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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 conferring their enzymatic activities. The enzymes produced by these clones will be characterized biochemically for their optimal pH and temperatures.

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024 - BACTERIAL RESISTOME ASSOCIATED WITH CERRADO STRICTO SENSU SOIL

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The general misuse of antibiotics promotes the selection of resistant microorganisms and allows mobilization and persistency of antibiotic resistance genes in the environment. Moreover, it is established that antibiotics and resistance genes play an important ecological role, as they are involved in signaling processes between

microorganisms and microbial communities. Hence, the unbalance in the concentration and selection of antibiotic resistance genes in the environment alter the signaling flow between microorganisms with unpredictable implications in the ecology of an ecosystem.

More information about the origins, reservoirs and movement of the antibiotic resistance genes is essential for the identification of new genes and assessment of their diversity, increasing the knowledge about the role of antibiotics and resistance genes in nature. The antibiotic resistome is the collection of antibiotic resistance genes of an environment. The main reservoir of these genes is the soil, a biodiverse and unappreciated environment in this context. Metagenomics has allowed for the access to a larger quantity of genetic material from environmental samples and, as such, allowed for the identification new genes and functions of culturable and not yet culturable microorganisms. The description of the soil resistome and the elucidation of the antibiotic resistance mechanisms provide more data about the role of resistance genes – and of antibiotics – in nature.

Two metagenomic libraries were previously constructed with soil samples from Cerrado *Stricto Sensu* collected in Distrito Federal. They differentiate in insert size and cloning vectors, as they were divided in small DNA insert and large DNA insert libraries, in pCF430 and pCCFOS vectors, respectively. The pCF430 library has median size of 8 Kb and the pCCFOS library has 35 Kb-size inserts.

The two libraries were screened for resistance to the following β-lactamic antibiotics: amoxicillin, ampicillin, carbenicillin, cefalexin, cefamandole, cefoxitin, ceftazidime, penicillin G and piperaciclin. In total, forty-four resistant clones were isolated to five of the nine antibiotics, in the two libraries. Of these, four clones from the small-insert library were selected for further tests and DNA sequencing. The implications of these new antibiotic genes in an ecological and biotechnological perspective will be discussed.

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