TITLE: MOLECULAR CLONING AND HETEROLOGOUS EXPRESSION OF A METAGENOMIC ESTERASE IN *E. COLI* AND *PICHIA PASTORIS*

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ABSTRACT:

Metagenomic libraries from diverse environments have been extensive sources of many lipases and esterases; nevertheless, most of these enzymes are biochemically uncharacterized. We built a metagenomic library from small-insert metagenomic library (insert size 3-8 Kb) constructed with DNA samples from Cerrado soil and tested it for lipolytic activity. In the present study, we identified 3 clones (Clones X, Y, and W) selected and sequenced from 500 clones of the metagenomic library, this alowed the identification of the 4 proteins: LipX, LipY,LipW and LamG.The protein from Clone X, LipX exhibited 79 % amino acid identity with the Lipase/Esterase from Bradyrhizobium sp [GenBank: WP024510238.1] and was classified into lipolytic enzyme family IV (the family with vast number of Patents). The protein was expressed in *E.Coli* BL 21(DE3) using as a vetor pET24 and in *Pichia Pastoris* X33 using pPICZαA. under the control of the AOXI promoter. The recombinant LipX have a molecular mass of ~34 kDa, which agrees with its predicted molecular mass and an pI 5.34. Biochemical characterization revealed that presents high activity in a wide range of temperature with an optimum pH of 8.0. The enzyme exhibited activity against p-nitrophenyl esters of different chain lengths and highest catalytic efficiency against p-nitrophenyl butyrato. Furthemore, the homology model of Lip X was built and compared to other esterases showed that the large alpha-beta domain is conserved. In summary, LipX is an esterase/lipase from Cerrado soil metagenomic library that has been cloned, expressed, and characterized for the first time in *Pichia pastoris* and its biotechnological applications will be discussed.

Keywords: esterase, soil, metagenome

Development Agency: CNPq, FAPDF and CAPES.