CHARACTERIZATION of β-GLUCOSIDASE PRODUCED by TRICHODERMA CITRINOVIRIDAE CULTURED on CHLORELLA VULGARIS

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Chlorella vulgaris promises as a biomass fuel, natural food coloring agent and dietary supplements in which their cell walls consist of multiple layers including cellulose. In order to utilize microalgae as a feedstock, the pre-treatment to breakdown their cell walls is important. A wide variety of bacteria and fungi produce cellulolytic enzymes able to hydrolyze cellulose. However, relatively few fungi and bacteria produce high levels of extracellular cellulase capable of solubilizing crystalline cellulose extensively. Trichoderma citrinoviride was found to be the most efficient producer of cellulases along with a high level of β-glucosidase. β-Glucosidase is a crucial enzyme for the efficient conversion of cellulose to glucose. In this study, the characteristics of the β-glucosidase produced by Trichoderma citrinoviride (ATCC 62394) cultured on microalga (C. vulgaris) were investigated. β-Glucosidase was purified to homogeneity by ion exchange and gel filtration chromatography with a recovery of 8.51% and specific activities of 168.74 U/mg. The purified enzyme was obtained as a single band with the estimated molecular masses of 95 kDa on SDS-PAGE. The optimum pH and temperature for enzyme activity and stability were 4.0 and 50°C, and 8.0 and 30°C respectively. Metal ions (Mg^{2+}, Zn^{2+}, K^+, Cu^{2+}) activated the enzyme activity, whereas EGTA and SDS inhibited it strongly and moderately, respectively. The $K_m$, $V_{max}$ and $K_{cat}$ values of β-glucosidase were 3.77 mM, 769.23 µmol min$^{-1}$ and 7.60 min$^{-1}$, respectively.