ENZYMATIC CHARACTERIZATION OF THE RECOMBINANT BETA-XYLOSIDASE XynB2

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Abstract— Enzyme research on thermophilic microorganisms has steadily increased given their high catalytic capabilities in extreme conditions, ideal for biotechnological as well industrial processes. XynB2, a β-xylosidase from Geobacillus stearothermophilus, and to date the only enzyme of this type displaying transglycosidase activity, offers attractive catalytic applications. Though previously biophysically and biochemically characterized, no detailed kinetics studies on the hydrolysis of various aryl-β-D-xyloside substrates have been reported. The goal of this study was to characterize XynB2 under maximal activity conditions (pH and temperature). XynB2 was expressed in E. coli C43 using the recombinant plasmid pJAVI91 which encodes the protein with a histidine tail (His-tag) at the stop codon, facilitating purification using Ni affinity chromatography, followed by size-exclusion chromatography. Purified XynB2 reached highest activity at 65 °C and pH 7.0, employing 4 methylumbelliferyl xylopyranoside as substrate. Calculation of kinetic parameters gave as result a KM =0.20 ± 0.04 mM, Vmax =0.57 ± 0.06 nmol/s, kcat =203 ± 21 (s-1) and kcat/KM = 1017 ± 230 (mM-1·s-1), under maximal activity conditions. The thermal stability of XynB2 was evaluated to be 69.0 ± 0.6 °C, with a t1/2 of 116 ± 10 min, corresponding to a first order reaction. These results correlate with others reported in the literature from similar thermophilic microorganisms. Complete analysis employing other substrates is being developed. These results may be useful for other applications, like enzyme immobilization, in order to perform catalysis over other type of substrates, like those coming from wastewater, sewage, or industrial residues rich in xylans and/or cellulose.
Keywords—aryl-β-D-xylosides, enzyme catalysis, kinetic parameters, xylanase.