TITLE: PRODUCTION AND PURIFICATION OF β-GALACTOSIDASE FROM *TRICHODERMA* sp. UNDER SUBMERGED FERMENTATION USING WHEY AS CARBON SOURCE

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ABSTRACT

The enzyme β-galactosidase (EC 3.2.1.23) hydrolyzes the disaccharide lactose to obtain the monosaccharides galactose and glucose as products. It has potential to be applied in pharmaceutical and food industries, and in effluent treatment of dairy industries. Additionally, the β-galactosidase also can be used for production of the prebiotic galacto-oligosaccharides (GOS) through the transgalactosylation reaction. This enzyme can be found in prokaryotes and eukaryotes, and among the latter, the filamentous fungi have claimed attention because their ability to produce extracellular enzymes, facilitating the recovery and enzyme purification process. According to this, the aim was to determine the best conditions of cultivation for obtainment of extracellular β-galactosidase by *Trichoderma* sp. under Submerged Fermentation (SbmF) and its purification. The SbmF was conducted using Nakao-lactose medium, pH 6.0, containing 3% (m/v) of different carbon sources separately (carbohydrates and agro-industrial by products) and 0.7% (w/v) of nitrogen sources separately (meat extract, yeast extract, peptone, ammonium chloride, ammonium nitrate and ammonium sulfate). The influence of the time of cultivation (24-480h) was also analyzed at 30°C and 120 rpm. After vacuum filtration, the extracellular cell-free filtrate was used as the source of enzyme. The β-galactosidase activity was determined using the glucose oxidase method. The best enzyme production (28 U/L) was obtained using whey and ammonium nitrate as carbon and nitrogen sources, respectively, for 312h of cultivation at 30°C. Improved conditions of SbmF increased the enzyme activity by 5.6-fold. After, the filtrate containing the enzyme was loaded into DEAE-cellulose chromatographic column and eluted with a linear gradient of NaCl (0-1.5M). Under this condition, the β-galactosidase was purified 2.4-fold, with 40.8% recovery. The enzyme partially purified presented maximal activity at 55°C and pH 5.0. In conclusion, the SbmF containing whey is an interesting option for production of β-galactosidase by *Trichoderma* sp. and the strategy of purification adopted is an alternative to initial isolation of the enzyme.

Keywords: Lactase, *Trichoderma* sp., galactooligossarídeos (GOS)

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