

**TITLE:** PROSPECTION OF CELULOLYTIC ENZYMES IN BACTERIA ISOLATED FROM THE GUT OF *Hypsipyla* spp. CATERPILLARS

**AUTHORS:** SILVA, Y. R. O.; SANTOS, A. V.; XAVIER, L. P.

**INSTITUTION:** UNIVERSIDADE FEDERAL DO PARÁ (RUA AUGUSTO CORRÊA, 1 – GUAMÁ, CEP 66075-110, BELÉM – PA, BRAZIL)

**ABSTRACT:** The use of enzymes in industrial processes has been increasing every year. Cellulolytic enzymes are extensively used in pulp and paper industry, textile industry, food processing and detergent industry, as well as in the production of ethanol from biomass, and there is a need for novel enzymes with different properties. The main predator of andiroba (*Carapa guianensis* Aublet) is the insect popularly known as Broca-da-andiroba, which comprises two species that feed on andiroba seeds, *Hypsipyla grandella* and *Hypsipyla ferrealis*. Little is known about the physiologies and digestive enzymes of both these insects and the microorganisms in their guts, although insect endosymbionts being known as promising but unexplored sources of hydrolytic enzymes. Thus, the objective of this study was to isolate and identify the gut bacteria of *Hypsipyla* spp. caterpillars, and evaluate their cellulolytic enzymes. Whole guts of caterpillars obtained from predated seeds were extracted and added to Luria-Bertani broth medium for cultivation, and the bacteria were isolated by spreading serial dilutions of the broth on Luria-Bertani agar plates. The microorganisms were identified using a MALDI Biotyper CA System, and the cellulolytic activities were screened by growing the microorganisms in liquid media and adding the extracellular supernatant to wells in solid media containing the substrate. The effect of pH on  $\beta$ -glucosidase activity was tested colorimetrically using p-nitrophenyl-glucopyranoside. Eight bacteria from the species *Proteus mirabilis*, *Pseudomonas taetrolens*, *Enterococcus casseliflavus*, *Klebsiella* sp., *Enterococcus* sp. and *Pseudomonas* sp. were isolated, and all of them presented positive results for  $\beta$ -glucosidase activity and negative results for endoglucanase activity. In six isolates, the highest  $\beta$ -glucosidase activity was detected in pH 8.0, while the other two presented highest activities in pH 7.0 and pH 8.6. Most  $\beta$ -glucosidases have a pH optimum in acidic pH (3.5 to 5.5 range), thus higher activities in alkaline and neutral pH may allow a broader application of this class of enzymes.

**Keywords:** *Hypsipyla* spp.,  $\beta$ -glucosidase, cellulolytic enzymes

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