XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

2 al 4 de agosto de 2017

San Miguel de Tucumán | ARGENTINA

SAMIGE

Asociación Civil de Microbiología General

MM-022

CONSTRUCTION AND STUDY OF *Pseudomonas putida* KT2440 MUTANTS, WITH REDUCED SALT TOLERANCE

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The aim of this work is to delve into stress salt tolerant mechanisms of *Pseudomonas putida* KT2440, which is a well known PGPR (Plant Growth Promoting Rhizobacteria). In order to evaluate this, transposon mutagenesis with mini-Tn5 (Km) was performed by triparental mating. The mutants obtained were screened for their reduced tolerance to salinity in solid media. After evaluating 1500 transposon mutants, only four showed less growth under saline conditions in solid media, in the preliminary screening: Mut10, Mut11, Mut50 and Mut59. Growth curves in different liquid culture media and spots assay, with and without saline conditions, were carried out. The curves showed that the mutants Mut11 and Mut59 grew markedly less with respect to the wild type, in saline conditions. Given these results, transposon insertion sites were determined by arbitrary PCR, followed by sequencing. The obtained sequences were analyzed and compared with genome databases. After analyzing the sequences for the Mut11, it was determined that the transposon was inserted into the gene PP_0024, coding for a membrane-associated metal-dependent hydrolase, involved in the synthesis of lipopolysaccharides. For the Mut59, the transposon was inserted into the gene PP_0003, which encodes a 16S RNA methyltransferase, whereby its growth is affected not only in saline conditions. For a better understanding of the importance of the correct synthesis of lipopolysaccharides in the stress salt tolerance, we studied the behavior of mutant bacteria (mus-40). This mutant is affected in *galU*, encoding UDP-glucose pyrophosphorylase and presents deficiencies in the synthesis of intact lipopolysaccharide. Growth curves and spots assay, in different media and in saline and non saline conditions, were carried out. As expected both mutants, Mut11 and mus-40 showed reduce stress salt tolerance, compared to the wild type. Also Congo red binding assay in saline and non saline conditions was performed, and the results showed rough and less red colonies for Mut11 and darker colonies for mus-40, compared to the wild type. Congo red is a dye with cellulose fibers affinity, therefore the more links of this type there are, the more red the colony will be. To evaluate EPS, calcofluor assay with and without saline stress was carried out, then calcofluor stainable EPS were visualized under UV light; mus-40 colonies were more fluorescent and Mut11 less fluorescent compared with the wild type colonies; the more fluorescence is an indicative for more EPS presence. Finally LPS extraction with phenol-chloroform technique, running in SDS-PAGE and visualized after silver stain; was performed. In the LPS profile the incomplete lipopolysaccharide structure can be observed, especially under saline conditions. The results presented in this work give an idea of the great importance of the study of polysaccharides to improve bacteria tolerance to saline stress.

MM-023

BIOINFORMATIC CHARACTERIZATION OF GENES ENCODING XYLAN DEGRADING ENZYMES IN THE *Paenibacillus* sp. AR247 AND *Cohnella* sp. AR92 GENOMES.

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The increasing interest to renewable lignocellulosic materials for the production of environment-friendly chemicals and biofuels boosts the search of new carbohydrate-active enzymes (Cazymes) and microbial strains. In this study, we analyze the draft genomes of two highly hemicellulolytic bacteria isolated from industrial liquor samples from the local paper industry to unravel their xylan degrading pathways. Gene annotations were carried out using Rapid Annotations Subsystems Technology (RAST) 2.0. BlastP was employed to find orthologous genes between the translated proteins from the predicted open reading frames of both genomes and a local database consisting on the translated proteins of the141 genomes of the *Paenibacillaceae* family members available on NCBI database up to date. Only bidirectional matches were considered. To identify potential Cazymes, translated proteins were submitted to the dbCAN database. The genome sizes were 7.1 Mb (*Paenibacillus* sp. AR247) and 6.0 Mb (*Cohnella* sp. AR92), which contained 7159 and 5439 coding sequences, respectively. 51 orthologous genes were found by the BlastP analysis, most of which corresponded to ribosomal genes. The phylogenetic tree built on the basis of those concatenated gene sequences showed *Cohnella* genus (including the strain AR92) as a monophyletic group within the paraphyletic group of *Paenibacillus* spp., while the strain AR247 was found to be related to *Paenibacillus* sp. P1XP2, *Paenibacillus* piniJCM 16418, *Paenibacillus* sp. IHBB 10380, forming a well supported clade. Both genomes displayed multiple genes encoding a broad variety of extracellular and cell-wall (SLH domians) of endo-b-1,4-xylanases (GHs 10; 11; 30 and 43), some of which also showed CBM domains (mainly CBM9; 22; 6). Sequences encoding potential intracellular exo-oligoxylanase (GH8) and b-xylosidases (GHs 39; 43; 51and 52) were identified, which might be responsible for processing the products released by the extracellular enzymes. Finally, the overall assimilation could beperformed by intracellular debranching enzymes a-glucuronidase (GH67), a-arabinofuranosidase (GHs 43 and 51) and acetylxylanesterase (mainly CEs 1 and 4). The redundancy of GH genes observed in the analyzed genomes, the predicted enzyme architectures and their cellular localization are in agreement with other well descripted *Paenibacillus* species. Therefore, the strains AR247 and AR92 might display similar strategies for the degradation of xylan.