

Identification of Clones with β -Glucosidase and Cellobiohydrolase Activities from a Goat Rumen Metagenomic Library

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The genomes present in an environmental sample can be accessed through metagenomic expression libraries. With this approach, the biotechnological potential of cultivated and uncultivated microorganisms in a biological sample can be exploited. Screenings of metagenomic libraries can be performed aiming the bioprospection of enzymes of biotechnological interest. The goal of this study was the pursuit of cellulases enzymes able to deconstruct plant biomass to be used in the production of second generation ethanol. A small insert metagenomic library was constructed using environmental DNA from the solid portion of *Moxotó* goats' rumen, a breed of goats native to the semi-arid region of Brazil. DNA with sizes ranging from 5-8 kb were cloned into a plasmid and transformed into *Escherichia coli*. Approximately 50,000 clones were obtained. A total of 10,839 clones from this library were screened for cellobiohydrolase and β -glucosidase activities in LB agar medium containing synthetic substrates. Three β -glucosidase and two cellobiohydrolase clones presenting strong phenotypes had their enzymatic activities confirmed by retransforming their plasmidial DNA into *Escherichia coli*. These clones were completely sequenced by primer walking. Similarity to other proteins was identified using ORFfinder, Blastp and Blastx tools at NCBI. One clone with β -glucosidase activity showed similarity to a major facilitator superfamily transporter. Another one aligned with a family 3 glycosyl hydrolase protein. The clones with cellobiohydrolase activity showed similarity to cellulases from the microbiota of other ruminant. Phylogenetic analyses of the genes identified will be presented. The kinetic properties of these enzymes will be studied after they are produced by heterologous expression.

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