Bioprospection of Clones with Endoglucosidase and Xylanase Activities from a Goat Rumen Metagenomic Library

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With a metagenomic library one can access genetic information of any microorganism directly from environmental samples. With this method, the biotechnological potential of cultivated and uncultivated microorganisms in a specific biological sample can be exploited. Screenings of metagenomic libraries can be performed aiming the bioprospection of enzymes of biotechnological interest. This study aimed at prospecting for plant cell wall hydrolases to be used in the industrial production of second-generation bioethanol. A small insert metagenomic library containing approximately 50,000 clones was constructed using environmental DNA from the solid portion of *Moxotó* goats' rumen, a breed of goats native to the semiarid region of Brazil. A total of 12,672 clones from this library were screened for endoglucanase and xylanase activities in LB agar medium containing the specific substrates. Four hundred and sixty two positive clones were identified in the primary screen: 340 clones with endoglucanase activity, 48 with xylanase activity and 74 with both activities. These clones were analyzed for the pattern of bands when digested with restriction enzymes to identify which were different clones. To confirm the enzymatic activities of clones the plasmid was extracted and used to retransform *Escherichia coli*. Clones whose enzymatic activity was corroborated are being sequenced. Similarity to other proteins has been investigated using ORFfinder, Blastp and Blastx tools at NCBI. Some clones with endoglucosidase activity showed similarity to glycosyl hydrolases proteins, while others showed similarity to proteins from the microbiota of other ruminants. Phylogenetic analyses of the genes identified will be conducted. In addition, these enzymes will be produced in heterologous expression systems so that they can be further characterized for their kinetic properties.

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