Extracellular Beta-Xylosidase from *Aspergillus ochraceus*: Purification and Biochemical Characterization

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Much attention has been given to xylan-degrading enzymes, including their potential industrial use in the animal feed, bread-making, and the paper and pulp industries. More recently, particular attention has been given to the enzymes involved in enzymatic degradation of arabinoxylan in relation to "second generation" biofuel process. β-Xylosidase attacks the non-reducing ends of short xylooligosaccharides to liberate xylose and catalyse the cleavage of xilobiose, and it is rate limiting in arabinoxylan hydrolysis. The aim of this work was to purify and to study the biochemical properties of the extracellular β-xylosidase from *A. ochraceus.* The enzymatic activity was measured by *p*-nitrophenol method. The enzyme was purified by sequential elution in DEAE-cellulose, Sephadex G-100 and Biogel P-60 chromatography columns. Analysis in SDS-PAGE showed a single protein band with a molecular mass of about 141 kDa. Maximal activity was achieved at pH 3.0-5.0 and at a temperature of 75ºC. The β-xylosidase was stable for one hour at 55ºC and at 60ºC the enzymes exhibited around 30% of activity after one hour of incubation. This enzyme also presented stability in all range of tested pH (2.5-8.0). Analysis of the hydrolysis products on TLC using birchwood xylan showed the presence of only xylose. The enzymatic activity increased significantly in the presence of $CaCl_2$ and KCl. Total neutral carbohydrate was estimated as 38.8%. The purified enzyme hydrolyzed mainly *p*-nitrophenyl-β-D-xylopyranoside, and in a minor extension *p*-nitrophenyl-β-D-glucopyranoside. The apparent Km and Vmax values were 0.66 mM and 39.3 U/mg protein.

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