TITLE: BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF A GH3 β-GLUCOSIDASE OBTAINED FROM *SYNTERMES WHEELERI* GUT METAGENOME

AUTHORS: LIMA, R.A.T.; SANTANA, R.H.; OLIVEIRA, G.M.; GARAY, A.V.; SOUZA, A.A.; BARBOSA, J.A.R.G.; FREITAS, S.M.; KRÜGER, R.H.

INSTITUTION: UNIVERSIDADE DE BRASÍLIA, BRASÍLIA, DF (UnB 1º ANDAR - ASA NORTE, BRASÍLIA - DF, 70910-900, BRASÍLIA – DF, BRAZIL)

ABSTRACT:

Cellulases are enzymes of the family Glycosil hydrolases (GHs) that are involved in the deconstruction of cellulose, being widely used in different industrial applications, contributing to the generation of value-added products. In this study we selected metagenomic sequences of *Syntermes wheeleri* gut, an endemic termite from the Brazilian Cerrado, for selection, production, purification and characterization of a cellulase. Among the sequences selected and produced in *Escherichia coli* BL21 (DE3), only one was chosen for the work, a β-glucosidase GH3 (β-G 72-26). This one was purified by size exclusion chromatography, having approximate size of 89.7 KDa on 12% SDS-PAGE gel. The enzyme was characterized in terms of pH and optimum temperature, when two ranges of optimum pH, pH 7.0 and 10.0, and an optimum temperature of 40 ºC were observed. By means of the kinetic parameters were observe better enzymatic activity at basic pH, but better affinity (K_m) for the pNPG substrate at neutral pH. Furthermore, at neutral pH the β-G 72-26 showed better catalytic efficiency (K_{cat} / K_m). The results of circular dichroism showed that the secondary structure of the enzyme is pH-dependent, with different percentages of alpha helix structures and beta sheets, being more structured at pH 10.0. In the results of thermostability were detected the denaturation of β-G 72-26 in both pHs, less in acid pH (4.0). As a structural analysis, the β-glucosidase in the study was aligned with structured sequences of PDB (Protein Data Bank) for analyzes of conserved domains and catalytic sites, when the presence of 3 domains was verified: (1) (α / β) 8-barrel (TIM barrel), (2) (α / β) 6 sheet (β-sandwich) and (3) Fibronectin III, with function not yet known. The conserved catalytic sites were Asp233 and Glu417. By means of the results obtained β-G 72-26 is a candidate for industrial applications, besides contributing to the characterization of a new β-Glucosidase GH3, which belongs to an endemic termite in the Brazilian Cerrado.

Keywords: β-glucosidase, metagenome, termite, *Syntermes wheeleri*

Development Agency: Fundação de Apoio a Pesquisa do Distrito Federal (FAPDF)