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7-44: Identification of glycosyl hydrolases in the microbial biodiversity of Brazil using a metagenomic approach

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One of the challenges of developing a process to make ethanol from biomass is to obtain enzymes that are efficient and cheap. The metagenomic approach can complement efforts directed at the culturable microbiota to identify such enzymes. The goat rumen and the Amazon forest soil have microbial communities that specialize in degrading plant biomass. To discover enzymes that can be used to deconstruct the plant cell wall, we have constructed and functionally screened three metagenomic expression libraries. The environmental DNA to construct these libraries was obtained from the rumen of the Brazilian goat breed 'Moxotó', and soil from two different locations in the Amazon region: one near the city of Manaus and the other near the city of Belém. The Moxotó goat rumen metagenomic library contained approximately 50,000 clones with small-sized inserts in the range of 3-8 kb. The metagenomic library from the Belém region contained approximately 70,000 clones in the 3-8 kb range, while the library from the Manaus region contained approximately 200,000 clones in the 30-50 kb range. Functional screens were optimized to identify endoglucanase, β-glucosidase, cellobiohydrolase and xylanase activities. A number of clones with endoglucanase, β-glucosidase, cellobiohydrolase and xylanase activities have been identified. After confirmation of phenotypes and restriction enzyme digestion to determine clone uniqueness some clones were fully sequenced. Sequence analysis revealed some ORFs similar to known glycosyl hydrolases, however, in a number of cases, no ORFs with similarity to this class of enzymes were found. Protein expression and enzyme characterization for chosen clones are underway.

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