Title: PRODUCTION OF XYLANASES BY THE MARINE-DERIVED FUNGUS *Aspergillus tubingensis* LAMAI 31

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Abstract:

Xylanases (EC 3.2.1.8) catalyze the hydrolysis of 1,4-beta-D-xyloside in xylans, which are the second most abundant polysaccharide found in nature, being the major constituent of hemicellulose from the plant cell wall. These enzymes have a wide range of industrial applications, including animal feed, food, textile, paper, biofuel, among others. The aim of the present study was to evaluate the production of xylanases by the marine-derived fungus *Aspergillus tubingensis* LAMAI 31, as well as to perform a preliminary characterization of the enzymes (crude broth). The fungus was selected due its xylanases activities in Mandels and Sternbergs medium (MS) added with xylan at 28 °C and 140 rpm after 7 days of cultivation. Experimental design was applied in order to evaluate the influence of different variables and optimize the conditions for the enzymatic production. The independent variables used were: pH, inoculum, salinity (artificial sea water), $(NH_4)_2SO_4$, peptone, sugar-cane bagasse, wheat bran, rice straw, sacarose, and xylan. Three experimental designs were performed, being two Plackett & Burman (PB) and one Fractional Factorial 2^{4-1}. Statistical analyzes were carried out using the software Statistica 7 (Statsoft Inc.) Xylanases activities were determined using xylan (birchwood) as substrate. Reducing sugar amounts were quantified by using the dinitrosalycilic method. Optimal pH (between 3-9) and temperatures (between 30-70 °C) for xylanases were determined. Temperature and pH stability were also evaluated. The fungus *A. tubingensis* LAMAI 31 was able to produce 49.41 U/mL of xylanases before the optimization in MS medium. After the conduction of the first PB design (10 variables) enzymatic activity reached 501.9 U/mL. In the second PB (5 variables) the enzymatic production was 629.6 U/mL (assay 2). From the third experimental design the production decreased considerably. Thus, the assay 2 of the second PB was validated, being the best production of the enzyme obtained after 96 hours of incubation (561.50 U/mL). In the validation assay, the higher specific activity was obtained after 72 h (209.26 mg/mL). Crude xylanases from *A. tubingensis* LAMAI 31 showed optimum pH and temperature of 5.0 and 55 °C, respectively. This enzyme showed to be stable in a range of 40 to 50 °C, and presented higher stability at pH 7.0. These results encourage further studies related to the xylanases characterization, production and application.

Keywords: Xylanases, Filamentous fungi, Marine biotechnology, Experimental design.

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