Screening for enzymes with biotechnological potential use for lignocellulosic bioethanol production

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Finding alternative ways to produce renewable energy is today a priority to a number of countries including Brazil. Second generation ethanol is one possibility. Its production process is based on the breakdown of complex carbohydrates found in biomass into simple sugar molecules that can be fermented into ethanol. Enzymes that degrade this cellulose are key in this process and these may come from microorganisms. Given that only 1% of the existing microbial diversity is known through culturing techniques, accessing the metabolic wealth of uncultured microorganisms using a metagenomic approach is an interesting strategy. The microbial community found in the soil of the Amazon forest in Brazil has a microbial community specialized in the degradation and recycling of plant cell wall carbohydrates from fallen leaves. The purpose of this work was to construct a large insert metagenomic library from the Amazon soil microbial community and to screen this library for hydrolytic enzymes useful in the production of second generation bioethanol. For this, DNA was extracted from the Amazonian native forest soil collected in the region of Moju (PA) in November 2009. The DNA was purified by Pulse Field Gel Electrophoresis and DNA fragments between 40 and 50 kb were selected for library construction. A fosmid library of 210,000 clones was obtained and screened for □-glucosidases, cellulases and xylanases. Two positive clones for β-glucosidase have already been confirmed and subcloning of fragments for sequencing is in progress. Furthermore, 10 clones with activity towards low viscosity CMC cellulose, 16 with activity towards high viscosity CMC cellose and 14 with activity towards xylanase have been identified. For β-glusosidase activity, the screening effort covered 50% of the library, while for the cellulase and xylanase, only 6% of the library has been screened. Our results suggest that the Amazon soil has an extensive amount of microorganisms that produce plant cell wall degrading enzymes that may be exploited for biotechnological purposes.

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