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Sección: Biotecnología y Fermentaciones

Modalidad: Poster

STABILITY ASSESSMENT AND IMPROVEMENT OF ENZYMATIC ACTIVITY OF THE ENDOGLUCANASES FROM *Bacillus* sp. AR03

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The lignocellulosic biomass is well known like a promising source to biorefinery due to its abundant and its renewable feature. Cellulose, the major compound of this material, needs the cooperative action of at least three types of enzymes to be degraded: exoglucanases, endoglucanases and β-glucosidases. Microorganisms and their enzymes are biotechnical tools that nature has designed to utilize biomass that is present in the habitat around them. In this sense, Bacteria are extensively considered as a source of novel cellulases because of their diversity and due to their higher growth rate and their extensive repertoire of glycoside hydrolases. The aim of the present work was to produce and to characterize endoglucanases from *Bacillus* sp. AR03, isolated from sugarcane bagasse liquor, to further generate lignocellulosic hydrolysates. The isolate AR03 was grown in a peptone broth amended with carboxymethyl cellulose 1% (w/v) and sucrose 1%(w/v) at 30 °C and 200 rpm. After 48 h, the culture supernatant was recovered by centrifugation and the endoglucanase activity was estimated by measuring reducing sugar released from CMC by the dinitrosalicylic acid (DNS) method. Zymograms of the culture supernatant were carried out by native PAGE. The effects of temperature, pH, cations and others additives such as EDTA, PEG, SDS and Tween 80 were assayed to assess their influence on the activity and stability of the endoglucanases produced. The enzyme production reached 3 IU/mL in the crude extract (culture supernatant) and the optimal endoglucanase activity was registered at 60 °C and pH 6.0. The evaluated enzymatic extracts showed that the enzyme activity was completely retained after pretreatments at temperatures £ to 40 °C, although it did not show thermal stability after preheating at 60 °C for one hour. Endoglucanases from AR03 isolate maintained approximately 80% of the total activity within a wide range of pH (3.0 to 10.0). The native PAGE revealed at least three bands with endoglucanase activity, having apparent molecular masses of 286, 208 and 157 kDa. Even when most of the effectors assayed did not affect significantly the enzymatic activity, the addition of Mn^{2+} and Co^{2+} (5 mM) to the enzymatic reaction mixture produced a noteworthy improvement of the endoglucanase activity from the crude extracts. The endoglucanase activity was upgraded as much as 150% and 80% when salts containing Mn^{2+} and Co^{2+} were added, respectively. Those increments were confirmed by means of HPLC measurements since it has been reported interference between some divalent cations and the DNS reagent. The results regarding the broad range of pH stability and the strong improvement of enzymatic activity by the presence of manganese are the most relevant features of the endoglucanases from *Bacillus* sp. AR03 to be considered as promising for further studies and for biotechnological applications.

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BIOETHANOL AND BIODIESEL PRODUCTION WITHIN A CYANOBACTERIA AND MICROALGAE BIOREFINERY

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Oleaginous microalgae have great potential as a feedstock for biodiesel and other biofuels. However, the current cost of producing biofuels from microalgae biomass is still high to envision massive and profitable commercialization in the near future. One of the drawbacks of implementing large-scale cultivation of these organisms is the unsustainable requirement of N-fertilizers. It is presumed, however, that co-production of higher value by-products in the frame of a biorefinery would increase the profitability of producing biofuels and co-products from microalgae. Recently, we showed the efficient conversion of N_2-fixing cyanobacterial biomass into oleaginous microalgae biomass. We further modeled an integrated bioprocess that would require no N-fertilizer other than air and would yield 7.000 - 10.000-l microalgae oil . ha^{-1}. $year^{-1}$ in raceway ponds placed in southeastern Buenos Aires. This estimated yield would be 2- to 20-fold higher than that reported for current oleaginous plant feedstocks heavily fertilized with conventional N-fertilizers. In addition to oil, this process would roughly produce from 2 kg of *Nostoc* biomass about 1 kg of *Nostoc* residues (mostly carbohydrates) and 1.0 kg of carbohydrates and 1 kg protein from