

## Purification of a Xylanase from *Penicillium sclerotiorum*

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Endo 1,4- $\beta$ -D xylanase (E.C. 3.2.1.8) is produced by a variety of microorganisms, being the fungi the most potent producers. This enzyme cleaves the  $\beta$ -1,4 glucosidic linkages between D-xylopyranosil residues from the xylan, the major hemicellulosic polysaccharide in plant cell walls. Interest in xylanases has increased in the recent years due to their biotechnological applications, especially in the paper, food and textile industries. The aim of this study was to establish a purification methodology for an extracellular xylanase from *P. sclerotiorum*. The fungus was cultivated in Vogel liquid medium, pH 6.5, at 30 °C, under stationary condition, for 5 days, with oat spelt xylan as carbon source. The supernatant was separated from mycelium by vacuum filtration. The crude extract was centrifuged (10.000g, 10 min), dialyzed (3x, 50mM Tris-HCl buffer, pH 9.0) and fractionated on DEAE Sephadex A-50 column equilibrated with the same buffer. Proteins were eluted with a linear gradient 0-1.0 M NaCl. The elution profile apparently showed two forms of xylanase. The adsorbed fractions corresponded to 63.78 % of the total activity and these fractions were pooled. The protein sample was dialyzed against 50 mM ammonium acetate buffer, pH 6.8, lyophilized and dissolved in this same buffer. After, it was chromatographed on size exclusion column Sephadex G-75 also equilibrated with 50 mM ammonium acetate buffer, pH 6.8 and fractions with xylanase activity were pooled. The sample presented electrophoretic homogeneity, with a 2.45-fold purification and 27.11% of activity recuperation. Molecular mass estimated by SDS-PAGE and gel filtration was 23.88 and 23.77 kDa, respectively.

Key words: enzyme purification, *Penicillium sclerotiorum*, xylanase

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