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MI-P33

DETECTION OF ACYLHOMOSERINE LACTONES IN CULTURES OF *Gluconacetobacter diazotrophicus* PAL5

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Gluconacetobacter diazotrophicus is an acid-tolerant nitrogen-fixing Alphaproteobacterium first found in association with sugarcane. It has also been isolated from rice, coffee and tea, among others crops. The recent sequencing of the *G. diazotrophicus* PAL5 genome shows the presence of one *luxI* homolog. These genes encode LuxI-type enzymes responsible for the synthesis of *N*-acylhomoserine lactones (AHLs), the main quorum sensing molecules in gram negative bacteria. The objective of this work was the detection and identification of AHLs produced by *G. diazotrophicus* PAL5. The strain was cultured aerobically, and extracts were prepared with acidified ethyl acetate. Samples were analyzed by thin layer chromatography developed with the biosensor *Agrobacterium tumefaciens* NTL4 (pCF218) (pCF372). Results show that *G. diazotrophicus* PAL5 produce at least two types of AHLs under the assayed conditions. Short-chain AHLs could be detected since early exponential growth phase, and medium-chain AHLs were detected in mid- and late-exponential growth phase. The results suggest that the *luxI* homolog in *G. diazotrophicus* PAL5 is expressed and quorum sensing molecules are produced and secreted. Similar to other bacteria, production of AHLs in *G. diazotrophicus* PAL5 could serves as a signaling mechanism among members of this genus or as inter kingdom signals.

MI-P34

SYNTHESIS OF QUORUM SENSING SIGNALS BY DIAZOTROPHS FROM THE RHIZOSPHERE OF STRAWBERRY PLANTS

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In the rhizosphere, interactions occur with both pathogenic and beneficial microorganisms. The latter group can stimulate plant growth by mean of phytohormone production, direct antagonism of pathogens or induction of systemic resistance in the plant. Quorum sensing (QS) systems from non symbiotic diazotrophic bacteria may play a role in the interactions with strawberry plants and with other organisms. The objective of this work was the characterization of *N*-acylhomoserine lactones (*N*-AHLs) produced by free living nitrogen fixing bacteria associated to roots of *Fragaria ananassa* Duch. cv. Camarosa. Strains were isolated and purified in nitrogen free semisolid media. Extracts were prepared with acidified ethyl acetate and samples were analyzed by TLC. *Chromobacterium violaceum* Vir07, *Agrobacterium tumefaciens* NTL4 (pCF218 pCF372) and *Pseudomonas putida* F117 (pKR-C12) were utilized as biosensor strains for the detection of QS molecules. Non-symbiotic diazotrophic bacteria producing QS molecules could be isolated from roots of strawberry plants. Production of short chain N-AHLs was detected in all strains. Only one strain produced long chain N-AHLs. Although biosynthesis of QS molecules is not widely distributed in diazotrophic strains from strawberry roots, secretion of short chain QS molecules seem to play a key role as signaling molecules.

MI-P35

PROTEOMIC ANALYSIS OF *Amycolaptosis tucumanensis* IN RESPONSE TO CHROMIUMN AND COPPER STRESS

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Heavy metal pollution is one the most serious environmental problems. Some heavy metals play an important biological role as trace elements but are toxic at higher concentrations. *Amycolatopsis tucumanensis* was isolated from contaminated sediments in Argentina. Its ability to remove Cu(II) and Cr(VI) has been reported.
The aim of this work was to analize the differential protein espression of *A. tucumanensis* under Cu(II) or/and Cr(VI) stress by two-dimensional polyacrylamide gel elctrophoresis.
Cells were incubated in minimal medium containing glucose and 10 ppm of Cu(II) or/and Cr(VI) during 72 h at 30ºC and 200 rpm. Cells grown in absence of heavy metals were used as control. The cells were chilled in liquid N2 and broken by physical technique. The supernatants of the cell lysates was used as protein sample. The proteome profiles were different, 18 spots were selected for the identification by mass spectrometry, and these included: dehydrogenase/reductase, alkyldroperoxidase Aph D, probable monoxigenase, RNA polimerase, cicloisomerase, some elongation factors, transcripcional regulator and several proteins with unknown function.
Cu(II) or Cr(VI) resistance mechanisms are not known clearly, for this reason is very important to study the functional analysis of these identified proteins and will allow to explain the tolerance of heavy metals by this bacterium.

MI-P36

DIFFERENTIAL REGULATION OF THE GENES INVOLVED IN RIBOFLAVIN BIOSYNTHESIS IN *Brucella abortus*

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Brucellosis is a worldwide zoonosis caused by closely related intracellular *Brucella spp* that affects livestock and humans. These species conserve an atypical riboflavin pathway, that presents the *ribH1* and *ribH2* genes, which encode enzymes with 6,7-dimethyl-8-ribityllumazine synthase activity, that synthesizes a riboflavin precursor. In *B. abortus*, *ribH2* is encoded in the chromosome II, contains an RFN riboswitch element in its 5' region, is expressed during the intracellular phase and is required for the virulence in mice; while *ribH1* is encoded in chromosome I and is dispensable for intracellular survival. These data suggest differences in the regulatory pathways for the *ribH* genes. In this work, we demonstrate that *ribH2* is specifically repressed by riboflavin and flavin mononucleotide. On the other hand, *ribH1* is transcribed polycistronically together with the riboflavin biosynthesis genes *ribD* and *ribE*, and the putative regulators *nrdR* and *nusB* genes. Overexpression of *nusB* produces a significative increase in the expression of *ribH1*, but not of *ribH2*. These results indicate the existence of two independent regulatory mechanisms for the *ribH* genes, probably acting at different stages of *B. abortus* life cycle.