FUNCTIONAL SCREENING FOR AMYLASES AND PROTEASES ACTIVITY OF A BRAZILIAN MANGROVE METAGENOMIC LIBRARY

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Mangroves are costal ecosystems located in estuaries shorelines with unique and specific characteristics where extreme environmental conditions are found, such as periodic flooding by tides and variations in salinity, temperature and nutritional availability. Those environments have intense biological activities and many of theirs valuable processes are directly related to their sediment microorganism's activity. Taking this into account, summed to the fact that the studies about mangroves are scarce; those ecosystems are promising source for the discovery of novel microorganisms and/or genes that can be used for various biotechnological applications. Among the possible applications is the field of enzymes, in which researchers have been searching for more effective molecules able to improve the current processes. It is also known that only a very small percentage of soil microorganisms are cultivated on the existing laboratory conditions while the functional metagenome approach allows to access uncultured microorganisms genes. Hence, the aim of this work was to study the enzymatic potential of a mangrove metagenomic library by performing a screening to select clones containing the genes for amylases and proteases. The metagenomic library was constructed using the extracted microbial DNA from sediment of the Jaguaribe River mangrove, Ceará. Afterwards, the obtained DNA was fragmented and the inserts ranging from 1 to 8 Kb were ligated in the vector pJET1.2/*blunt* and used to transform *Escherichia coli* TOP 10F'. A total of 1152 clones were tested in Luria Broth (LB) agar containing ampicillin and supplemented with 1% skimmed milk, for the protease assay; or 1% of starch, for the amylase assay. The protease activity was detected by the formation of cleared zones (halos) around the colonies after 48 h of incubation at 37°C. The amylase activity was detected by the formation of cleared zones around the colonies after adding 1% Lugol's iodine solution (I_2KI). A number of 24 (2.08%) clones positive for amylase and 88 (7.63%) clones positive for protease were selected. Those results are very encouraging and are a starting point for better understanding of the mangroves potential for novel enzymes. The next steps involve the gene sequencing, the high yield expression and purification of the obtained enzymes.

Keywords: mangrove, functional metagenome, amylase, protease

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