Biotechnological Potential of Secreted Cellulases from Cultivable Aerobic Bacteria Isolated from the Giant Snail *Achatina fulica*

Pinheiro, G.L.¹,²; Corrêa R.F.C.¹; Lourenço, D.C.¹; Rosa, M.S.¹; Lima L.S.¹; Cardoso A.M.¹; Garcia E.S.¹,³; Souza, W.¹,² and Carvajal, S.F.¹,².

¹Laboratório de Biotecnologia (Labio), Instituto Nacional de Metrologia, Normalização e Qualidade Industrial (Inmetro), RJ, Brazil; ²Laboratório de Ultraestrutura Celular Hertha Meyer, Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, UFRJ, RJ, Brazil; ³Instituto Oswaldo Cruz, Fiocruz, RJ, Brazil.

The enzymatic hydrolysis of cellulose by cellulases is one of the major limiting steps in the conversion of lignocellulosic biomass to yield bioethanol. Great efforts have been done to identify novel cellulases. The snail *Achatina fulica* is a gastropod with high cellulolytic activity, mainly due to the abundance of glycosyl hydrolases produced by the animal and the resident microbiota. The main focus of our work was to isolate cellulolytic bacteria from the gastrointestinal tract of *A. fulica* and evaluate the biotechnological potential for their secreted cellulases. First, the luminal content was collected and plated onto solid minimal media containing carboxymethylcellulose (CMC) as unique carbon source. The resulting colonies were isolated and screened for cellulase activity. 42 cellulolytic bacterial strains were isolated and identified. The secreted enzymes from the strain with the highest cellulolytic activity were characterized by enzymatic assays and the optimal temperature, pH and thermo stability were performed. Zymograms of concentrated bacteria culture supernatant showed the presence of a multimeric enzyme with high CMCase activity and an estimated molecular mass of 215 kDa. To identify these secreted CMCase components, enzymes were separated by SDS-PAGE and four selected spots were subjected to mass spectrometry analysis. Two protein spots were positively identified based on the Mascot search engine (100% homology) as *Streptomyces* sp. bacterial cellulases belonging to the glycosyl hydrolase family 6 (GHF-6). Our results indicate that *A. fulica* is an important model to isolate cellulolytic bacteria with biotechnological potential in biomass ethanol production.

Word Keys: Cellulolytic Bacteria, Cellulase, Bioethanol, *Achatina fulica*.

Supported by: Inmetro, Petrobras/Cenpes, CNPq and