TITLE: CHARACTERIZATION OF A NOVEL GH3 β-XYLOSIDASE FROM A CAATINGA GOAT RUMEN METAGENOMIC LIBRARY

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ABSTRACT:

Hemicellulose is the second most abundant polysaccharide in plant biomass. One of the most common components of hemicellulose is xylan, which is β-1,4-linked xylose residues that may be chemically modified by acetyl-, arabinofuranosyl- and glucuronyl- groups. Endo-1,4-β-xylanases (EC 3.2.1.8), which cleaves the xylan backbone, and β-D-xylosidases (EC 3.2.1.37), which cleave xylobiose into xylose monomers, are two fundamental enzymes in the degradation of hemicellulose. Thus, β-xylosidases have application in plant biomass deconstruction, and the released sugars can be fermented into a number of chemically valuable industrial products, ranging from the biofuel ethanol to the sugar substitute xylitol. Screening of metagenomic libraries is an effective approach for the identification of novel enzymes. Here we report a functional screen of a goat rumen metagenomic library using esculin as a substrate, and the identification of a gene from an unknown bacterium encoding a novel GH3 enzyme named BGL11. Most of the kinetically characterized β-xylosidases belong to the GH43 family, and were characterized using the synthetic substrate ρNPX (ρ-nitrophenyl-β-D-xylopyranoside). This enzyme was expressed in *Escherichia coli* and purified using affinity chromatography. Enzyme activity was tested with 12 different substrates, some synthetic and some natural, and results showed that this enzyme is a β-xylosidase, being most active toward xylobiose. None of the closely related genes have been previously characterized. Optimal pH was determined to be 5.6, and the optimal temperature for enzyme activity was 50 °C. Enzyme stability, an important parameter for industrial application, was investigated. Using a Plakett and Burman method analysis, it was possible to identify five parameters affecting BGL11 enzymatic activity, including four monosaccharides, a uronic acid, and a salt. Enzyme kinetic parameters of Km and Vmax using xylobiose will also be presented, and were compared to others from previously characterized β-D-xylosidases. Until today, few studies used natural substrates to characterize the enzyme kinetic parameters and they are more relevant for industrial applications.

Keywords: xylobiose, biochemistry, GH3.

Funding Agency: CNPq, FAP-DF, Embrapa.