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Enzymatic hydrolysis of xylooligosaccharides monitored in real-time by mass spectrometry

Paulo R Heinen
University of São Paulo, Brazil

The present study describes the one-step purification and biochemical characterization of an endo-1,4-β-xylanase from Aspergillus tamari Kita. Extracellular xylanase was purified to homogeneity 7.43-fold through CM-cellulose. Enzyme molecular weight and pI were estimated to be 19.5 kDa and 8.5, respectively. The highest activity of the xylanase was obtained at 60°C and it was active over a broad pH range (4.0–9.0), with maximal activity at pH 5.5. The enzyme was thermostable at 50°C, retaining more than 70% of its initial activity for 480 min. The K0.5 and Vmax values on beechwood xylan were 8.13 mg/mL and 1,330.20 μmol/min/mg of protein, respectively. The ions Ba^{2+} and Ni^{2+}, and the compounds β-mercaptoethanol and DTT enhanced xylanase activity, while the heavy metals (Co^{2+}, Cu^{2+}, Hg^{+}, Pb^{2+} and Zn^{2+}) strongly inhibited the enzyme, at 5mM. Enzymatic hydrolysis of xylooligosaccharides monitored in real-time by mass spectrometer showed that the shortest xylooligosaccharide more efficiently hydrolyzed by A. tamarii Kita xylanase corresponded to xylopentaose. In agreement, HPLC analyzes did not detect xylopentaose among the hydrolysis products of xylan. Therefore, this novel GH11 endo-xylanase displays a series of physicochemical properties favorable to its application in the food, feed, pharmaceutical and paper industries.

Biography

Maria de Lourdes Teixeira de Moraes Polizeli has graduate at Faculdade of Farmacia and Odontologia from Ribeirao Preto at São Paulo University (1982), master and PhD degree in Biochemistry from São Paulo University (1986 and 1991). Has experience in cell biology, microbiology, biochemistry and microorganisms physiology, working mainly with the following subjects: bioprospecting, enzymatic production, purification and characterization of amylases, lipases, xylanases, cellulases, ligninases, pectinases, phosphatases and phytase of thermotolerant filamentous and thermophilic fungi. As well as enzymes application from wild and recombinant fungal lines by heterologous expression, bio bleaching of cellulose pulp, second generation ethanol and in ruminant and monogastric feed.

polizeli@ffclrp.usp.br

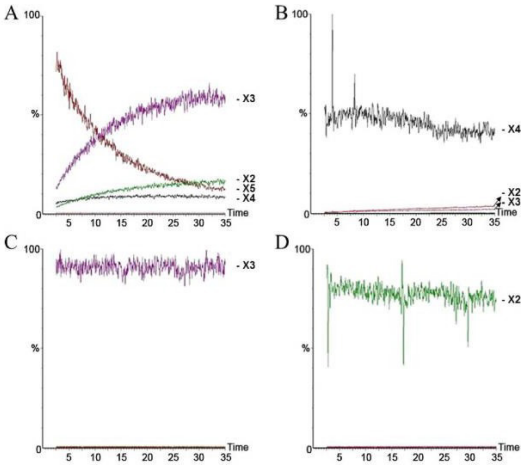

Fig 1. Analysis of endo-xylanase activity monitored in real- time by mass spectrometry using different XOS as substrates: (A) xylopentaose-X5; (B) xylotetraose-X4; (C) xylotriose-X3 and (D) xylobiose-X2.

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