XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

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San Miguel de Tucumán | ARGENTINA

SAMIGE

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BB-010

MICROARRAY DATA TO ELUCIDATE HYDROCARBONS DEGRADATION CAPACITIES OF *Amycolatopsis tucumanensis* DSM 45259

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The analysis of catabolic capacities of microorganisms is currently often achieved by cultivation approach and by the analysis of genomic or metagenomics datasets. Recently, a microarray system designed from curated key aromatic catabolic gene families and key alkane degradation genes was designed. The collection of genes in the microarray can be exploited to indicate whether a given microbe or microbial community is likely to be functionally connected with certain degradative phenotypes, without previous knowledge of genome data. Herein, this microarray was applied to capture new insights into the catabolic capacities of polycyclic aromatic hydrocarbon (PAH) degrading actinomycete *Amycolatopsis tucumanensis* DSM 45259. *Aromadeg* was used as data base for reconstruction of the catabolic pathway. Furthermore, to validate the predictions, removal of hydrocarbons was performed in minimal medium (MM) supplemented with 500 mgL-1 of each compound. Target analysis by Liquid Chromatography-Mass Spectrometry (LC-MS) was further used to confirm the consumption of the initial substrates. The formation of key degradation intermediates in test cultures was compared to the abiotic (culture without cells) and biotic (culture without the aromatics) control cultures. As result, a total of 23 genes related with hydrocarbon catabolism were detected. The array data supported the presence of key catabolic genes in the DSM 45259 strain genome that confer the capacity to degrade aromatic hydrocarbons (naphthalene, biphenyl, phenanthrene, anthracene, pyrene, isopropylbenzene, ethylbenzene, tetralin and benzene), heterocyclic or substituted aromatic hydrocarbons (anthranilate, aniline, quinoline, 2-chlorobenzoate, 2,4-dinitrotoluene), single alkanes (n-decane and n-tetradecane) and several intermediates of the degradation of the mentioned compounds. The detected genes allow proposing the presence of the catechol pathway, the salicylate pathway and the phthalate pathway, as well as hydrocarbon degradation lower pathways. The presumptive ability of DSM 45259 strain to use the single alkanes, benzoate, phthalate and phenol as sole carbon sources would be inferred, which was experimentally validated by cultivation and mass spectrometry. Degradation occurred in the absence of glucose as co-substrate that was previously reported to be required for the degradation of naphthalene and phenanthrene. Interestingly, while *alkB* gene encoding an alkane hydroxylase is most likely highly similar to that found in other actinomycetes, the genes encoding benzoate 1,2-dioxygenase, phthalate 4,5-dioxygenase and phenol hydroxylase were homologous to proteobacterial. This occurrence suggests that strain DSM 45259 contains catabolic genes distantly related to those found in other actinomycetes. Together, this study not only provided new insight into the catabolic abilities of strain DSM 45259, but also suggests that this strain contains genes uncommon within actinomycetes.

BB-011

PRODUCTION OF DELTA ENDOTOXINS BY *Bacillus thuringiensis* USING COMPLEX SUBSTRATES

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The *Bacillus thuringiensis* delta endotoxins are widely used as insecticidal proteins. These crystalline inclusions show a wide range of specificity for different insect orders such as Lepidoptera, Coleoptera, Diptera and also to nematodes. Moreover, productivity of crystal proteins can be regulated by optimizing the concentration of complex substrates yielding an economic medium. On the other hand, agro-industrial raw materials and waste products are constantly produced being their final disposal sometimes associated with several environmental problems. Thus, bioconversion of these cost-effective substrates that are also locally available is turning in an interesting and a useful approach. In this connection, we previously applied a sequential optimization strategy involving a Plackett-Burman and a full factorial experimental design to maximize the production of crystal proteins; as a result the following culture medium formulation was obtained (in g/l): milk serum 7.5, starch 3.0 and soybean meal 10.0. In this work, both the supplementation of 10.0 g/l of vinasse (X1) and the ratio reactor volume/working volume (X2) were evaluated using a full factorial design. The native *Bacillus thuringiensis* RT from our own culture collection was used throughout this study. The crystal protein (Cry) concentration was determined by the method of dye elution in SDS-isopropanol using bovine serum albumin as a standard. Fermentations were carried out during 72 h at 30 °C and 200 rpm. Our results indicated that X1 has a significant and a positive effect on the production of both Cry 1 Ac ($p=0.008$) and Cry 2 Ab ($p<0.001$). While, X2 ($p<0.001$) and the interaction X1-X2 ($p<0.001$) only have a significant impact on the Cry 2 Ab production. In addition, the adequacy of each model was verified by the R2 (> 88.67) and the Adj R2 (> 80.17) coefficients indicating the percentage of variability that is explained by the model. Thus, our finding revealed that a careful balance of culture conditions should be established to increase delta endotoxins production. In addition, the biological activity of the improved product against *Spodoptera frugiperda* was also discussed.