ENZYMATIC ENGINEERING OF MICROBIAL LIPASES AND PHOSPHOLIPASES AND THEIR USE BIOCATALYSTS FOR FOOD APPLICATIONS

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ABSTRACT

Background and aim: Currently, chemicals from different industries pose serious problems for human health and the environment. Biotechnology has enabled these sectors to develop new or better products and be more environmentally friendly. Microorganisms, play a fundamental role in the functioning of natural ecosystems through to a considerable number of active molecules. Among these, lipolytic enzymes, biocatalysts of a protein nature having catalytic properties. They constitute a biological alternative to chemical agents traditionally used in industries, oil mills and vegetable oil refineries.

Methods: During this work we were interested in the production, purification and biochemical characterization of a lipase and phospholipase of bacterial origin as well as the evaluation of their potential in some biotechnological applications.

Results: A thermostable phosphatidylcholine-specific phospholipase C encoding gene from *Bacillus thuringiensis* strain IL14 (PC-PLC$_{Bt}$) and *Serratia* sp. strain W3 lipase (rSmL) were expressed in *E. coli* and purified to homogeneity. The biochemical and interfacial kinetic for the native and recombinant enzymes was studied. Interestingly, both PC-PLC$_{Bt}$ forms were found to be able to hydrolyze negatively charged phosphatidylglycerol film with a more pronounced rate of hydrolysis for the native form. The ability of both PC-PLC$_{Bt}$ forms to hydrolyze the PG remains exclusive compared to all known *Bacillus* PLCs. Using olive oil emulsion as substrate, the specific activity of the rSmL was 3530 U/mg, twelve times higher than that of native SmL. The results proved that SmL exhibited a high penetration power and was found to be able to hydrolyze the ester bond at *sn*-1 and *sn*-3 positions with a clear regio-preference toward the *sn*-3.

Conclusion: The construction of overproducing strains of lipase and phospholipase enzymatic activities, as well as the optimization of the conditions of production, stabilization and formulation of these enzymatic preparations and making available to vegetable oil refineries an enzymatic cocktail (of phospholipases and lipases) fairly stable under unconventional conditions of temperature, pH, or in the presence of organic solvents and detergents, which can be partially or completely substituted for conventional methods of degumming and the modification of lipids.

Keywords: PLC, lipase, thermostability, substrate specificity, refined vegetable oils, oil degumming