PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A THERMOSTABLE ENDOXYLANASE FROM COLLETOTRICHUM GRAMINICOLA

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Agro-industrial residues, rich in cellulose and xylan, are highly convenient sources for the production of lignocellulosic ethanol and xylooligosaccharides. The economic viability of both processes is highly dependent on an efficient hydrolysis of xylan, raising a great interest in xylanases with attractive properties for industrial applications. Xylan hydrolysis involves various hydrolases acting synergistically on the main and side chains of the polysaccharide, including the endoxylanases (EC 3.2.1.8). In this study an endoxylanase (CGEX) from Colletotrichum graminicola produced under solid-state fermentation was purified and biochemically characterized. The activity was assayed at 65°C in McIlvaine buffer, pH 5.5, containing 1% (w/v) beechwood xylan. The enzyme was purified 4.9-fold with a yield of 34% by a procedure which involved hydrophobic interaction chromatography in Phenyl-Sepharose CL-4B followed by ion exchange chromatography in DEAE-Fractogel. The purity of the enzyme preparation was confirmed by the single band revealed both in PAGE and SDS-PAGE. CGEX was a glycoprotein with a total carbohydrate content of 98% and an apparent molecular mass of 20 kDa. Optimal pH and temperature were 5.5 and 65°C, respectively, and CGEX was stable for 24h at 4°C at pH 3.0-10.0. The pure enzyme in water was fully stable at 50°C and maintained 72% of its initial activity at 60°C after 60 min. CGEX hydrolyzed xylan with $V_M = 497.8 \pm 35.0 \text{ U.mg}^{-1}$ and $K_{0.5} = 3.7 \pm 0.26 \text{ mg.mL}^{-1}$. Thin-layer chromatography analysis of the reaction products showed the presence of xylobiose and short xylooligosaccharides only, with a minimal content of xylose. The good stability of the enzyme to temperature and pH, the elevated specific activity for xylan hydrolysis and the nature of the reaction products suggest that CGEX may be attractive for application in industrial processes for the production of xylooligosaccharides and lignocellulosic ethanol.

Keywords: thermostable endoxylanase, Colletotrichum graminicola, ethanol and xylooligosaccharides
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