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Cloning and Expression a Gene of β-Glucosidase from Penicillium echinulatum in Pichia pastoris

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Abstract : Bioethanol is one of the most promising biofuels and able to replace fossil fuels and reduce its different environmental impacts and can be generated from various agroindustrial waste. The Brazil is in first place in bioethanol production to be the largest producer of sugarcane. The bagasse sugarcane (SCB) has lignocellulose which is composed of three major components: cellulose, hemicellulose and lignin. Cellulose is a homopolymer of glucose units connected by glycosidic linkages. Among all species of Penicillium, Penicillium echinulatum has been the focus of attention because they produce high quantities of cellulase and the mutant strain 9A02S1 produces higher enzyme levels compared to the wild. Among the cellulases, the cellobiohydrolases enzymes are the main components of the cellulolytic system of fungi, and are also responsible for most of the potential hydrolytic in enzyme cocktails for the industrial processing of plant biomass and several cellobiohydrolases Penicillium had higher specific activity against cellulose compared to CBH I from Trichoderma reesei. This fact makes it an interesting pattern for higher yields in the enzymatic hydrolysis, and also they are important enzymes in the hydrolysis of crystalline regions of cellulose. Therefore, finding new and more active enzymes become necessary. Meanwhile, β-glycosidases act on soluble substrates and are highly dependent on cellobiohydrolases and endoglucanases action to provide the substrate in the hydrolysis of the biomass, but the cellobiohydrolases and endoglucanases are highly dependent β-glucosidases to maintain efficient hydrolysis. Thus, there is a need to understand the structure-function relationships that govern the catalytic activity of cellulolytic enzymes to elucidate its mechanism of action and optimize its potential as industrial biocatalysts. To evaluate the enzyme β-glucosidase of Penicillium echinulatum (PeBGL1) the gene was synthesized from the assembly sequence from a library in induction conditions and then the PeBGL1 gene was cloned in the vector pPICZαA and transformed into P. pastoris GS115. After processing, the producers of PeBGL1 were analyzed for enzyme activity and protein profile where a band of approximately 100 kDa was viewed. It was also carried out the zymogram. In partial characterization it was determined optimum temperature of 50°C and optimum pH of 6,5. In addition, to increase the secreted recombinant PeBGL1 production by Pichia pastoris, three parameters of P. pastoris culture medium were analysed: methanol, nitrogen source concentrations and the inoculum size. A 23 factorial design was effective in achieving the optimum condition. Altogether, these results point to the potential application of this P. echinulatum β-glucosidase in hydrolysis of cellulose for the production of bioethanol.

Keywords : bioethanol, biotechnology, beta-glucosidase, penicillium echinulatum
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