023 -CHARACTERIZATION OF HYDROLYTIC ENZYMES ISOLATED FROM AN AMAZON SOIL ENVIRONMENTAL DNA LIBRARY

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Current environmental and political problems related to diminishing oil stocks have pushed for the search of renewable alternatives to fossil fuels. An attractive candidate for fuel source is plant biomass, which is abundant, cheap and renewable. Plant matter can be used to produce second generation biofuels, such as bioethanol derived from lignocellulosic biomass. Nonetheless, plants have cell walls, which have very complex structures that require a great deal of energy input to break its bonds, making the process of producing second generation biofuels expensive and thus ineffective. On the other hand, microbial enzymes have long been used for industrial purposes and these could be used to break the complex bonds in plant cell walls. The process of deconstructing the plant cell wall requires the synergism of many enzymes, including endo-and exoglucanases, xylanases, β-glucosidases, amylases, proteases and others. In the quest for new microbial hydrolytic enzymes, the Brazilian Amazon soil was chosen to be studied for two main reasons. First, the soil contains organic matter and it is believed that there are a great number of microorganisms
participating in the biogeochemical cycles associated to it. Also, there are few studies related to the Amazon biome and even less studies related to its microbiota. Since only about 1 % of microorganisms can be readily cultured by traditional methods, culture-independent molecular techniques such as metagenomics are more adequate for biotechnological applications. In this study, a small insert metagenomic library was constructed using *Escherichia coli* as a host by cloning environmental DNA from the Brazilian Amazon soil into a vector. The library generated has about 70,000 clones and their insert size varies from 3 to 8 kb. Over 123,000 clones of this library were screened for several enzymatic activities, such as amylase,
β-glucosidase, endoglucanase, exoglucanase, lipase, protease and xylanase. A total of seventeen positive clones with various enzymatic activities were isolated, but only five –one amylase, three β-glucosidase and one endoglucanase –were chosen for further testing. These clones are currently being sub-cloned and sequenced to identify the location of the genes conf erring their enzymatic activities. The enzymes produced by these clones will be characterized biochemically for their optimal pH and temperatures.

Financial Support: CNPq, FAP-DF, CAPES.