Abstract

The present study investigates the purification and biochemical characterization of an extracellular thermostable xylanase (called XYN35) from Caldicoprobacter algeriensis sp. nov., strain TH7C1^{T}, a thermophilic, anaerobic strain isolated from the hydrothermal hot spring of Guelma (Algeria). The maximum xylanase activity recorded after 24 h of incubation at 70 °C and in an optimized medium containing 10 g/L mix birchwood- and oats spelt-xylan was 250 U/mL. The pure protein was obtained after heat treatment (1 h at 70 °C), followed by sequential column chromatographies on Sephacryl S-200 gel filtration and Mono-S Sepharose anion-exchange. Matrix assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF/MS) analysis indicated that the purified enzyme is a monomer with a molecular mass of 35,075.10 Da. The results from amino-acid sequence analysis revealed high homology between the 21 NH_2-terminal residues of XYN35 and those of bacterial xylanases. The enzyme showed optimum activity at pH 11 and 70 °C. While XYN35 was activated by Ca^{2+}, Mn^{2+}, and Mg^{2+}, it was completely inhibited by Hg^{2+} and Cd^{2+}. The xylanase showed higher specific activity on soluble oat-spelt xylan, followed by beechwood xylan. This enzyme was also noted to obey the Michaelis-Menten kinetics, with K_m and k_{cat} values on oat-spelt xylan being 1.33 mg/mL and 400 min^{-1}, respectively. Thin-layer chromatography soluble oat-spelt xylan (TLC) analysis showed that the final hydrolyzed products of the enzyme from birchwood xylan were xylose, xylobiose, and xylotriose. Taken together, the results indicated that the XYN35 enzyme has a number of attractive biochemical properties that make it a potential promising candidate for future application in the pulp bleaching industry