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A novel β-xylosidase from *Anoxybacillus* sp. 3M towards an improved agro-industrial residues saccharification

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ABSTRACT

An intracellular β-xylosidase (AbXyl), from the thermoalkaline *Anoxybacillus* sp. 3M, was purified and characterized. The homodimeric enzyme (140 kDa) was optimally active at 65 °C and pH 5.5, exhibited half life of 10 h at 60 °C, 78 and 88% residual activity after 24 h, at pH 4.5 and 8.0, respectively. Fe^{2+}, Cu^{2+}, Al^{3+}, Ag^{+} and Hg^{2+} inhibited the enzyme; the activity was moderately stimulated by SDS and not influenced by β-mercaptoethanol. In the presence of *p*-nitrophenyl-β-D-xylopyranoside, AbXyl exhibited Km of 0.19 mM, Kcat of 453.29 s^{-1}, Kcat Km^{-1} of 2322 s^{-1} mM and was moderately influenced by xylose (Ki 21.25 mM). The enzyme hydrolyzed xylo-oligomers into xylose and catalyzed transxylosilation reactions also in presence of alcohols as acceptors, producing xylo-oligosaccharides and alkyl-xylosides. Finally AbXyl was applied towards a statistically optimized process of brewery's spent grain bioconversion, highlighting the important role of this biocatalyst in reaching high yields of fermentable sugars.

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1. Introduction

Xylan is, after cellulose, the most abundant renewable carbon source present in wood and agricultural residues. Xylans consist of β-(1,4)-linked D-xylopyranose residues substituted with α-L-arabinofuranose, 4-O-D-methyl-glucuronic acids and acetyl groups [1]. In nature the complete hydrolysis of xylan occurs by the synergistic action of several enzymes, among which the *endo*-β-1,4-xylanases and 1,4-β-xylosidases play the major role. The xylan deconstruction starts with the action of debranching enzymes such as α-L-arabinofuranosidases, α-D-glucuronidases and acetyl xylan esterases which remove the side chains that hinder the xylanases attack onto the polysaccharide. The internal β-1,4-xylosidic linkages in the xylan backbone are cleaved by the *endo*-β-1,4-xylanases, yielding soluble xylo-oligosaccharides which are hydrolyzed to D-xylose by 1,4-β-xylosidases that proceed from the non-reducing ends [2,3]. These biocatalysts are also necessary to reduce the end product inhibition that usually affects the *endo*-β-1,4-xylanases and for this reason are rate-limiting in xylan degradation [4]. Currently, growing interest in xylan bioconversion has arisen due its potential applications in several agro-industrial processes. These include the conversion of hemicellulosic materials for second generation biofuel production in which the enzymatic depolimerization of xylan represents an eco-friendly alternative to the physicochemical extraction of lignin and hemicellulose [5]. Moreover the recovery of xylose in a free form from lignocellulosic wastes as substrates is significant for the overall efficiency of bio-ethanol production process [6]. Hemicellulases have also many other applications on industrial scale. A complete biodegradation of xylans is one of the goals for the paper industry [7] and the exploitation of xylanolytic activities is also relevant in feed industries to hydrolyze the hemicelluloses in cereals for enhancing the availability of nutrients and promoting their absorption [2]. Xylanases are employed also in food sectors to improve bread dough quality [8], to promote the release of aroma during wine making process [9] and simultaneous recovery of free D-xylose [10]. Another industrial application of particular interest for β-xylosidases is the synthesis of xylo-oligosaccharides, valuable compounds with prebiotic and drug functions [11]. New xylosidic linkages can be catalyzed by retaining-xylosidases in transxylosilation reactions and the alkyl-xylosides, that can be obtained by this process, are non-ionic surfactants endowed with relevant properties to be utilized in pharmaceutical, cosmetic and food industries [12].

Anoxybacillus has currently been studied as a genus comprising important species from the perspective of biomass saccharification. A cellulose degrading strain *A. flavithermus* BTN7B, isolated from Egyptian soils [13] was reported to reach an high cellulase production in presence of sucrose as carbon source. Recently, sequenced genomes of *Anoxybacillus* spp. revealed the presence of several genes coding for

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