Title: Molecular cloning of a lipase from the deep-sea marine bacteria *Marinobacter excellens* LAMA842

Authors: Linhares, B., Cavalett, A., Odisi, E.J., Pinto, J., Montemor, J.E., Silva, M.A.C., Lima, A.O.S.

Institution: UNIVALI - Universidade do Vale do Itajaí (R. Uruguai, 458 - Itajaí - SC)

Abstract:

The physical, chemical and biological characteristics of bacteria that inhabit deep-sea environments are adapted to such extreme conditions. Therefore, it potentially express enzymes with different characteristics than those found in terrestrial counterparts. Those bacteria may also produce industrially important lipases that are widely applied in detergents, food goods, organic synthesis, biodiesel transesterification, etc. Recently, the Applied Molecular Genetic research group of the UNIVALI university isolated the strain *Marinobacter excellens* LAMA842 from a marine sediment sample (5,000m deep). This microorganism stands out because of its lipase activity on substrates p-nitrophenylpalmitate and Tween (20, 40, 60 and 80), indicating great production potential. Once characterized, low temperature active lipases where identified, leading us to belive that this organism is a valuable reservoir fore psychrophilic coding gens. Thus, its complete genome was described previously and in the present work, the lipase genes were exploited. For this purpose, genomic data was evaluated (CLC *Genomics Workbench*) for selection of lipase genes. Therefore, the predicted proteins for *M. excellens* were compared (Blast) to data from the NR *GenBank* (*NCBI*), they were also evaluated by BLAST2GO and their secondary structures evaluated by *Phyre* software. Once the target gene was established – a lipase with 930 amino acids and containing the conserved domain esterase-lipase superfamily – a pair of specific primers was designed to amplify the target gene. By means of PCR the gene was amplified and then digested with restriction enzymes (*Xho*I/*Xba*I). Once the PCR product was digested and cleaned, it was ligated to the expression vector pBADMycHisB (Invitrogen) using T4 DNA Ligase and, finally introduced into chemically competent *Escherichia coli.* Among the transformation colonies, six were selected in order to confirm by colony PCR the presence of the target gene. Among the positive ones, one was selected and its plasmid was extracted and submitted to sequencing. The next steps will be the production of lipase as well its profile characterization (pH and optimum temperature) against chromogenic substrates (e.g. p-nitrophenyl palmitate). As product of this research we hope to obtain the partial characterization of a new psychrophilic lipase that will be amble to meet commercial demand.

Key words: bioprospection, cloning, marine biotechnology

Fomenting agent: CNPq, FAPESC, CNPq – INCT Mar COI, CAPES, Governo do Estado de Santa Catarina.