XII CONGRESO ARGENTINO DE MICROBIOLOGIA GENERAL

2 al 4 de agosto de 2017

San Miguel de Tucumán | ARGENTINA

SAMIGE

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BF-008

PRODUCTION OF A PROTEASE ACTIVITY FROM *Bacillus thuringiensis* RT IN A COMPLEX MEDIA

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Bacillus thuringiensis is a Gram-positive, facultative anaerobic bacterium that produces crystal proteins with insecticidal properties. In addition, it is an excellent producer of proteases and other lytic enzymes that can increase the insecticidal effect by acting in synergy with the crystal protein. In this work, the production of proteases was carried out by submerged fermentation using a complex media for possible use in different industries such as food, chemical, textile and bioinsecticides. The strain *Bacillus thuringiensis* RT (Genebank: EF638795, 16S partial sequence) was used. The production was carried out in a Labfors 3 fermenter (Infors HT, Bottminguen, Switzerland) in 3000 ml at 30°C and free pH. A complex culture media called M5 developed from economical substrates was used; it contains (g/L): cerelosa 2, whey 10, skim milk powder 5, vinasse 5, sucrose 2, starch 2 and soybean meal 7. Protease activity was determined in triplicate in culture supernatant every 24 h during 5 days. One hundred and twenty ml of supernatant was mixed with 480 ml of azocasein (1% w/v) in phosphate buffered saline. One unit of activity is defined as the amount of enzyme that produces an increase in 0.01 OD420 in 30 min at 30°C. The values of protease activity during fermentation were (U/L): 0.13 ± 0.02 x 106 (24 h), 0.26 ± 0.02 x106 (48 h), 0.35 x106 ± 0.01 x106 (72 h), 0.98 ± 0.07 x106 (96 h) and 1.36 ± 0.09 x106 (120 h). Thus, the maximum value of protease activity was obtained at 120 h (a = 0.05) displaying a volumetric productivity of 11421.97 U/Lxh. In addition, the final pH value of the culture was 9.51. In conclusion, this lab-scale study gives useful information to continue with the scaling-up of the process. This work was supported by FONCyT (PICT 2011-2158 and PICT 2015- 2596), CONICET (PIP 339) and UNT (PIUNT E548/3).

BF-009

IDENTIFICATION AND HETEROLOGOUS EXPRESSION OF *Enterococcus faecalis* ESTERASES FOR THE PRODUCTION OF SHORT CHAIN FATTY ACIDS COMPOUNDS THAT CONTRIBUTE TO FLAVOR GENERATION IN CHEESES

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Enterococcus strains usually dominate the non-starter flora of traditional cheeses such as Mozzarella and Fontina. They contribute positively on the development of flavor compounds during ripening. The most important enzymatic activities of non-starter lactic acid bacteria involved in flavor production are proteolysis and lipolysis. As the applications of enzymes in the food industry are expanding in this project we have performed a screening of *E. faecalis* esterases in order to produce flavor-enhancing esterases in a GRAS host. First, twenty-three hydrolases with possible esterase activity were identified in the available genome of *E. faecalis* JH2-2 using *in silico* tools. In an attempt to identify wall-anchored or secreted enzymes the program SignalP 4.0 was subsequently used. Hence, nine out of twenty-three hydrolases showed to have a signal peptide indicating possible secretion. Four of these hypothetical esterases coding genes, named *estA*, *estB*, *estC* and *estD,* were cloned in pET 28b and expressed in *Escherichia coli* DH5a. Then, cell fractions of IPTG-induced recombinant strains were obtained and analyzed. The presence of EstB and EstC was only observed in the cytoplasmic fractions which suggests that neither of the putative enzymes could be recognized or transported by *E. coli* secretion system. On the other hand, EstD was not detected in soluble form under tested conditions. Interestingly, EstA putative esterase could be recovered in the periplasmic fraction which indicates that the hypothetical signal peptide is being recognized and the protein secreted by the *E. coli* Sec system. In an attempt to corroborate the hydrolytic capability of the putative enzymes, the esterolysis of p-nitrophenyl (pNP) monoesters of fatty acids were evaluated. EstA showed to hydrolyze only short chain acyl pNP derivatives (C4), while EstC over C4, C16 and C18 acyl pNP derivative. Noteworthy, short-chain free fatty acids are indicators of quality and source of flavor in cheese. In order to broaden the knowledge of EstA and EstC regarding its origin, a phylogenetic analysis was performed. As a result, EstA and EstC were identified in all *E. faecalis* strains analyzed and in lesser extent within the genus. Finally, in order to study the contribution of EstA in the production of cheese sensorial relevant compounds, constructions of GRAS EstA–producing strains were conducted. In order to do so, a codon optimized version of *estA* was synthetized and cloned in pNZ8048, a NICE (nisin-controlled expression) system vector, which derives from the *nis* operon (*nisABTCIPRKEFG*). As hosts, *L. lactis* NZ9000 and a derivate strain deficient in ClpP and HtrA major proteases were employed. As an alternative expression tool, the promotor of NICE system was also exchanged by the promotor of P170 expression system which is up-regulated as lactate accumulates in the growth medium. Currently, the best combination of host, promotor type, and expression conditions are under evaluation.