a sorting platform component of the type III secretion system in enteropathogenic *Escherichia coli*

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Enteropathogenic *Escherichia coli* (EPEC) belongs to a family of bacterial pathogens that induce a characteristic lesion on intestinal cells known as attaching and effacing (A/E) lesion. EPEC employs a type III secretion system (T3SS) or injectisome to deliver effector proteins into host cells. The assembly of this molecular nanomachine involves a highly coordinated process that ensures the ordered secretion of three classes of proteins: early substrates (inner rod and needle subunits), middle substrates (translocators) and late substrates (effectors).

The injectisome and the flagellum are evolutionarily related structures that share several homologous proteins. The EscQ protein is an essential component of the EPEC injectisome. EscQ forms a ring-like oligomeric structure at the cytoplasmic base of the T3SS that is similar to the flagellar cytoplasmic ring.

In other bacteria such as *Salmonella,* it has been proposed that the SpaO protein (EscQ in EPEC) serves as a basal core component that -together with OrgA and OrgB, (EscK and EscL in EPEC, respectively)- establishes an appropriate substrate hierarchy for the secretion process, so this complex has been named sorting platform. In *Shigella* an important role for the Spa33 protein (EscQ) in the formation of the T3SS and protein translocation has been reported. Moreover, in *Yersinia* it has been show that the YscQ protein (EscQ) exists as a dynamic basal complex with a constant exchange of subunits that could aid in substrate secretion.

Given the importance of the EscQ protein for T3SS assembly and because its functional role has not been completely elucidated, we carried out a search for potential EscQ protein interactions and additionally analyzed the EscQ regions important for these interactions. To this end, we selected several conserved residues between EscQ and its homologs in other T3SSs and performed site-directed mutagenesis on these amino acids. In addition, we evaluated protein-protein interactions with other T3S components by yeast two-hybrid and pull down assays.

In this work we identify several residues that are important for EscQ function and report a novel T3 interaction partner of EscQ. We discuss the possibility that this novel interaction could be involved in docking of substrates to the sorting platform in EPEC.

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Expression analysis of the Diguanylate Cyclase Cdg-A from *Azospirillum brasilense* Sp7 in the transition from the motile to the sessile life style.

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The transition from planktonic (motile) to sessile (adherent) state is thought to be essential to colonize the root of plants. The mechanism by which bacteria regulates the transition between motile and sessile state is modulating and monitoring the intracellular level of the second messenger cyclic di-guanylate monophosphate (c-di-GMP). In bacteria, proteins containing GGDEF domains (diguanylate cyclases enzymes) are involved in the production of the second messenger c-di-GMP. Our research group had reported that the *cdgA* gene encoding the diguanylate cyclase A (CdgA) is involved in biofilm formation and exopolysaccharide (EPS) production in *Azospirillum brasilense* Sp7[1]. Herein we showed that CdgA is functional *in vivo* and *in vitro* by analysis of *cdgA* mutant and a His-tagged recombinant CdgA protein respectively.

The *in silico* analysis predicted that the 1623 bp *cdgA* gene encode a protein (CdgA) of 540 amino acids, with a predicted molecular weight of 62 kDa. The N-terminal of CdgA contains three putative PAS domains and the carboxy-terminal has the GGEEF domain. In addition, next and downstream to *cdgA* gene it was found two ORFs; the first one a protein of unknown function with a PAS/ PAC domain (AZOBR_140290 or ORF2), while the second one showed homology to a methyl-accepting chemotaxis (MCP) sensory transducer protein (AZOBR_140291 or ORF3), which previously study shown that is functional [2]. The presence of ORF2 and ORF3 downstream from the *cdgA* gene raised the question, if these two genes could play a role in a signal transduction mechanism, linking an input signal from the PAS/PAC domain protein (ORF2) to an output response involving the signal detection by the MCP chemoreceptor protein (ORF3). To this end, we analyzed the CdgA protein expression in planktonic and biofilm conditions by western blot assays. We obtained rabbit polyclonal antibodies against the His-tagged CdgA recombinant protein. Our preliminary data indicated that CdgA begins its expression to the end of the logarithmic phase of growth (18 h), following to until stationary phase both in planktonic as in biofilm growing conditions. With these exciting results, it is tentative to speculate that c-di-GMP synthesis is critical and locally micro compartmentalizes, in order to ensure the c-di-GMP signaling pathway. Further studies are needed to better carefully test this idea.

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Characterization of the small regulatory RNA RsmY belonging to the Rsm system in *Azotobacter vinelandii*

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Aztobacter vineladii is a Gram negative bacterium that produces three secondary metabolites alginates, poly-hydroxybutirate and alkylresorcinols, controled by the GacS/A-RsmA/Z1-8/Y regulatory cascade. The Rsm system controls at post-transcriptional level the expression of their targets (*algD*, *arpR* and *phbR*). RsmA is a repressor protein and their counterparts are a family of small regulatory RNAs (sRNAs) which possesses several stem loops structures that counteract the RsmA activity. In Pseudomonadaceas this non-coding RNAs are divided into three families according to primary and secondary structures: RsmX, RsmY and RsmZ. *A. vinelandii* has eight belonging to RsmZ family and one belongs to RsmY family. RsmY originally was reported two alleles of *rsmY* sRNA (called *rsmY1* and *rsmY2*). These genes are contiguous located in the same intergenic region, but only the *rsmY1* regulatory region has the conserved -10 conserved box in *rsm*-sRNAs genes and GacA binding box in their regulatory region commonly found in all *rsm*-sRNAs. The above mentioned suggest that RsmY1 and RsmY2 constitute a single sRNA. The *rsm*-sRNAs in Pseudomonas usually are ranging between 100-120 pb and have three of four stem-loops; the *A. vinelandii* RsmY non-coding RNA has 330 pb and presents nine stem loops. By RT-PCR analysis Hernández-Eligio et al (2012) suggested the existence of a single RsmY sRNA, however yet there is not any analysis of their functionality in vivo. In this study we present a genetic complementation analysis to confirm that *rsmY1* and *rsmY2* constitute a single gen. Also we proved that RsmY controls the *algD* expression and we show that the RsmY overexpression restores the alginate production in a *gacA* mutant. Finally we studied the regulatory role of RsmY over the alkylresorcinols synthesis, other metabolite controlled by GacA.

Generation and characterization of double mutants in *rsm*-sRNAs genes in *Azotobacter vinelandii*

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Azotobacter vinelandii is a bacterium with biotechnological interest due to its capacity to produce secondary metabolites as PHB and alginates. PHB is a biodegradable and biocompatible polymer with similar proprieties to the plastics derived from petrochemicals. The alginate is a heteropolymer with gelling capacity which can be used in the food and pharmaceutical industry as a thickener or gelling [(1)].

The production of both polymers in *A. vinelandii* has been vastly studied; the polymers are co-regulated by the GacS/A two component system and the Rsm post-transcriptional regulatory system. The *rsm* system possess nine isoforms of small RNA regulators (sRNAs), eight belonging to RsmZ family and one to RsmY family. Single mutants in rsmZ1 decrease the alginate synthesis. To deepen in the study of the system is necessary to generate sRNAs-*rsm* multiple mutants for which would be necessary to have several useful markers of resistance. In our model are useful few resistance markers, thus became necessary implement a mutagenesis strategy that allows eliminating multiple genes using a single resistance marker.

A mutagenesis system designed for *Burkholderia pseudomallei* that use a site-specific recombination of flippase system [(2)] was modified and adapted to be used in *A. vinelandii*. Using this FRT system we generate two double mutants, the first, carrying mutations in *rsmZ1* and *rsmZ2* sRNAs, and a second double mutant in the *rsmZ2* and *rsmY* genes. We measured the alginate and PHB production in the generated mutants. As expected, both double mutants decrease the alginate production to lower levels of the single *rsmZ1* mutant and even more than of the wild-type strain.

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ZTP dependent autophosphorylation of response regulators in *Escherichia coli*

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Abstract

Bacterial two component signal transduction systems comprise a membrane bound histidine kinase protein (HK) and a cytosolic response regulator protein (RR). Signal perception by the HK stimulatesan ATP-dependent autophosphorylation at a conserved histidine residue, which then donates the phosphoryl group to an aspartate residue in the cognate RR. Phosphorylated RR acts as a transcriptional regulator and modulates the expression of a specific group of genes. In the absence of the cognate HK, RRs have been shown to autophosphorylate at the expense of the high-energy phosphate compounds acetyl phosphate and carbamoyl phosphate.

In this study we report that ZTP (5-amino 4-imidazole carboxamide riboside 5'-triphosphate), an intermediate of the purine synthesis, can also be used by RRs to autophosphorylate in a HK-independent manner. Thus, ZTP is presented as a novel low molecular weight phospho-donor for RRs.

Identification of a putative operon involved in chemotaxis from *Azospirillum brasilense* Sp245.

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One of the most studied *Azospirillum* speciesin terms of basic research and agricultural applications is *Azospirillum brasilense.* Beneficial plant–microbe associations play critical roles in plant health. Bacterial chemotaxis provides a competitive advantage to motile flagellated bacteria in colonization of plant root surfaces, which is a prerequisite for the establishment of beneficial associations. Chemotaxis signaling enables motile soil bacteria to sense and respond to gradients of chemical compounds released by plant roots. This process allows bacteria to actively swim towards plant roots and is thus critical for competitive root surface colonization.

The objective of the present study was to determine the contribution of a putative operon form *A. brasilense* Sp245 strain constituted by three genes (Figure 1). The genes likely encoding for a putative diguanylate cyclase E, a histidine kinase (HhkB) and CheY-like protein are involved in motility to several chemo-attractants.

The RTPCR determination shown that *dgcE* and *hhkB*arecotranscribed, and bioinformatics analysis indicated that *cheY*-like gene is overlapped with *hhkB* gene. We constructed three mutants: The *dgcE*::Km^{R}, Δ*dgcE*, and Δ*hhkB* mutants. For comparison of chemotaxis responses in the soft agar assay, bacteria were inoculated into MMAB solidified with 0.25% (w/v) agar (soft agar plates) and supplemented with 18.7 mM ammonium chloride and 10 mM malate, succinate, proline, and glutamate. Chemotactic rings were measured after 48 h incubation.All mutant shown decrease motility, suggesting that this operon is in involved in motility. In order to observed the contribution of Δ*hhkB* to colonization to wheat, the mutant was tagged withm Cherry protein and determined by confocal microscopy the colonization. Taking in account the data obtained we concluded that the DgcE and Hybrid histidine kinase are involved in chemotaxis.

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Effect on the inhibition of Quorum-dependent virulence factors in *P. aeruginosa* by pyrazino-isoquinolines

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Pseudomonas aeruginosa a Gram negative bacterium of clinical significance. is an opportunistic human pathogen that can propagate in the abnormal human airway, burn-damaged skin, and artificially implanted organs, and can also cause hospital-acquired secondary infections in patients with compromised immune reactivity.[1] The misuse and abuse of antibiotics are recognized to create selective pressure, resulting in the widespread development of resistant bacterial strains. Antibiotics are also known to kill good/beneficial indigenous bacteria, which may have protective role against pathogenic bacteria. Facing these limitations of antibiotics, there is an increasing need for the discovery and the development of antimicrobial agents that present novel or unexplored properties to efficiently control and manage bacterial diseases. Inhibition of bacterial virulence and/or biofilm formation by targeting nonmicrobicidal mechanisms are examples of increasingly explored antipathogenic approaches.[2] Quorum sensing (QS) coordinates the expression of multiple virulence factors in *Pseudomonas aeruginosa*; hence its inhibition has been postulated as a new alternative to treat its infections. QS interference approaches claim that they attenuate bacterial virulence without directly decreasing bacterial growth and suggest that *in vivo* the immune system would control the infections.[3]

Diketopiperazines have antibiotic effect as well as well as a remarkable inhibitiory activity of the QS-dependent virulence factors in *Pseudomonas aeruginosa* and *C. violaceum*.[4]

In this work we evaluated four different pyrazino isoquinolines in the inhibition of alkaline protease, elastase, pyocyanin, alginate and biofilm, using concentrations of 5, 30 and 50 microgram / ml, obtaining results of up to 90% for inhibition of pyocyanin, 40-50 on the remaining virulence factors.

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Effect of various solvents in addition of curcumin on the inhibition of virulence factors dependent on quorum sensing in *Pseudomonas aeruginosa*.

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Due of the multi-resistance to antibiotics, alternatives have been proposed to combat bacterial infections, among these is the inhibition of virulence, by blocking processes that coordination such as the perception of quorum (QS or Quorum Sensing), which coordinates the expression of multiple virulence factors such as exoproteases, phenazines, toxins, exopolysaccharides, biosurfactants, siderophores, etc. In response to an increase in cell density.For the inhibition of QS, there are strategies that either involve blocking the binding of QS signals to receptors, which inactivate receptors, while others decrease the production of signals, or promote their degradation.Probably the most abundant inhibitors of QS are the small molecules isolated from natural products, such as bromate furanones, fatty acids, flavonoids, some antibiotics such as azithromycin, or compounds such as anacardic acid or curcumin (1).
Due to the great structural diversity of these compounds different solvents are used to administer them, among them methanol, ethanol, dimethylsulfoxide (DMSO), dimethylformamide (DMF) etc. And although it is generally assumed that these solvents do not affect the expression of virulence factors, we consider that a systematic characterization of the possible effects of solvents on the expression of the virulence factors regulated by the QSis indispensable, with the objective of identifying the appropriate vehicles for the use of QS inhibitory compounds.Especially if we know that in *P. aeruginosa* the addition of ethanol affects the expression of QS dependent and independent virulence factors. Hence we are evaluatingthe addition of the solvents: methanol, ethanol, dimethyl sulfoxide (DMSO) dimethylformamide (DMF) and acetate (2) in the inhibitory effect of curcumin over QS using the main reference strains PAO1 and PA14 evaluating the production of exoproteases, pyocyanin, alginate, and biofilm formation.

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Conjugative transfer of rhizobial plasmids under diverse environmental conditions and during symbiosis

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Rhizobium etli CFN42 is a Gram-negative bacterium of great agricultural importance, able to establish symbiosis with *Phaseolus vulgaris*, through the formation of nitrogen fixing nodules. The genome of *R. etli* CFN42 is constituted by one chromosome and six large plasmids. Among these, pRet42a has been identified as a conjugative plasmid, which can be transferred at high frequency (10^{-2}). Transfer is regulated by quorum sensing (QS) involving a *traI* gene encoding an acil homoserin lactone, and two transcriptional regulators (TraR and CinR), which induce the transcription of transfer genes in response to TraI (Tun-Garrido *et al.*, 2003).
The aim of this work is to determine the effect of environmental conditions on the transfer of conjugative plasmids, including factors related to soil environment (temperature, low oxygen, nutrients) as well as elements related directly to the symbiosis (seed exudates). To do these experiments, we used the strategy of double fluorescent labeling described by (Nancharaiah *et al.*, 2003). We constructed a donor containing a red fluorescent protein (RFP) marker inserted in the chromosome, and a green fluorescent protein (GFP) marker in the plasmid (Torres Tejerizo *et al*., 2015). Our results showed that the frequency of conjugation increases when it is performed in the presence of the plant or of a plant exudate (naringenin). Also we were able to visualize transconjugants in nodules, opening the possibility of conjugation taking place inside the plant. Different strategies were employed to analyze this hypothesis. In the first one, we used transcriptional fusions of the promoter regions of *traI* and *traA* genes to a reporter gene (*traA*::GFP and *traI*::GFP), to determine the activity of genes involved in conjugation. In the second strategy, we inhibited conjugation outside the plant, using a *traM* antiactivator, allowing conjugation when the bacteria enter the symbiotic process. In the third strategy, the *traI* gene was placed under the *nifH* promoter, allowing expression of transfer genes only under conditions that allow nitrogen fixation Overall, our results strongly suggest that conjugation may occur inside the plant, in addition to transconjugants generated on the surface. Conjugation in this conditions is also dependent of quorum sensing.

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Methylotrophic bacteria isolated from *Neobuxbaumia macrocephala* mainly possess *xoxF* and metabolize methanol in presence of Ce^{3+} and Ca^{2+}.

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Methylotrophic culturable bacteria was isolated from plant surface, rhizosphere and inside the stem of *Neobuxbaumia macrocephala*. They included Actinobacteria, Bacteroidetes, Alpha-, Beta- and Gamma proteobacteria. Translated amino acid sequences of methanol dehydrogenase of strains of Actinobacteria, Alpha-, Beta- and Gamma proteobacteria showed high similarity to lanthanides-dependent XoxF methanol dehydrogenases, specifically to the cluster XoxF5. The sequences included Asp^{301}, the lanthanide-coordinating amino acid, present in all known XoxF dehydrogenases. Anumber of the isolates showed positive hybridization with a *xoxF* probe. Isolates of all the groups showed methylotrophic growth in the presence of Ce^{3+} or Ca^{2+}. The ubiquity of methylotrophic bacteria carrying *xoxF*from the xerophytic plant *N. macrocephala*is discussed.

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Production and purification of human beta defensin (HβD3) with antimicrobial activity again strains isolated from diabetic foot.

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Since the development of penicillin in the 1940s, the synthesis and use of different antibiotics have positively impacted human health. However, in recent decades resistance to antibiotics has been generated, so it is currently difficult to combat infections generated by resistant bacteria [1].Diabetes mellitus is a common, chronic, debilitating and sometimes fatal endocrine disease with constant growing prevalence, skin infections are common in diabetic patients and the diabetic foot ulcers is a serious diabetes mellitus type 2 complication. Approximately 25% of diabetic patients have cumulative life-time risk for foot and these ulcers can get infected easy in 40-80% of the case [2].

The current state of resistance of microorganisms to conventional antibiotics has led to the search for new alternatives. Antimicrobial peptides have emerged in recent years as a family of substances with great potential for clinical use, due to their multiple mechanisms of action, wide spectrum of activity and low resistance potential [1,3]. Antimicrobial peptides can be classified according to their secondary structure and composition, with human beta defensin being a peptide containing 6 cysteine residues and forming 3 disulfide bonds with a stable beta folded structure [2].The study peptide is beta defensin human type 3 (HβD3-M).

In this word we performed the characterization of human beta defensin in combination with commercial antibiotics again strains of *Staphylococcus aureus (UPD13)* and *Pseudomonas aeruginosa(UPD3)* that were isolated from diabetic foot, also we tested the S. aureus (ATCC29213) and P. aeruginosa (ATCC27853) strains such as reference strains.

The results obtained in this word showed that the minimal concentration inhibitory (MIC) of the HβD3-Magainst both strains ATCC27853 and UPD3 of *P. aeruginosa*were 125µg/mL and against both strains ATCC29213 and UPD13 of *S. aureus* were >250µg/mL. For other side, the best combination of the defensin with antibiotics were amoxicillin, sulfamethoxazole and vancomycin showed an effect near to synergistic again *P. aeruginosa* UPD3. Furthermore, the best combination of the defensin with antibiotics were amoxicillin and sulfamethoxazole showed an effect near to synergistic and synergisticrespectively again *S. aureus* UPD13.

These results are an alternative for the treatment of the diabetic foot ulcer.

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Study of the adaptation strategies of *Salinibacter ruber* to the conditions of Europa's ocean

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Terrestrial life exhibits a huge number of adaptations to extreme conditions, allowing a certain type of organisms to proliferate on extremes environments. These organisms are called extremophiles.Some extreme environments possess at least one physical or geochemical condition like those described for the planetary bodies of astrobiological interest in the Solar System. So, the study of terrestrial extremophiles provides clues to understand iflife, as we know it, can adapt tosome of the environmental conditionsfound in a planetary object different from Earth.One of thesescenariosis the ocean located on the subsurface of the Jovian satellite Europa, enriched with sulfate salts such as $MgSO_4$ and Na_2SO_4. A good biological model to understand the habitability potentialof this extraterrestrial ocean are the halophiles, those organisms that require high concentrations of salts, usually expressed in terms of NaCl, to proliferate.

Salinibacter ruber, an extreme halophilic bacterium whose growth in different concentrations of NaCl and $MgSO_4$ have been previously studied in our group, was exposed to different concentrations of Na_2SO_4 and equimolar concentrations of Na_2SO_4 and $MgSO_4$. Bacterial growth curves were used to determine the optimal growth conditions, growth rate and duplication time. The presence of compatible solutes was evaluated by quantitative NMR, and the expression of proteins in different saline stress conditions was identified by electrophoresis and shotgun proteomic.

The experimental evidences will be used to propose arguments about the habitability of the ocean of the satellite Europa, and about the tolerance and adaptation strategies that can be used by this or other halophiles in an extraterrestrial scenario.

Selection of *Pseudomonas aeruginosa* quorum sensing by pyocyanin

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Quorum sensing (QS) in *Pseudomonas aeruginosa* coordinates the expression of virulence factors such as exoproteases and siderophores, that are public goods utilized by the whole population of bacteria regardless if they invested or not in their production[1]. In addition, the production of toxic compounds such as hydrogen cyanide (HCN) is also controlled by QS, but unlike being a public good, HCN is a policing compound that restricts the selection of QS deficient mutants, likely by a lower expression of a cyanide insensitive cytochrome oxidase, which is also controlled by QS[2]. Phenazines are toxic QS controlled metabolites produced by *P. aeruginosa*, among them pyocyanin is the most common, being produced by several clinical and environmental strains, pyocyanin is a redox active compound that promotes the generation of reactive oxygen species as well as the depletion of glutathione pools in mammal cells, it also possesses antibacterial properties and increase fitness in competition with other bacterial species. Since QS deficient individuals are less able to tolerate oxidative stress we hypothesized that the pyocyanin produced by the wild-type population could act as a policing mechanism to select functional QS systems in this bacterium and demonstrate by competition experiments that indeed this compound increase the fitness of the cooperative QS proficient individuals.

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Two plasmids from the bean-nodulating *Sinorhizobium fredii* strain GR64 regulate each other's conjugation genes

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Rhizobial strain are interesting because of their ability to form nitrogen fixing nodules in symbiosis with plant roots, there is evidence suggesting that conjugative transfer has impacted on the diversity of rhizobial strains. *Sinorhizobium fredii* GR64 was isolated from bean nodules in Granada, Spain. It contains three plasmids in addition to the chromosome: pSfr64a (183 Kb), the symbiotic plasmid (pSym) pSfr64b (460 Kb), and megaplasmid pSfr64c (>1000 Kb). Analysis of the regulatory mechanism of the conjugative transfer ability of plasmid pSfr64a showed that it is transmissible at high frequency, regulated by quórum-sensing (QS) through elements encoded in plasmid pSfr64a (TraRa, TraIa), in the symbiotic plasmid (TraIb, TraRb) and in the chromosome (ngrI, ngrR). Also, a mutation in a gene encoding a hypothetical protein, located next to *traH* showed a decrease in transfer.

In spite of the fact that the genomic sequence of GR64 strain showed that the pSym contains a complete set of *tra* genes, transfer of the pSym was found to depend on the presence of pSfr64a. To elucidate what causes 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.

The results showed that the TraIa and TraRa encoded in pSfr64a are required for expression of the transfer genes encoded in this plasmid, although the *traIa* mutant was still able to transfer the plasmid, indicating that the pSym encoded TraIb is able to substitute TraIa. Mutations in *traIb, traRb* and *traRa* impaired transfer of both plasmids, and the three genes are required to allow expression of the genes from pSfr64b. Additionally, expression of *traIa* requires the pSym encoded TraRb-TraIb.

Overall, the results indicate that a regulatory circuit is formed between both plasmids, becuse the expression of the homoserine lactone synthatese encoding TraI gene from each plasmid, also requires the TraR transcriptional regulator from the other plasmid. Additionally, the expression of the tra genes from each plasmid, are regulated by the TraI-TraR complex encoded in the same plasmid.

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S1 protein promotes the translation of A- or U-rich downstream mRNAs

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S1 is the largest ribosomal protein of the 30S subunit,and it is known for its high affinity for single-stranded AU-rich RNA stretches. Additionally, S1 is involved in the docking of structured mRNAs to the 30S subunit. On the other hand, adenine and uracil nucleotides downstream of the initiation codon enhance the binding of mRNA to 30S subunits and the protein synthesis. Thus, r-protein S1 could be pivotal for recognition of downstream A-or U- rich mRNAsby 30S subunits and to promote their translation.In an attempt to understand the mechanism by which downstream adenine and uracil nucleotides promote the translation. First, we tested the role of S1 in the mRNA-30S interaction by filter binding assays, by incubatinglow structured mRNAs containing different nucleotide content in the second and third codons with wild type (wt) 30S subunits, S1-deficient 30S subunits or S1-deficient 30S subunits reconstituted with either wt or truncated formsof S1.As expected, synthetic A- and U-rich mRNAsbound stronger to 30S subunit than messengers with guanines at the same positions. Interestingly, compared with the G-rich mRNA the enhanced binding of the adenine- or uracil-rich mRNAs was reduced to a greater extent when the ternary complex was formed with S1-deficient 30S subunits. The binding affinity was restored to wild type levels when S1-deficient subunits were reconstituted with wtS1 but not with an S1 mutant lacking domains 1-2 or 1-3. Although it has been reported that A/U rich sequences upstream the SD sequence promote translation by binding to anS1 protein, it was necessary to assess whether the mRNAs tested in the ternary complex formation assays showed the same binding capacity with S1 protein alone outside the 30S subunit context. The formation of binary complexes between wild type or mutant S1 proteins and the synthetic mRNAs was analyzed usinggel shift assays. S1 alone showed a higher affinity for A- or U-rich mRNAs compared G-rich RNA messengersas it happened in the formation ofternary complexes. Interestingly,deletion of the first 3 domains ofS1abolished its binding to A-rich mRNA, and the loss of the domains 1-2 and 1-3also prevented its binding to U-rich mRNA. Remarkably, S1 did not show binding to G-rich mRNA.To further explore whether S1 not only enhances A- or U-rich mRNA binding to 30S subunits but also the translation of these messengers, an S1-defficient in vitro transcription-translation assay was developed. Compared with wt in vitro reactions, S1 deficiency affected dramatically the translation of A- or U-rich downstream mRNAs. The in vitro reconstitution of S1 not only did restore the translation from A- or U-rich mRNAs but also stimulated their translation.These results indicate that S1 promotes protein synthesis from A- or U-rich mRNAsby enhancingtheirbinding to 30S subunits.

Self-similarity in bacterial regulatory networks: insights into a novel organizational property conserved during evolution

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It is known that regulatory networks (RNs) are highly plastic, evolving faster than structural genes. Nevertheless, the way evolution shapes RNs functional architecture and their systems is poorly explored. To address this issue, a wide number of reconstructed RNs for different organisms is required. Abasy Atlas, developed by our group, provides an extensive collection or RNs across bacteria. An analysis of this data shows that not only RNs degree and clustering coefficient distributions follows a power law, but also the module sizes distribution, as identified by the natural decomposition approach, has the same behaviour. The recurrent emergence of a power law behavior could suggest that self-similarity is a principle governing RNs architecture. Self-similarity, invariance against changes in scale, is a property of many natural systems exhibiting fractal features. Although, fractality has never been explored nor observed in RNs and even its existence has been controversial. On the other hand, we have found that density is strongly conserved across RNs, raising the question of which other topological parameters are conserved and how their limited range of possible values delimit the RNs organizational landscape. In this study, we explored the fractal nature of 52 bacterial RN reconstructed and available in Abasy Atlas. We implemented an optimized version of the box-counting algorithm to obtain RNs fractal dimension. We found that fractal dimension is conserved and constrained into a small range across all analyzed RNs despite their genomic coverture of the RN**.** We then compute the fractal skeleton supporting RNs topology by implementing a decomposition algorithm based on edge betweenness and minimum spanning trees. Our results suggest that fractality, as well as density, is an organizational property of bacterial RNs that could impose constraints to their evolutionary dynamics and can be exploited to improve our current evolutionary models.

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Novel plant growth promoters affects the microorganism populations present in rhizosphere

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Soilsare a dynamic environment, where a large number of interactions are carried out between the different elements that form this ecosystem. Abiotic factors play an important role in the fluctuation and behavior of interactions occurring between microorganisms and the plants un the rhizosphere. Some microorganisms are capable topromote plant growth, others inhibit this growth and some have no effect, however arise several questions of what happens in microbial populations present when added a plant growth promoter compounds. Weanalyzed the effect of the addition of plant growth promoting compounds on the fluctuation of microorganisms present in soil and their sporulation capacity.

Microorganismsisolated from rhizosphere samples of maize plant treated with two different growth promoting compounds were characterized under microscope by Gram stain and phase contrast.Spores formation was determined through kinetics of sporulation time of each isolated strains.

The amount of isolated microorganisms from rhizosphere samples per gram of soil was for the treatment A: 3 $X10^8$ cfu, for the B: $1X10^9$cfu and for the treatment C: $7X10^8$cfu,64 strains of sporulating microorganisms and 2 of non sporulating microorganisms were isolated, all were Gram positive, 97% belonged to the genus *Bacillus*. In the sporulation analyzes, the strains showed different behaviors, since some delayed the formation of spores or their efficiency resulted affected. The addition of plant growth promotors un maize, could impact and change the environmental conditions in which the bacteria carry out biological processes such as sporulation in the case of bacteria belonging to the genus *Bacillus spp*.

Key words: Rhizosphere, microorganism populations, sporulation

Characterization and production of Acyl Homoserine Lactones (AHLs) produced by *P. aeruginosa* isolated from cured vanilla beans

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Post-harvest processing and curing of the vanilla beans in Mexico is an artisan work that allows vanilla pods to get their quality requirements such as aroma and flavor, which are determined mainly of by the content of vanillin. The contribution of microbial activities on the curing process of vanilla can be regulated by the mechanism of communication mechanism called Quorum Sensing (QS). Several auto inducer molecules, name lyacylhomoserinelactones (AHLs), participate in QS *P. aeruginosa* is a AHLs producing specie, isolated from cured vanilla beans. However, the role of this bacteria in the traditional curing process in Mexico is unknown. In order to describe the potential contribution of *P. aeruginosa* on the development of vanilla flavor, the production of pectinolytic enzymes were evaluated by cup-plate as say supplemented with 0.5% pectin, well technique, reported in enzymatic hydrolysis rates, as well as the bioconversion off erulicacid to vanillin were evaluated by cup-plate as say supplemented with 0.01% ferulicacid, well technique, reported inenzymatic hydrolysis rates off erulicacid, vanillin resistance (spectrophotometric method and was reported as MIC), and and the production capacity of AHLs was determined in growth kinetics of *P. aeruginosa* in TSB medium, for 24h, incubation at 37°C, using bioassays and the biosensor *A. tumefaciens* NTL4 (pCF218) (pCF372). As results were found that *P. aeruginosa* presented pectinolytic activity (3.2± 0.60IU), can transform ferulicacid to vanillin (1.1± 0.3 IU) and is resistant to vanillin (1562 µg/mLMIC). The maximum production of AHLs was greater achieved at 8 hincubation time with avalue of 11.08 induction indices (IID). The results obtained in this work are useful for understanding the role of *P. aeruginosa* on the curing process. Never the less, future experiments are necessary to completely uncover the mechanisms by which AHLs are involved with the mechanism of communication QS and the processes related to the aromatic profile of vanillin.

Using the organizational principles of regulatory networks to identify global regulators in bacteria

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Regulatory networks are groups of macromolecules, mostly regulatory proteins and genes, interacting to control the level of gene expression in a certain organism. The increasing information in databases has made possible the large-scale representation of these networks, which depict the circuitry enabling bacteria 'making decisions' to adapt to different media conditions.Regulatory proteins are transcription factors consisting of a DNA-bindingsite and an allosteric–metabolite interaction,which perform as a link in the transduction of signals from extracellular and intracellular stimuli to several biological processes. A small number of regulatory proteinsexhibit pleiotropic effects when its corresponding genes are mutated,they are named global regulators (GRs). It is mainly due to their capability to influence the expression of a large number of transcription units, showing that they regulate different metabolic pathways and subsystems. Oppositely, local regulators are a wide number of specific transcription factors regulating only a few transcription units.Besides, transcriptional regulation in bacteria is carry out by a catalytically competent core RNA polymerase (RNAPc) that requires an additional sigma factor for specific promoter recognition and correct transcriptional initiation. This RNAPc is not able to selectively bind to anysigma factor, but different sigma factors have different affinities and competefor RNAPc dramatically reprogramming the transcriptional machinery. Although diverse diagnostic criteria have been proposed to identify GRs, they consider a wide number of parameters. Conversely, the *kappa*-valueis a mathematical criteria based solely on two apparently contradictory behaviors occurring in hierarchical-modular networks, modularity and hubness. Here, we assess the effectiveness of the *kappa*-valueto identify GRs in bacteria by analyzing different reconstructions of the regulatory networks of *Escherichia coli*,*Bacillus subtilis* and *Corynebacterium glutamicum*. We also evidence the relevance of sigma factors for the hierarchy of regulatory networks and their possible role as GRs.

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Searching for proteins from *Alicycliphilus* sp. BQ1, a bacterium capable to attack polyurethane, with capacity to break the urethane group

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Polyurethane (PU) is a synthetic polymer with raised versatility, highlighting its use as foam, elastomer, adhesive, paint, coating and elastic fiber. Its production is based on the high reactivity of the isocyanate double bond that easily binds either ester or ether polyols, generating the urethane/carbamate groups, by a condensation reaction. PU physicochemical properties and chemical structure give it such a design to resist degradation under environmental conditions, that its overall decomposition is difficult and very slow. With the aim of developing technologies for PU biodegradation, several microorganisms with capacity to attack PU have been identified, and most of the activities correlated with the attack have been esterases. However, no enzymes have been correlated with the attack to the carbamate group, the most recalcitrant of the PU structure. In our laboratory, we work with the bacterial strain *Alicycliphilus* sp. BQ1 able to grow in a minimal medium supplemented with Impranil DLN, a polyester-PU varnish, as the only carbon source. We characterized BQ1 growth in Impranil by oxygen consumption, and quantified the amount of cytosolic and membranal protein, observing a log phase from 12 to 28 h. Impranil DLN clearance has been reported as indicative of its degradation, we observed that BQ1 produced 82% of Impranil clearance in 28 h of culture. Moreover, by FTIR spectroscopy, we demonstrated that BQ1 is able to hydrolyze Impranil DLN ester and urethane linkages, which suggest that esterase and amidase activities must be involved in PU degradation. Therefore, searching for these activities, temporal analyses quantifying both activities were performed, observing their maximum at 28 h, which correlates with the changes observed in FTIR spectroscopy. By a novel zymography assay using ethylcarbamate (EC) as a substrate, a protein band of 150 kDa, capable of breaking the EC amide group has been detected. Experiments are undertaking to identify and characterize this protein.

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Characterization of isolated microorganisms of extreme environment with enzymatic capacity

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Introduction: Several industrial biotechnological processes employ a large amount of microorganisms to obtain products for human interest, such as enzymes, biopolymers, antibiotics and various secondary metabolites. At present, the use of microorganisms isolated from extreme environments represents an area of opportunity for obtaining enzymes, since these microorganisms are adapted to very particular conditions of environmental stress, reason why their metabolism and the synthesis of enzymes are of Importance for industrial biotechnology. Objectives: Isolation and microbiological, molecular and enzymatic characterization of strains isolated from the zone of Iztaccihuatl volcano, Puebla. Materials and methods:A total of 93 strains of microorganisms were isolated, characterized by Gram staining, enzymatic activity (Amylaseand protease) was determined, and strains with both hydrolytic capacities were characterized by 16 S RNA sequencing. Results: Since 93 isolates, 75 strains showed only 1 activity and 9 did not exhibit any enzymatic activity, while 13 strains showed considerable capacity to produce both amylasesand proteases at pH 7. Gram stains indicated that all isolates were Gram positive and mostly belonging to the genus *Bacillus sp*. Discussion: Microorganisms isolated from samples obtained from an extreme environment were found to have an enzymatic capacity capable of being used in several biotechnological processes, because most isolates belong to the genus *Bacillus sp*, we can infer that the physiological characteristics of the bacterium as sporulation favors its prevalence in this environment.

Key Words: enzymatic capacity, extreme environment, microorganisms

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Assessing bacterial consortiaactivity against polyurethane contamination

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Polyurethanes (PUs)are plastics ubiquitous in different aspects of modern life. They are found in furniture, coatings, paints, adhesives, fibers,shoe soles, construction materials and medical devices. Paradoxically,PUs versatilityand long durability exert a major environmental threat: their accumulation in dumpsites because they cannot be degraded or re-cycled.Nevertheless, since environmental microorganisms are constantly changing, bacteria able to attack PUs, have been naturally selected. This attack depends on the chemical groups present in the material, being polyether polyurethanes more recalcitrant. Several works using monocultures to investigate the mechanisms PUsis attacked,have been published.However, the biological capacities of bacterial communities to biodegrade this polymer, have not been exploited. The present work aims to study the biodegradative activity of two microbial consortia (BP6 and BP8), on a polyether polyurethane (PE-PU)varnish (Poly Lack®),which besides the acrylic/urethane copolymer, it contains other xenobiotic compounds such as N-methyl-2-pyrrolydone, 2-butoxyethanol, isopropanol, dipropylene glycol butyl ether and dipropylene glycol monomethyl ether.

BP6 and BP8 consortia were able to growdiauxically along 20 days,in a mineral medium with Poly Lack® (0.3%), as the only carbon source, indicating that microorganisms present in the consortia metabolizedmore than one component of the PE-PU varnish. PU degradation was quantified by turbidimetric analysis, finding consumptions of 64 and 40%, by BP6 and BP8, respectively at 20 days of cultivation. Cell-PU interactions were studied during the biodegradation process, by measuring hydrophobicity of consortia cells.Cell hydrophobicity was variable along the cultures, oscillating between 58 – 80%. This variability suggested that bacteria of the consortia are able to modify its capacity to interact with PU. Cells attached to the hydrophobic substrate could be responsible for PU degradation, whileless hydrophobic cells could be degrading theless hydrophobic varnish components. By FTIR spectroscopy and thermogravimetry,we observed that,besides the easily degraded carbonyl group, the highly recalcitrantPU functional groups (aromatic, urethane and ether), were reduced by the consortia biodegradativeactivity.

In order to identify the enzymatic activities involved in PU biodegradation, radial diffusion and spectrophotometric enzymatic assays for esterase, urease, urethanase and protease were performed in membrane, cytoplasmic and extracellular fractions from cultures of the two consortia.For the BP8 consortium, esterase, urease and urethanase activities were mainly detected in cytoplasmic and membrane fractions, and protease activity was extracellular. We are on the way to identify the PE-PU degradative activities expressed by these consortia, and to demonstrate their action over the functional groups of the polymer.

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Detection of *Listeria monocytogenes* from samples of ham by PCR technique

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Introduction.*Listeria monocytogenes* is an intracellular bacilli, which can cause infections in man invasive and survives readily in media inanimate, adapting to changes in environmental conditions, which explains its large transmission capacity. It causes damage in pregnant women, newborn infants, elderly and people with compromised immune systems. At the beginning you can take the form of intestinal clinical with fever, abdominal pain, diarrhea and headaches in healthy individuals, but they do not determine the diagnosis of the disease. Foods such as raw meat and cured, raw and pasteurized milk, cold meats, cheese and vegetables have been the main vehicle of input of l. monocytogenes to the body. **Objective.** The presence of *Listeria monocytogenes* in samples of ham using PCR NORGEN kit for the detection of *Listeria monocytogenes*. **Methods.** The sample was according to the NOM - 102-SSA1-1994, for the identification of *L. monocytogenes* was taken as a basis the NOM-143-SSA1-1995, using the enrichment NORGEN BIOTEK to *L. monocytogenes* from which the DNA extraction was performed. For molecular analysis was followed the Protocol established in *Listeria monocytogenes* PCR Detection Kit. **Results**. Of 20 samples analyzed of ham, three was detected in L. monocytognes and identified bacteria belonging to the Enterobacteriaceae family by conventional microbiological methods.**Conclusion**. It is detected to *L. monocytognes* in this work gives us a reference to that due to the characteristic of the bacteria adapt to extreme environmental conditions, can be found in raw foods.

Genome assembly of *Alicycliphilus* sp. BQ1, a polyurethane degrading bacterium, by bioinformatics and PCR

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We have identified a bacterium, *Alicycliphilus* sp. BQ1,capable of growing in a mineral medium with different types of polyurethane (PU) varnish as the only carbon source. This strain, besides to attack the carbonyl, urethane and ether groups of PU degrades N-methyl pyrrolidone (NMP) present as additive in some varnishes. Close relatives, *Alicycliphilus denitrificans* strains K601 and BC, even that are able to degrade xenobiotic compounds, do notattackPU, neither degrade NMP. In order to identify the genetic differences between BQ1 and the *Alicycliphilus denitrificans* strains K601 and BC, we will compare their genomes. In this work, we report the results of our first step, the sequencing of BQ1 genome.

BQ1 DNA was sequenced by different NGS methods. By Illumina, libraries of 200, 300, 2000 bp sequences were analyzed; we also performed 454 Pyrosequencing and Ion Torrent sequencings. With these sequences and using the program SPAdes 3.9.0, an assembly of 15 contigs was generated. More than one assembly was generated, using different genome assemblers and libraries; however the assembly of 15 contigs was the most efficient, getting the least number of contigs and the largest number of open reading frames.

Alicycliphilus sp. BQ1 hasa GC content of 69% andthe genome coverage obtained was 496X. The size of the genome was 4.6 Mb, similar to genomes of *Alicycliphilusdenitrificans*strains BC (4.8 Mb) and K601 (5.0 Mb).The probable order of the contigs was obtained through alignment of sequences using *Alicycliphilusdenitrificans* K601 and BCas reference genomes, consideringan identity greater than 90%. The order of the contigs was: 1, 5, 6, 10, 4, 2, 7, 9, 3 and 8. Because of their small size (2100-511 bp) and low identity (55%), contigs 11-15 were not considered.Based on the proposed order, primers were designedto amplify by PCR,regions between the predicted consecutive contigs, using BQ1 DNA as template. Nine reactions were tested to amplify inter-contig regions. Five amplicons of: 5100 bp for GAP4-2; 500 bp for GAP9-3; two bands close to 1400 bp for GAP6-10;300 bp forGAP5-6, and a 800 bp for GAP3-8, were obtained.Cloning and sequencing of the amplified inter-contig fragments is being carried out. These will provide information to reduce the number of contigs in the genome assembly, which will allow a better comparison of the BQ1 genome with the K601 and BC genomes, in order to identify BQ1 specific genes that may be involved in PU biodegradation.

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The community dynamics of oligotrophic bacteria in a microcosm environment

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Microbial communities are known for their impressive ecological and evolutionary dynamics. Such activity is the resultof the interaction of a big number of biotic and abiotic elements. To analyze changes in these systems, researchers quantify modifications in their composing populations, nonetheless, in natural settings, neither biotic nor abiotic elements can be reliably controlled, so assumptions made to associate the observed dynamics to specific causes may not hold. Additionally, detailed long-term microbial community studies are still scarce.
A particularly intriguing ecosystem is the Churince pond in the Cuatrocienegas basin (Coahuila, Mexico), where microbial communities have thrived for millions of years although being exposed to demanding circumstances constantly. To analyze the community dynamics of these bacteria, we designed *in vitro* microenvironments (microcosms) for sediments from two sites having different intrinsic bacterial content. Triplicates per site and their combinations were made and then incubated for two years on constant temperature, photoperiod, and water level. During this time, multiple samples were extracted, increasing the sampling time continuously and mixing the content each time, then, bacterial diversity of the colony forming units on plates was measured, and characteristic colonies were identified by 16S rRNA gene sequencing. Finally, this diversity was compared to metagenomic results from the initial and final times.
Our results suggest that the microcosms successfully maintained a community of mixed autotrophs and heterotrophs. Differences in abundance among treatments were of orders of magnitude, and the diversity of the initial populations changed, with a large number of unregistered phenotypes being observed at final times. Communities seemed to reach an equilibrium as the perturbation times were prolonged. The composition and structure of all the microcosms (individual and mixed sites) diverged in time, to the point that the original abundance and diversity did not resemble the initial community. In conclusion, these sediment communities, even when maintained isolated and in a relatively constant abiotic environment showed no resilience to initial perturbations caused by mixing nor heat, but self-reshaped to have different populations abundances and diversity.

Analysis of Lsr2 proteins in *Streptomyces*

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The nucleoid-associated proteins (NAPs) are small, mostly basic proteins with DNA-binding ability. They play an important role in the structure of the bacterial nucleoid and contribute to chromosome compaction, which impacts DNA replication, recombination and gene regulation.

Lsr2 is a NAP of *Mycobacterium tuberculosis* is a basic, small protein (12.1 kDa) ., Lsr2 binds preferentially to AT-rich regions, and is involved in several cellular processes, including cell-wall lipid biosynthesis, and metabolic fuctions in *M. smegmatis*. Anull mutation lackingLsr2 in *M. smegmatis* exhibited altered colony morphology, and reduction biofilm formation. However, Lsr2 cannot be deleted from the *M. tuberculosis*chromosome suggesting that it is essential. In addition, Lsr2 shows limited sequence and structural similarity to H-NS (one of the major NAPs of *Escherichia coli*) but has analogous biochemical properties and is functionally interchangeable with H-NS in *E. coli.*

Lsr2 homologues are present in all sequence mycobacterial genomes, as well as in most of theActinobacteria,including members of the*Streptomyces*genus. Two copies of Lsr2-like proteins are present in the chromosome of *Streptomyces coelicolor*, encoded in genes*sco3375*and*sco4076*, but these proteins are not yet characterised. Orthologues of these genes are present in other *Streptomyces*species. Moreovera domain homologous to Lsr2 is located at the C-terminus of the M.SalImethyltransferase of *Streptomyces albus*, but not in other homologous DNAmethylases..

The aims of this work are to determine the functionality of genes *sco3375*and*sco4076*, and the characterization of proteins encoded by these genes, and domain Lsr2 of M.SalI. As a first step single knockouts of gene *sco4076* were obtained, indicating that this gene is not essential for *S. coelicolor*. It has not been possible to knock out gene *sco3375*, suggesting that this gene is likely to be essential. The Lsr2 proteins encoded by these genes will be expressed in*E. coli* BL21 (DE3) and purified for DNA-protein interaction experiments. In addition, a variant of the M.SalImethylase lacking the C-terminal Lsr2 domain has been constructed, and preliminary experiments indicate that it is importante for the full function of this methylase.

Bacterial diversity in the hemiparasitic oak/mistletoe association

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Keywords: diversity, plant-bacteria interactions.

Bacterial diversity is considered enormous and only a fraction is known. Bacteria is a group of microorganisms that thrive in all ecosystems like soils, bodies of water, subsurface earth and organisms. Beneficial plant-bacteria associations can help plants to obtain nutrients, can also protect plants from disease, and promote plant growth as well. Bacterial communities can inhabit all tissues in the plant and are involved in essential processes like carbon and nitrogen cycles. The host invasion of mistletoesis produced by a xylem-xylemconnection denominated austorium. In this association, the mistletoe obtains all the water and minerals from it shost, which in high degrees of infestation may cause death. Since a few decades the use of 16S rDNA gen has been an efficient tool to describe differences between bacterial microorganisms, not only for culture-dependent approaches, but also for culture-independent approaches. In this work, we utilized two different culture media to isolate culturable bacteria associated to both plants, oak and mistletoe, and a total DNA extraction from leaves of both plants was carried out for culturable-independent approach. The goal of this work was to know and compare bacterial communities associated with its two hosts. So far, we have sampled fourmistletoe-infested oak individuals. The isolated bacteria in the oak belong to the phylum Actinobacteria and Firmicutes, whereas the isolated ones from mistletoe are mainly Alphaproteobacteria.

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The production of novel tRNA “halves” isstimulatedby translation arrest due to deficiency of an amino acylable tRNA

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We have demonstrated recently that mRNAs translation in *Escherichia coli* generates two types of tRNA byproducts: peptidyl-tRNAs and tRNAs “halves” (f-tRNAs). Based on indirect evidence we speculate that both derivatives result from interruption of cell mRNA translation by sporadic limitation of aminoacylated tRNAs, the basic supplies for protein synthesis. To test this conjecture, we induced the expression of minigenes,a way to deplete the pool of specific amino acylable tRNAs. We used minigen variants harboring the 3’-end sequence AGA AGA UAA at different positions relative to the initiation codon AUG in the mini-ORFs, eitherlocated immediate to the initiator codon, or displaced to increasing distal positions. Upon induced expression the shorter minigenes accumulated pep-tRNA(Arg4) but not f-tRNA(Arg4), onthe contrary, longer ORF minigenes with AGA AGA UAA at 5-7 and farther positions accumulated f-tRNA(Arg4) but not pep-tRNA(Arg4). Previous evidence shows that short pep-tRNAs are released from ribosomes and their mRNA remains associated to the ribosomes for multiple rounds of translation but not of longer minigen mRNAs translation. In this last case, it is expected that tRNA(Arg4) remains attached to the AGA codon of the mRNA in the P site of the ribosome. We speculate that this tRNA is eventually processed in halves of RNAs. This inference is consistent with the result that the expression of long AGA AGA minigenes generates specific f-tRNAs for other amino acids. We suggest that the ribosomal pauses by Arg-tRNA(Arg4) limitation result in processing of stuck tRNAs in the P site. Other type of evidence that supports this proposal will be discussed.

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DEAD-box RNA helicases in *Bacillus subtilis* as a case study of evolution of duplicate genes

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The presence of duplicated genes in organisms is well documented, and there is increased interest in understanding genetic interactions among paralogous family members. Often, the different members are dispensable, so the question remains of why paralogous genes are retained. In the Firmicutes, the ancient and conserved DEAD-box RNA helicase (DBRH) gene family increased the number of its members through gene duplication events. *B. subtilis* possesses four DBRH genes, *cshA, cshB, deaD*, and *yfmL*. We explored the genetic interaction of the *B. subtilis*DEAD-box RNA helicases by analyzing the effect of manipulating gene expression and cross complementation on growth. Increased transcription of the DBRH genes in the different helicase mutant backgrounds was observed; however, this does entail compensated DBRH's function, as no cross-complementation was observed upon overexpression of paralogous genes, except for marginal complementation of YfmL in the *cshB* mutant background. Positive epistasis (amelioration of the defective growth) was only observed for *cshA, cshB,* and *yfmL*. We also observed that spore development was delayed in all DBHR mutants, and this was more severe for the*deaD* mutant, for which previously no phenotype had been reported. Chimeras from the smalles helicase YfmL and the carboxy terminal negatively affected growth, but only on mutant backgrounds, suggesting a specific negative interference. Our findings suggest that the long time divergence between DEAD-box RNA helicase genes has resulted in specialized activities in RNA metabolism and that cannot replace one another so that their pleiotropic effect is explained by the interconnected RNA functions they perform.

Immunoinformatics approach to determine the immunogenic role of enolase from *Haemophilus influenza,* as a vaccine candidate.

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Introduction: *H. influenzae* is a Gram-negative, coccobacillary bacterium. This bacterium is a major cause of invasive diseases as meningitis, sepsis and mucosal infections such as acute otitis media, sinusitis, conjunctivitis, bronchitis, community acquired pneumonia and exacerbations in chronic obstructive pulmonary disease in adults (COPD). *H. influenzae* mainly affects children under 5 years of age and adults over 65 years and immunocompromised hosts.

Objective: To determine the immunogenic role of enolase from *H. influenzae* through bioinformatics and immunological experimental assays.

Materials and Methods: Bioinformatics analyses of enolase were performed to determine their possible immunogenic role. Oligonucleotides were designed to obtain the gene encoding to the protein. *eno* gene was obtained by PCR reaction, and the product obtained was cloned into plasmid pCR® 2.1-TOPO® TA cloning and sent to sequencing. Subsequently, the gene was subcloned into the plasmid pRSET-A, obtaining pRSETA::HiEno; this recombinant plasmid was transformed into *E. coli* Bl21 pLysS cells. Purification of the recombinant protein was performed by nickel affinity chromatography, and immunization protocol was done to obtain polyclonal antibodies against rHiEno; the antibodies generated were used to identify rHiEno and wild enolase in total extracts of *H. influenzae* by immunoassays.

Results: By BepiPred 1.2 and NetCTL 1.0 programs were predicted immunogenic epitopes that activate B and cytotoxic T lymphocytes, and a putative transmembranal region was identified. The purified rHiEno was obtained with a molecular weight of approximately 52 kDa. The polyclonal antibodies obtained were capable to recognized rHiENO and the wild protein in Western blot assays.

Conclusions: rHiEno is capable of trigger an immune response. These results indicate that rHiEno could be studied as a possible candidate for the development of a vaccine against *H. influenzae*.

Co-infections Between Microorganisms That Cause Cervicovaginal Pathologies.

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Cervicovaginal infections (CI) can be acquired by endogen (EI), iatrogenic (IT) and sexual transmitted infection (STI). Bacteria that can cause this kind of infections are *Chlamydia trachomatis*, *Mycoplasma hominis*, *M. genitalium*, *Neisseria gonorrhoeae*, *Ureaplasma urealyticum,* and *Gardnerella vaginalis*. It has been described that the presence of human papillomavirus (HPV) is associated whit bacterial infections (Gillet et al., 2011), in Mexico, there is not reports about this topic.

In this work, 112 cervicovaginal exudates were analyzed to identify and stablished the frequency of bacteria and co-infections whit HPV that can cause CI. The methodologies employed were traditional and molecular biology techniques. Traditional were: Amsel´s criterion, Nugent´s criterion, *G. vaginalis* and *N. gonorrhoeae* culture, fresh examination, chromogenic medium culture, gallery Genital AF System for *M. hominis*, *and U. urealyticum* and Papanicolau staining. While molecular ones were: reverse hybridization by the INNOLiPAR system for HPV detection and single and multiple PCR for *C. trachomatis*, *M. genitalium*, *N. gonorrhoeae*, *M. hominis* and *U. urealyticum*.

Human papillomavirus was founded in 88.4%, bacterial vaginosis 26.8%, *C. trachomatis* 10.7%, *M. hominis* 9.8%, *M. genitalium* 3%, *U. urealyticum* 3.6% and *N. gonorrhoeae* 0.9%. The most frequent co-infection was between HPV and bacterial vaginosis (28.3). In this study, no statistically significant relationship was founds between HPV co-infection with STI and EI causing by bacteria.

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The *repABC* plasmids replicate with a theta mechanism

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The *repABC* plasmids take their name because all the necessary elements for replication, segregation and regulation are encoded in a single operon: the *repABC* operon. RepA and RepB are the segregation responsible proteins and they regulate negatively the transcription of their own operon. RepC is the replication initiator protein and within its sequence the origin of replication is located. *RepABC* plasmids also possess a small antisense (ctRNA), that regulates RepC levels postranscriptionaly. Through regulation of the RepC protein, each cell maintains a constant plasmid copy number, in this case of one copy per cell.

The replication initiator proteins, like RepC, recognize the origin of replication, destabilize double-stranded DNA, and recruit the replisome, which is the machinery directly involved in DNA replication.

Replicators *repABC*, are found mainly in high molecular weight plasmids and in some secondary chromosomes of alpha-protobacteria such as Rhizobiaceas

To identify the direction and type of replication carried out by the *repABC* plasmids, a suicide vector pSEC-1 derivative from pSRK-Gm was constructed, where the repC gene from p42d of *Rhizobium etli* CFN42 strain was cloned under an inducible promoter. The plasmid pSEC-1 was able to over-replicate in *Rhizobium etli*, when RepC was induced.

The replication intermediates of the pSEC-1 plasmid were purified and analyzed by two-dimensional agarose gel electrophoresis. It was determined that these *repABC* plasmids replicate by a bidirectional and asymmetric theta mechanism, in which the replisome extends preferentially the leading strand in the direction of the transcription of *repC*.

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Tolerance of *Prosopis laevigata* plants and rhizobia symbionts to heavy metals exposure.

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SUMMARY: *Prosopis laevigata* is a native flora of Central America and Mexico, being able to tolerate drought, alkaline pH, elevated temperatures, high concentrations of salt, and heavy metals exposure (Felker *et al*., 1983). Essential trace elements play biochemical and physiological functions in plants and animals, taking part in redox reactions, electron transfer and structural functions in nucleic acid metabolism. Some of the heavy metal are strongly toxic to metal-sensitive enzymes involved in the metabolism, detoxing and repair of cell damage (Wang and Shi, 2001), resulting in growth inhibition and death of organisms (Wang and Shi, 2001; Chang *et al*., 1996; Wang and Shi, 2001; Beyersmann and Hartwig 2008). Heavy metals are significant environmental pollutants, and their toxicity is a problem of increasing significance for ecological, nutritional and environmental reasons.*Prosopis laevigata*nodules were collectedin Chietla, Puebla, Mexico, obtaining89 isolates directly from nodules. 15 isolates were tolerant to 2000 ppm of Zn, some isolates (12) were capable to tolerate 2000 ppm of Fe (III), and some other isolates (9) were tolerant to 10,000 ppm of Cr(VI). Analysis by Wavelength dispersive X-ray fluorescence (WDXRF) shows that *P. laevigata* is capable to accumulate in tissues high concentrations of elements such as Cu, Al, Cl, Ti, Zn and Fe. We found 765 μg Cu/g (765 ppm) in pod and 729 μg Cu/g (729 ppm) in root. Al concentrations were found in pod ($1.4X10^4$ ppm, 1.4%), leave ($4.3X10^3$ ppm, 0.43%) and stem ($9X10^3$ ppm, 0.9%). High Cl levels were found in pod($1.07x10^4$ ppm, 1.07%), leave ($1.14\ x10^5$ppm, 11.4%) stem ($7.8\ x10^3$ ppm, 0.78 %) and root ($1.24x10^4$ppm, 1.24%). Amounts of Br were also found in pod (460 ppm) and leave (1400 ppm). Trace amounts of Ti were found in pod (1600 ppm, 0.16%), and Zn was found in pod (2500 ppm), leave (928 ppm) and root (638 ppm). High levels of Fe were also found in pod ($1.4X10^4$ ppm, 1.4%), leave ($4.3X10^3$ ppm, 0.43%), stem ($3.2X10^3$ ppm, 0.32%) and root ($9X10^3$ ppm, 0.9%). WDXRF results showed that *Prosopis* trees are able to tolerate and accumulate heavy metals such Cu, Al, Cl, Ti, Zn and Fe; demonstrating that symbiotic interaction*Prosopis laevigate*-rhizobia could be a good alternative for the phytoremediation of contaminated areas with heavy metals and other pollutants.

Keywords: *Prosopis laevigata,* Heavy metals, Symbiotic association, Rhizobia.

RAMbio mixer as a high oxygen supply shake flask system alternative: culture of *Azotobacter vinelandii*

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Azotobacter vinelandii is a soil nitrogen fixing bacteria that produces two kinds of polymers with a wide variety of applications:alginate and PHB. This bacterium has shown that its growth, alginate and PHB production change depending on the oxygen availability when growing in submerged liquid cultures [1-3].

For years, the orbital agitation has been the mixing option for cultures growing in flasks.However, for *A. vinelandii* cultures orbitally shaken flasks usuallyoperateunder oxygen limited conditions [3-4]. As an alternative, new mixing technologies are an interesting solution. Recently, the resonant acoustic mixer (RAMbio) exhibited an enhanced performance to supply oxygen for shaken flasks cultures of *E. coli* BL21 DE3 gold when compared with the orbital mixed[5].

As previously reported, the RAMbio system generates a high intensity axial mixing pattern of the culture inside the flasks, along with small bubbles and droplet formation [5]. Consequently the acoustic mixing couldsustain a high oxygen supply to the liquid broth.However, it is also necessary to consider its effect over the rheological features of the cultures due to the exopolysaccharides accumulation.

In the present work, we aimed to evaluate the *A. vinelandii* culture performance under resonance acoustic mixing at different shaking frequencies. We found differences of the growth, sucrose consumption, PHB and alginates production, as well as differences DOT profiles between these cultures and the orbital shaken flasks cultures previously reported. For example, a non-oxygen limited culture in a shaken flask was observed in the cultures growing at the highest shaking frequency (20 g) which was accompanied with a high alginate accumulation (~9g/L). Furthermore, rheological features of the culture broth are being analyzed.

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Growth of halophilic and psychrophilic bacteria in environmental conditions similar to the Europa's ocean

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Many organisms on Earth have evolved and developedstrategies to cope with the extreme conditionsthey find in their environment. The discovery of the extremophiles, as they are identified, has made more plausible the understanding of the possibility that life can succeed outside Earth. We are interested in the adaptation strategies displayed by halophilic bacteria when exposed to laboratory-controlled conditions that represents the salinity, temperature, and available oxygen conditions of the ocean of Europa, one of Jupiter's satellites.

The adaptation strategies displayedby *Bacillus pumilus* (a non-halophilic bacterium),*Cobetia marina* (a moderate halophile), and *Psycrobacter cryohalolentis*(a psychrohalophile) when exposed to different sodium chloride (NaCl) and magnesium sulfate ($MgSO_4$) concentrationsthat simulate the salt content of the liquid water ocean of Europa were studied. Betaine, a compatible solute reported to be present in halophilic bacteria, was added to the modified bacterial media in order to determine its incorporation mechanism, and its role as osmoprotectant. The effect of temperature on bacterial growth was studied at30 °C (optimal growth temperature), 20 °C, and 10 °C.

With the set of results obtained, the possibilities of adequacy of the bacteria under study for the conditions prevailing in the salty ocean of the Europa satellite will be determined.

"Resistance to quinolones in commensal and enteropathogenic *Escherichia coli* in a human-animal health interface model"

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Objective. To identify and compare the genetic elements associated with quinolone resistance in commensal and enteropathogenic *E. coli* in a one health (humans-swines) interface model.

Methodology. Isolates of swines (piglets, sows and stallions) were obtained from a farm located in the municipality of Jiutepec, Mor.; also human isolates corresponding to farm workers and their home contacts, and patients with diarrhea to attend at health centers near the site of the farm. From the populations mentioned (humans and swines) a representative sample of *E. coli* was selected that presented some of the different combinations of resistance and susceptibility to nalidixic acid (NA) and ciprofloxacin(CIP). In these isolates the genes, *qnrA, qnrS, qnrB* and *qepA1* were amplified by PCR. A phylogenetic tree was constructed with *gyrA* sequence.

Results. To date, a total of 86 *gyrA* sequences are analyzed. According to the phylogenetic tree,two main heterogeneity clades were identified in the groups. The most of the isolates did not show changes in the QRDR region of the *gyrA* gene (Humans 42%, Swines 51%). The detected mutations were S83L (Humans 32%, Swines 23%), S83A (Humans 3%), D87Y (Humans 3%, Swines 6%) and S83L/D87N (20% both populations). Isolates without mutations in *gyrA*, mostly correspond to *E. coli* commensals (Humans 29%, Swines 27%). When the variability of the QRDR region with respect to the resistance phenotype is analyzed, it is observed that the S83L change occurs more frequently in the NA^rCIP^s phenotype (Humans 29%, Swines 18%), whereas the S83L/D87N mutation was exclusive of the NA^rCIP^r phenotype in humans. When evaluating RQMP, a higher prevalence of qnr's in commensal *E. coli* was observed (Humans 34%, Swine 35%) with the NA^r/CIP^s phenotype. The prevalent gene in both populations corresponded to *qnrB* (Humans 39%, Swines 27%). With respect to the *qepA1* gene, only two isolates (2%) were positive.

Conclusions. According to the phylogeny study the humans and swines*E. coli* isolates are heterogeneous. The changes identify in the QRDR region of the *gyrA* gene corresponded to those already reported in the literature, except for S83A. It is interesting the fact that the higher frequency of qnr's was observed in commensal *E. coli*, in both populations, constituting a reservoir of resistance for other microorganisms. In addition, the similarity of results found in humans and swinessuggests that resistance to quinolones is present in genetic elements that are shared in both populations.

Phenotypic characterization of non-toxigenic *Clostridium difficile* isolated from patients in Mexico.

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*Clostridium difficil*e is a Gram positive, anaerobic bacteria that sporulated to survive in aerobic conditions. This bacteria has been reported as the first cause of nosocomial diarrhea, ranging from 10% to 35% of the diarrhea cases associated to the excessive use of antibiotics. C. difficile was isolated from human feces in an asymptomatic patient and then considered part of the intestinal microbiota, until 1970 when it was recognized as the causal agent of pseudomembranous colitis. Recently, the incidence and severity of C. difficile infection (CDI) are increasing as a result of the emergence of new hypertoxigenic strains, which have new virulence factors.

There are two principal virulence factor needed for CDI, the enterotoxin TcdA, and the cytotoxin TcdB. Both toxins are located in the pathogenic island PaLoc (Pathogenesis Locus). TcdA and TcdB target the Rho/Ras GTPases thorugh a plicolutarion ar Thr35 and Th37 residues, inactivating them, as result occurs a dysregulation of actin, cell damage, and also a caspase activation that cause apoptosis.

However there are some strains that are unable to produce this toxins, and are denominated Non-toxigenic *Clostridium difficile* (NTCD).The evolutionary history of NTCD has not been cleared, and the relation of these strains with illness remains in doubt. So, the aim of this work is to analyze the phenotype of NTCD strains isolated from clinical cases from a hospital in Mexico, and analyze whether the heterogenicity of these strains, may be useful to differentiate NTCD strains from the toxigenic strains.

Prospection of hydrocarbonoclastic marine bacteria toward Polycyclic Aromatic Hydrocarbons present on the Baja California Coast

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Polycyclic aromatic hydrocarbons (PAH) are very toxic compounds that persist in the environment and could cause severe damage in organismsleading to ecological implications.At least, 16 PAHhave been catalogued as high-priority pollutants by the Environmental Protection Agency (EPA). Coastal areas are prone to contain elevated PAH concentration as a result of several anthropogenic activities, suchas sewagedischarge, marine traffic of ships, andfuel loading and unloading. Significant levels of PAH have been found
in marine sediments of the Rosarito Port, Baja California, Mexico. The characterization of specific metabolic pathways and mechanisms that microorganisms, such as marine bacteria, display toward PAH biodegradation, has given relevant knowledge to design efficient, viable and “green” strategies to eliminate PAH of marine environment.
Therefore, the main aim of this work is to isolate marine bacteria from Rosarito Port, Baja California, Mexico, and explore their potentialhydrocarbonoclastic activity toward different PAH. So far, 50 bacterial strains have been isolated frommarine sedimentsand surface seawater by using the enrichment method in Bushnell Haas broth supplemented with pyrene [25 mg/L], dilution and spread on plate. Different colonial and color morphologies have been found. One of the bacterial isolates named MSA14, was selected for being positive to indole test andpositive growth in Bushnell Haas broth supplemented with pyrene, as its only source of carbon and energy. Depending on the pyrene concentrationtested, MSA14 shows turbidity in the broth in a period of 7 to 14 days of incubation. In addition, preliminardata suggest that MSA14 could produce biosurfactants.
These results suggest that in the coastal area of Rosarito Port there are bacteria with high potential to degrade PAH and some of them could bear the ability to produce biosurfactants. This is the first data derived from a research project which pretends to contribute to the characterization of the marine bacteria present in this coastal area and their potential in minimizing the PAH presence in water bodies and effluents impacted by human activities.

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 kinasesenses 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 and inactivating its kinase activity. The implications of our findings on the spatiotemporal regulation of the barA kinase activity will be discussed.

Characterization of the biodegradative activity of bacterial consortia able to grow in polyurethane varnish as the sole carbon source

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Polyurethanes (PUs) are synthetic polymers widely used due to their low degradability and high versatility of forms and textures. Because of their diverse chemical composition, recycling is complex and toxic, generating accumulation in landfills. Microbial degradation of PUs by individual bacterial and fungal species has been extensively analyzed. However, there are few reports on microbial consortia able to degrade and assimilate PUs as carbon source, even though they seem to be more efficient than axenic cultures to degrade some xenobiotics. The aim of this work was to explore the ability of bacterial consortia to grow in polyester-PU varnishes (Bayhydrol A 2470 (Bhy) or NeoRez R-9637 (Neo)), as the sole carbon source.

Three bacterial consortia, BP1, BP3 and BP7, were obtained by culture enrichment in mineral medium (MM) with a PU varnish as the sole carbon source, inoculated with PU foams collected in a dumpsite. Growth, changes in the FTIR spectroscopy patterns of PUs after incubation with consortia, extracellular enzymatic activities related to PU biodegradation (esterase, protease, urease), and morphology of some of the isolated colonies were analyzed in cultures grown in MM-Bhy or MM-Neo. Identification of bacterial diversity was carried out by sequencing the V3 region of the 16S rDNA gene from the different isolated colonies.

The three consortia showed more growth in MM-Neo than in MM-Bhy. After 21 days of culture in Bhy, consortium BP1 decreased the signals of alcohol, amine, ether, and ester groups. In Neo, consortium BP7 generated the major decreases in signals of amide, urea, carbonyl, ester, and ether groups. In accordance, consortia BP1 and BP7 exhibited extracellular esterase, urease, and protease activities, which showed a tendency to increase over 21 days of cultivation. Isolated members from BP1, BP3, and BP7 showed greater morphological diversity in MM-Neo than in MM-Bhy. The growth of isolated members was lower than the growth of whole consortia, an effect more pronounced in consortium BP7. Consortium BP1 consisted of four members identified as *Acinetobacter baumannii* whereas the twelve members from BP7 belonged to the species *Acinetobacter* sp., *Acinetobacter baumannii, Paracoccus* sp., *Hydrogenophaga* sp., and *Microbacterium* sp.

Our results indicate that bacterial consortia BP1, BP3, and BP7 are able to degrade both Bayhydrol and NeoRez to assimilate the compounds derived, as the sole carbon source, and to sustain their growth. Thus, the three consortia could be useful to develop technological processes for PS-PUs biodegradation.

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Green synthesis of silver nanoparticles using a *Bacillus subtilis* strain with overexpressed nitrate reductase gene.

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Biosynthesis of nanoparticles is the major division in the field of applicable Nanoscience and nanotechnology. Nanoparticles are playing an important role in biomedical and various applications. Synthesisof silver nanoparticles using microorganism have been reported, but the hypothesis about the formation of nanoparticles still remain unexplained. There is a couple of works where *Bacillus subtilis* has been able to produce nanoparticles. Nevertheless it has been suggested that nitrate reductase is a key component of the silver nanoparticle formation although this process israther slow. We decided to take all the information available and developed a strain of *B. subtilis* that was transformed with an extra chromosomal copy of the Nitrate Reductase gene for the synthesis of silver nanoparticles.

Synthesis of silver nanoparticles was rapid and stable analyzed by UV-visspectrophotometer. Most of the particles were spherical in shape and size ranges from 65-70 nm analyzed using ScanningElectron Microscope (SEM). Finally the nature of the nanoparticles was identified by Elemental analysis (EDX). This method is extremely rapid, requires no toxic chemicals and the nanoparticlesare stable for several weeks. The main conclusion is that the bio-reduction methodto produce nanoparticles is a good alternative to other non-green chemistry methods.

Modulating the Phenotype and the Membrane Properties of Bacterial Cells by Combinatorial Engineering of Ornitine Lipids

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Phospholipids such as phosphatidylethanolamine (PE), phosphatidylglycerol (PG), cardiolipin (CL), and phosphatidylcholine (PC) are considered the basic building blocks of bacterial membranes. Many bacteria can also form other membrane lipids like for example hopanoids, sphingolipids, diacylglycerol *N,N,N*-trimethylhomoserine (DGTS), sulpholipids, glycolipids or ornithine lipids (OLs). The capacity to form OLs is widespread in eubacteria, but OLs have not been found in eukaryotes or archaea. They are structurally composed of a 3-hydroxy fatty acid that is bound to the alpha-amino group of ornithine by an amide bond. A second fatty acid is ester-bound to the 3-hydroxy group of the primary fatty acid. Several modifications of OLs such as hydroxylations in different positions and an *N*-methylation have been described in recent years. One hypothesis is that by modification of OLs the bacteria can change their properties membranes without de novo lipid synthesis, which would allow for a quicker response to changing and adverse conditions. These modifications might also change the immunogenic properties of the membranes, play an important role in protecting the bacteria against abiotic stress conditions and/or during the interactions with eukaryotic hosts. We constructed *Escherichia coli*, *Agrobacterium tumefaciens* and *Rhizobium tropici* strains synthesizing different types of OLs. Currently we are characterizing and comparing these strains with respect to biophysical properties, abiotic stress resistance and interactions with eukaryotic hosts to see how the presence of different OLs affects their membrane properties and the resulting macroscopic phenotypes.

*Pseudomonas stutzeri*A1501 andPQQ. Mechanism for plant growth promotion?

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Pirroloquinolinequinone (PQQ) is a compound synthetized in stationary phase of bacterial growth, which could act as a cofactor. This compound can be produced by plant growth promoting rhizobacteria (PGPR) and has been involved with different phenotypes like phosphate solubilizing and biocontrol. *Pseudomonas stutzeri* A1501 is a PGPR which can associate with rice roots, promoting its growth, nevertheless the mechanisms involved in this process are unknown. In order to get insights into the mechanisms of PQQ to promote rice growth by *Pseudomonas stutzeri* A1501, in this work,a mutant with a transcriptional fusion was constructed in the *pqq*A gene (which codes for precursor for the biosynthesis of PQQ) and examined its capacity of biofilm formation, chemotaxis, phosphate solubilizing and plant growth promotion using different carbon sources. Chemotaxis of *pqq*A fusion in presence of glucose, sucrose, succinate, citrate and methanol, is lower than WT and the complemented strain, opposite case in malate and ethanol. In biofilm formation, when grown in presence of glucose, succinate and methanol, the complemented strain shows more activity than WT and *pqq*A fusion. However, under sucrose, malate, citrate and ethanol conditions, the activity of *pqq*A fusion is lower than WT and the complemented strain, opposite case in malate and ethanol. In phosphate solubilizing tests, the solubilization is almost null in all cases, suggesting that PQQ might not participate on this process. In plant growth promotion assays, *pqq*A fusion shows a decrease in colonization as well as roots and aerial part length, in contrast to what is observed with WT and the complemented strain. The β-glucoronidase activity tests confirm our findings since this activity is null in the mutant, but not in the complemented strain, restoring this activity in this strain when exogenous PQQ is added to the medium. This indicates that PQQ could be regulating *pqqA* transcription. These results show that PQQ produced by *Pseudomonas stutzeri* A1501, is involved in rice growth promotion. The mechanisms are related with the colonization of the roots by biofilm formation and polysaccharide production, which result in an increase of roots and aerial part length in rice plantlets.

Isolation of Bacterial Strains from Soils Affected by Salinity and Analysis of their Potential use as Biofertlizers in Common Bean and Maize

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Maize and common bean represent an important part of the protein source in the Mexican population diet. The production of common bean (*Phaseolus vulgaris*) is severely affected by drought: more that 60% of the world production comes from arid zones with poor water availability. As an example, it has been calculated that only during the year 2012, drought was the cause for a drop of about 60% in the production of common bean in México, which represent around 800 milliondollars in lost.The application of Rhizobiaceaebacteria as biofertilizers can improve the performance of *P. vulgaris* in regards to grain production, biomass, N content, and a moderate better response to abiotic (including drought and salinity) and biotic factors, among other benefits.Several strategies have been successfully used to improve rhizobium-induced plant tolerance to drought: rhizobium overexpressing the trehalose-6-phosphate synthase gene in order to increase the trehalose content (1), enhanced expression of cbb3 oxidase to increase respiratory capacity(2), and use of validamycin as a trehalase inhibitor(3). Unfortunately, because of regulatory and economic facts, the use of these strategies on field is not viable at the moment. Thus, it is necessary the hunting for novel autochthonous rhizobium strains adapted to extreme climate conditions for its potential use as biofertlizers. Here, we show the isolation of wild-type rhizobium strains from arid and saline areas from the central México, an analysis of their ability to grow under stressing conditions, as well as its molecular classification and N fixation capacity in symbiosis. Moreover, preliminary results showing the effect of these strains on the ability of *P. vulgaris*and *Zea mays*to tolerate salinity and drought will be presented.

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