XI CONGRESO ARGENTINO DE MICROBIOLOGÍA GENERAL

5 al 7 de Agosto de 2015
Córdoba, Argentina

SAMIGE

Asociación Civil de Microbiología General

analyzed by TLC, and spot intensities were quantified with ImageJ software. The amount of lovastatin was quantified by RP-HPLC. In a second stage, selected lovastatin hyperproducing mutants were subjected to another mutation cycle and further evaluated. The first obtained 164 putative mutants were comparatively analyzed against the wild-type (WT) *A. terreus* MEC strain and, according to TLC results, 28 mutants produced 20% or less than it, whilst 20 out of the 164 produced 20% (or higher) more lovastatin than WT. These results, as confronted to the HPLC analyses, confirmed 6 mutant strains with 20%-lower production than WT strain, while only one showed a hyperproducing phenotype. This latter mutant, named C10´-27, produced 168% more (2.35 g/L) lovastatin than WT strain. After a second mutation cycle of *A. terreus* C10´-27, 157 putative mutants were analyzed. Lovastatin production, as witnessed by RP-HPLC, increased by 20% or higher than the one for C10´-27 for 5 of the obtained mutants. The highest lovastatin titer was achieved by mutant *A. terreus* S12,5´-9 with a 40% increase over the already improved production of *A. terreus* C10´-27. These results pave the way to a more efficient lovastatin production by using the selected mutant and may additionally open new perspectives for reducing its production costs.

Código de Resumen: BF-024

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

FIBRINOLYTIC ENZYMES PRODUCTION BY *Bionectria* sp. LY 4.1: THE RELEVANCE OF INOCULUM HOMOGENIZATION AND PH-CONTROLLED CONDITIONS AT FERMENTER SCALE

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Fibrin accumulation in blood vessels usually results in thrombosis, which can thereafter lead to myocardial infarction and other cardiovascular diseases. Fibrin is the primary protein component of blood clots and is physiologically formed from fibrinogen by the catalytic action of thrombin. During fibrinolysis, the insoluble fibrin fiber is hydrolyzed into fibrin degradation products by plasmin. Nowadays, fibrinolytic enzymes of microbial origin have attracted more attention than typical thrombolytic agents used for thrombolytic therapy. This choice is based on the high price and the undesirable side effects of the latter. The aim of this work was to study the production of fibrinolytic enzymes by *Bionectria* sp. LY 4.1, a wild fungus isolated from Las Yungas rainforest and already described in our group. In this case, we focused on the fermentation process upstream optimization in order to increase fibrinolytic enzymes production at fermenter scale. At first, inoculation process was standardized by hand-blender-aided homogenization of mycelial suspensions and evaluating the influence of power input and the number of pulses. The production of fibrinolytic enzymes was also preliminary evaluated at different initial cultivation pHs, from 4 to 8, at shake-flask scale and by using an optimized production medium based on glucose, soy peptone, NaCl and $MgSO_4$. Subsequently, batch cultures were carried out with a 1-L working volume fermenter either at uncontrolled pH or by controlling culture broth pH (with 1 N NaOH) at the optimal value obtained in previous assays, and results were comparatively assessed. Fibrinolytic activity was determined by the fibrin plate test and by using a plasmin standard curve. Inoculum standardization showed that, at 48 h of fermentation, inoculum homogenized with a higher number of pulses allowed to obtain an increased fibrinolytic activity (495 U plasmin/ml) as compared to the process started with a slightly homogenized inoculum (170 U plasmin/ml). The screening for optimum cultivation pH in shake-flasks assays revealed that a 20% higher production was obtained at pH=8 and accordingly, this value was selected for testing at fermenter scale. Batch fermentations were comparatively run under free pH (firstly set in culture medium at 6.6 and left uncontrolled afterwards) and with automatic control at pH=8. Further operative conditions were set as follows: agitation, 200 rpm; temperature, 25°C and airflow rate, 1.5 vvm. Fibrinolytic enzymes titers reached 1888 U plasmin/ml at pH-free conditions *vs.* 2437 U plasmin/ml under controlled pH. These findings provide first clues into the possibilities for the upstream fermentation process optimization through the automatic pH controlling strategy. Following studies will be focused on the use of a different pH controlling agent and further operative conditions. The elucidation of these optimal parameters will then be useful for the subsequent scaling-up.

Código de Resumen: BF-025

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF THE INFLUENCE OF CARBON SOURCES ON CELLULASES AND XYLANASES PRODUCTION BY *Microbacterium* sp. AR462-2

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The lignocellulose is one of the main constituents of vegetable biomass. Besides being a renewable and abundant source of energy, it has a high potencial for bioconversion to value-added bioproducts. Among its components, the hemicellulose portions (mainly xylans) represent around 25% to 40% of their composition after cellulose that constitute the main component which represents between 50% and 70% of the total. In adition to the lignin, both the cellulose and hemicellulose, make its structure to be resistant to the enzymatic degradation. Thus, it is necessary the cooperative and synergistic effect of various enzymes, including cellulases and xylanases, to reach higher yields for its degadacion. In the present study *Microbacterium* sp. AR462-2, a cellulolytic and xylanolytic actinobacteria isolated from intestines of wood beetle larvae, was characterized. The effect of various carbon sources was evaluated on cellulases and xylanases production into Omeliansky mineral culture medium. The substrates assayed as sole carbon source were xylan, lactose, glucose, sucrose, fructose, maltose, carboxymethylcellulose (CMC) and filter paper. This strain was taxonomically identified as a member of *Microbacterium* spp. according to the sequence analysis of the 16S ribosomal gene. Microscopic observation of AR462-2 strain showed small yellow rods (RAL 1014). Physiological studies showed that this strain did not produce melanic nor soluble pigment. Growth was observed between 5.0 and 8.0 pH units and showed halotolerance and lysozyme resistance. It was able to grow in presence of various carbon sources. *M.* sp. AR462-2 secreted cellulases when glucose 1% (w/v) was used as a sole carbon source which activity measured was 0.1 U/ml. Also, this strain showed evidence of mild degradation of filter paper in liquid mineral medium. Xylanolytic activities were detected in all culture medias, but the highest xylanase production was recorded when lactose 1% (w/v) was added individually in Omeliansky´s medium. The highest xylanase activity measured was 1.03 U/ml within 7 days using lactose, while in presence of CMC was 1.14 U/ml but after 14 days of culture. Further assays were performed in order to improve both growth and production, by adding casein peptone. Production rates were similar although growth was significatively shortened to 3 days. These results suggest that lactose could be used for enzyme production by *Microbacterium* sp. AR462-2 as an inexpensive carbon source, since lactic whey is an abundant industrial by-product.

Código de Resumen: BF-026

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

EVALUATION OF THE INTERACTION AMONG YEAST β-GLUCAN AND POLYPHENOLS DERIVED FROM CINNAMIC AND BENZOIC ACIDS

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The β-(1-3)-D-glucan is a homo-polysaccharide of D-glucose with insoluble alkali-acid characteristics. It is widely used to activate the immune system, for its antiviral and antimicrobial properties. Considering its use by the food industry, the FDA (Food and Drug Administration-USA) has been recognized as GRAS. One of the most important sources of β-glucan is the cell wall of yeast, especially of *Saccharomyces cerevisiae.* Phenolic compounds are secondary plant metabolites occurring in fruits and vegetables. They are involved in the defence system against invading pathogens (including *Escherichia coli* and *Campylobacter jejuni*). Many of these phenolic compounds have valuable chemical and biological activities, as antioxidants, metal chelators as well as antimicrobial and antiviral agents. This study focuses on obtaining biomaterials with metal complexing and antioxidants properties based on secondary interactions between β-glucan from *S. cerevisiae* and a series of phenolic compounds (caffeic, ferulic, and gallic acids). The aim of this work is to develop antioxidant solid filters to remove free radicals and metal ions to be used in environmental remediation and food and pharmaceutical industry. YPD growth medium was supplemented with EDTA and adjusted to pH 4.00 with tartaric acid. Cells were incubated at 30 °C with shaking at 200 rpm for 24 h. The number of viable cells was analysed using the McFarland turbidity standards. The cell wall was obtained by cell autolysis at 50 °C for five days. The alkalis-acid soluble components of the cell wall were removed by alkali treatment (incubation in NaOH at 90 °C) and acidic treatment (incubation in H_3PO_4 at room temperature). Mannoproteins removal was performed by autoclaving at McIlvane pH 7.00 buffer for 90 min. The β-glucan extracted was analysed by IR and NMR. The interactions were assessed by monitoring the UV-Vis spectral changes for different ratios of polyphenol/glucan mass at pH 5 to 8 and its stability for one hour. The presence of $1{,}5 \times 10^{12}$ viable cells/mL was observed. Amounts of 0.31 g of glucan per liter of culture were obtained. The yield obtained is low considering the physical size of the microorganism, as well as the energetic conditions used during extraction. The quantification of viable cells was null after autolysis. The purity of the material was analysed by IR and NMR spectroscopy, and results were satisfactory. For interactions between β-glucan and polyphenols the UV-Vis spectral changes indicated the existence of interaction between β-glucan and caffeic acid as well as between β-glucan and gallic acid. The evaluation of changes in the antiradical activity and metal complexing abilities when the polyphenols were combined with β-glucan was carried out. Further studies of high biological ongoing interest are in progress concerning the chemical modification of β-glucan to enhance the interaction with polyphenols.