XI CONGRESO ARGENTINO DE MICROBIOLOGÍA GENERAL

5 al 7 de Agosto de 2015
Córdoba, Argentina

SAMIGE
Asociación Civil de Microbiología General

health, as well as for the agricultural and food industry; although inhibition of phytopathogenic fungi was not observed. Maximum inhibitory activity was obtained in CSM medium at 20°C, showing that bacterial antagonism was neither related to siderophore- nor biosurfactant-like compounds, although both activities were detected for living-cell and cell-free supernatants. The antimicrobial showed similar properties, such as positive net charge, resistance to proteases and the UV-Vis spectrum, to those of pseudobactin, a well-known siderophore produced by species of *P. fluorescens*,. However, the non-siderophore nature of the produced antimicrobial makes difficult to relate it with pseudobactin. Further studies including HPLC-MS/MS and NMR are expected to help at elucidating the *P. yamanorum* 8H1^T antimicrobial structure and its potential novelty.

Código de Resumen: BF-022

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

IMPACT OF MANGANESE ON THE PRODUCTION OF A BIOMASS ASSOCIATED β-D-GLUCOSIDASE ACTIVITY USING A THERMOPHILIC *Bacillus licheniformis* STRAIN

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The β-Glucosidase (EC 3.2.1.21) constitutes a group of well-studied hydrolases. This enzyme catalyzes the hydrolysis of arylglucosides, alkylglucosides, cellobiose, and cellooligosaccharides. The interest in this biocatalyst centers on its roles in the enzymatic hydrolysis of cellulose. The rate of cellulose hydrolysis can be improved by supplementing commercial cellulases with immobilized β-D-glucosidase, which usually has high stability and can be recovered and reused. In addition, for industrial saccharification of cellulosic materials, β-glucosidases from thermophilic bacteria are also of particular interest due to their increased stability. In this work, we study the influence of manganese on the production of a biomass associate β-D-glucosidase activity using a thermophilic *Bacillus licheniformis* strain. Assays were performed at 45 °C in 500 ml Erlenmeyer containing 200 ml of LB medium supplemented with 0 - 1.0 mM $MnCl_2$. The β-D-glucosidase activity was also determined at 45 °C using 3.6 mM p-nitrophenyl-β -D-glucopyranoside (Sigma) as substrate. Cells were harvested by centrifugation and washed twice with 100 mM Tris-HCl buffer (pH 7). The pellet was resuspended in the same buffer, and it was directly used as the β-D-glucosidase source. The mixture was shaken at 1000 rpm. Then, the absorbance of the supernatant was measured at 405 nm and the enzyme activity calculated and related to the biomass dry weight. One unit of the enzyme was defined as the amount needed to release 1 µmol *p*-nitrophenol per min. Thus, dose-response experiments showed that in the presence of 0.3 mM $MnCl_2$ the enzyme production was increased by about 20%. Under this culture condition, a specific activity value of 19.99 U per mg of dry weight was obtaining after 4 h of cultivation. Finally, these results could be of relevance to the bioethanol industry where lignocellulosic material is used as feedstock for fermentation and, which should be treated enzymatically. The use of naturally bound enzymes is an important immobilization technique. This type of biocatalyst system is potentially cost-effective because the biomass can be directly utilized in the treatment.

Código de Resumen: BF-023

Sección: Biotecnología y Fermentaciones

Modalidad: Poster

Aspergillus terreus STRAIN IMPROVEMENT FOR ENHANCED LOVASTATIN PRODUCTION

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Cholesterol plays a vital role in body metabolism and membrane transport, and acts as precursor for the synthesis of several key biomolecules. Nevertheless, changes in cholesterol level lead to cardiovascular disorders, like atherosclerosis and hypercholesterolemia, which are currently the main causes of death. This is why controlling cholesterol by inhibition of its biosynthesis is a promising approach. Cholesterol is synthetized from acetyl-CoA through a complex pathway, where the rate-limiting step is the conversion of HMG-CoA to mevalonate, catalyzed by HMG-CoA reductase. This key enzyme is selectively and competitively inhibited by lovastatin, a fungal secondary metabolite used as a hypocholesterolemic which can therefore reduce the risk of cardiovascular diseases. Lovastatin production is normally carried out using selected *Aspergillus terreus* strains, however industrial process yields may be improved by strain manipulation. Accordingly, the aim of this work was to develop a lovastatin-hyperproducing *A. terreus* strain. To this end, 10^7-spores/mL suspensions of lovastatin-producer *A. terreus* MEC were exposed to UV radiation for different times ranging from 5 to 15 min. Spores were kept in the dark for 30 min, plated onto PDA plates and incubated at 25°C for 48 h. Isolated colonies were transferred to an optimized lovastatin production medium (SQop) containing cheese-whey as substrate and incubated at 25°C for 14 days. Lovastatin was extracted from fungal colonies by using ethyl acetate and converted to its β-hydroxyacid form by alkaline hydrolysis. Organic extracts were preliminary

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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