TITLE: PROSPECTION AND OVEREXPRESSION OF NOVEL ESTERASE FROM THE METAGENOMIC LIBRARY OF A MANGROVE SOIL

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ABSTRACT:
Mangroves are costal ecosystems with unique ecological dynamics, where extreme environmental conditions are found such as periodic flooding, variations in temperature and high contents of salt and metal ions. Therefore, mangrove habitats are considered valuable source for the discovery of novel microbial enzymes that tend to be resistant to those harsh conditions. Lipases and esterases constitute the most important group of biocatalysts being used in various biotechnological applications like detergent production, leather processing, cosmetic production and use in perfumes and biodiesel. In this context, the current work aimed the screening and posterior overexpression of lipases/esterases from a metagenomic library from previously constructed using DNA from mangrove sediments of Jaguaribe, Ceará, Brazil. A total of 1152 clones were screened in LB agar supplemented with 1% (v/v) tributyrin and ampicillin (100 µg/ml) by the formation of a clear halo around the colonies. The positive clones were subjected to plasmid extraction and sequencing. The selected lipase/esterase genes were subsequently cloned in an expression vector (pET302-NT) and inserted in different expression *Escherichia coli* strains. A number of 3 clones (LipG6, LipG7 and LipH7) showed lipolytic activity presenting an insert DNA size of approximately 4.0, 2.9 and 0.8 Kb, respectively. The sequences were analyzed by performing Blastx against the non-redundant database from NCBI. Complete sequence of the clone LipG7 (1179 bp) reached 80% identity with 1.4-butanediol diacrylate esterase from *Porticoccus hydrocarbonoclasticus*. The low amino acid sequence identity with a bacterial esterase previously described, suggests that could be a novel esterase. Plasmidial DNA from the clones LipG6 and LipH7 revealed no homology to any known lipase or esterase, and the gene responsible for their lipolityc activity could not be annotated. The overexpression of LipG7 esterase was tested in the different expression strains (*E. coli* BL21, Rosetta-gami and Artic Express), under various IPTG concentrations (0.1, 0.5 and 1mM) and temperatures (13, 20, 30 and 37° C) at 200 rpm, for optimal expression conditions. All expression tests led to the achievement of the recombinant LipG7 esterase in inclusion bodies. The results so far, support the idea that mangroves are promising sources for novel genes and the next steps involve refolding and purification assays of the recombinant LipG7 esterase.

Keywords: bioprospecting, *Porticoccus*, lipase, tributyrin, metagenomics.

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