

## Purification of a Cell-associated $\beta$ -Xylosidase from *Penicillium sclerotiorum*

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$\beta$ -xylosidases (E.C. 3.2.1.37) are hydrolytic enzymes, which play an important role in xylan degradation, hydrolyzing xylobiose and xylooligosaccharides to xylose from the non-reducing end. In recent decades,  $\beta$ -xylosidases have been used in many industrial processes, such as pulp and paper, food, beverage and textile. The aim of this study was to establish a purification strategy for a cell-associated  $\beta$ -xylosidase from the fungus *P. sclerotiorum*. The fungus was cultivated in Vogel liquid medium, pH 5.0, at 30 °C, under stationary condition, for 6 days, with oat spelt xylan as carbon source. The mycelium was washed with distilled and sterilized water, frozen and ground with sand in McIlvaine buffer pH 4.0. The suspension was centrifuged at 3.900g, 4 °C and the supernatant was used as a cell-associated enzyme source. Partial purification of  $\beta$ -xylosidase was achieved by fractional precipitation with ammonium sulfate. The supernatant of 60% ammonium sulfate saturation contained the highest enzyme proportion. This fraction was dialyzed against 50 mM ammonium acetate buffer, pH 6.8 and lyophilized. The protein sample was dissolved in this buffer and chromatographed on Sephadex G-100 column also equilibrated with 50 mM ammonium acetate buffer, pH 6.8. The molecular exclusion profile showed only one  $\beta$ -xylosidase form. Fractions with this activity were pooled and the sample submitted to SDS-PAGE analysis in which two bands with molecular mass of 81.59 and 42.36 kDa were verified. In non-denaturing PAGE, electrophoretical homogeneity was observed, revealing this protein possesses dimeric structure. A native molecular mass of 127.06 kDa was estimated by gel filtration. From this protocol a 4.85-fold purification was obtained and 46.07% of activity was recovered.

Key words: xylanase, enzyme purification, *P. sclerotiorum*

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