XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

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San Miguel de Tucumán | ARGENTINA

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MM-028

ANTIBIOFILM EFFICACY OF BACTERIOCINS AGAINST THE EMERGENT PATHOGEN *Listeria monocytogenes*

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Listeria monocytogenes is a foodborne pathogen able to survive in a wide range of environments even at refrigerated conditions. Moreover, some strains of *L. monocytogenes* can form biofilm facilitating their persistence in the food processing environments. Recently, major advances have been made in the prevention and control of pathogens biofilm by lactic acid bacteria (LAB) or their bacteriocins. The aim of this work was to investigate the ability of bacteriocins produced by *Lactobacillus curvatus* CRL705 and *L. curvatus* CRL1532 to compete with *L. monocytogenes* FBUNT during biofilm formation on polystyrene microplate. Biofilm formation of Listeria strains (*L. monocytogenes* FBUNT, CECT 4031T, Scott A) with and ithout bacteriocins using crystal violet method after 6 days of incubation at 10 °C was determined. The strain *L. monocytogenes* FBUNT was selected due to its high biofilm-forming capacity in the control samples. Both bacteriocins inhibited biofilm development of pathogen microorganism being the bacteriocin produced by *L. curvatus* CRL705 the most effective. In addition, the presence and expression of genes related to *L. monocytogenes* biofilm formation were studied by PCR and real time-PCR, respectively. *L. monocytogenes* FBUNT showed to harbor *luxS* and *pfs* genes encoding enzymes that catalyze S-ribosyl homocysteine and genes of *agrBDCA* system. During sessile growth, the expression levels of *agrB* and *luxS* genes were 2-fold higher than under planktonic growth. In presence of both bacteriocins a higher expression of *agrD* gene was observed. Expression levels of *agrB* and *luxS* genes decreased at higher concentrations of bacteriocins assayed which could indicate that bacteriocin addition affected expression of key genes involved in biofilm formation. These results evidence the potential use of the bacteriocins produced by *L. curvatus* CRL705 and CRL1532 as inhibitors of *L. monocytogenes* biofilm formation in refrigerating conditions.

MM-029

DIFFERENTIAL EXTRACELLULAR ENZYMES EXPRESSION BY THREE *Paenibacillus* STRAINS USING GEL-FREE PROTEOMICS ANALYSIS

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Three *Paenibacillus* strains, identified according to their 16S rDNA gene sequence and named as AR247, AR460-1 AR489, were selected due to their ability to produce glycoside hydrolases (GH) for second generation ethanol and other biotechnological applications. The assessment of extracellular enzyme production was previously approached by utilizing a mineral-based medium, MM0.2, added with agricultural by-products. These substrates are low-cost and abundantly available carbon sources for biotechnological purposes. Among the tested carbon sources, an alkali pretreated sugarcane bagasse (OH-SCB) was the one that better promoted the production of extracellular xylanases for all strains. The aim of this work was to study the differential extracellular enzymes expression by these strains through gel-free proteomic analysis method. Firstly, crude extracts were obtained by centrifugation after 72 h of cultivation in MM0.2 - OH-SCB 1% medium. Then, samples were concentrated by lyophilization and digested by using trypsin for further mass spectrometry analysis. Tryptic peptides obtained were analyzed using 2D nano-Ultra Performance Liquid Chromatography, coupled to tandem mass spectrometry. Bioinformatics analysis for protein identification was performed by searching against Swiss-Prot database, using Mascot server and ProteoIQ v2.8. Peptide summary report provided by Mascot evidenced hemicellulases which are active not only over b-1,4-linkages of xylose units but also on substituents of xylan. An endo-b-1,4-xylanase with 20.1 kDa molecular weight and 9.2 isoelectric point (pI) was found exclusively in crude extract samples of strain AR247. This enzyme, identified as a GH11, was also detected as a band between 18-20 kDa in zymograms and showed a pairwise similarity of ³ 99 with an homologous from *Paenibacillus* sp. Y412MC10. In addition, a second b-1,4-xylanase was identified in the secretome samples, yet not detectable through zymography, from both AR247 and AR489 strains. It was of 140.9 kDa, 4.7 pI (potential GH10), and showed to be closely related to a b-1,4-xylanase from *P. glucanolyticus*. Moreover, additional extracellular xylanase were found, which were related to a GH30 detected in *P. favisporus* and to two GH25 from *P. polymyxa*. Other proteins related to xylan utilization identified were: S–layer protein; sugar ABC transporters and sequences from substrate-binding proteins towards beta glucans. On the other hand, strains AR460-1 and AR489, revealed sequences corresponding to a carbohydrate binding module type CBM54 and an ABC transporter protein. Finally, a chitosanase belonging to GH8 family was only found in strain AR489. In conclusion gel-free proteomics analysis proved to be a useful methodology to achieve a widespread knowledge of the enzymatic repetory contributing to better quality and quantity of results.