Title: Prospection and cloning of proteases from matagenomic data collected from deep-sea sediment – South Atlantic Ocean

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

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

Abstract:
Proteases are enzymes that hydrolyze peptide bonds between amino acids. These enzymes represent 40% of the industrial enzymes commercialized, being applied in the production of detergents, food goods, drugs, leather, bioconversion of fish wastes, etc. Depending on its application, these enzymes must be active in extreme conditions such as temperatures, pH, pressure, etc. Therefore the prospection of better adapted enzymes is economically relevant. Due to the extreme conditions of the deep sea, marine bacteria from this environment stand out as a reservoir of enzymes with unique characteristics of which there is little knowledge. In this context, the prospection and cloning of protease genes collected from environmental DNA of ocean sediment (4.200m, São Paulo Plateau, South Atlantic Ocean) by means of a Japanese manned submersible - Shinkai 6500, was conducted. The data from the environmental DNA (1Gb) was screened, permitting access to both cultivable and non- cultivable microorganisms. Bioinformatic procedures (software: CLC *Genomics Workbench*) were used to identify the protease genes: trimming of the data, developing of the contigs (*Denovo assembly*), prediction of the genes (*ORF finder*), translation of the proteins and notation of the genes (Blastp). Of the complete protease genes identified, six were selected (criteria: size, commercial interest, novelty) for the design of primers, amplification (PCR), cloning in the pBADMycHisB vector and expression in *Escherichia coli* Top10. Moreover, of these six attempts, three were amplified successfully. The amplification products end the vectors were digested by two different restriction enzymes and then linked by *T4DNA ligase.* The resulting plasmids were introduced into competent *E. coli* Top10 cells by means of thermal shock and calcium chloride. From the transformation colonies, amplification by PCR was conducted and the clones of interest were recognized. The plasmid DNA of these clones was recovered and will be sequenced. The next step will be the evaluation of the heterologous protease expression, and their characteristics, of the selected clones. It is hoped that the product of this process will prove that the combination of effective metagenomic study of the biodiversity of the deep-sea environment can lead to the acquisition of unique proteases biotechnologically relevant and commercially viable.

Key words: environmental DNA, marine bacteria, heterologous production

Fomenting agent: CNPq, FAPESC, CNPq – INCT Mar COI, CAPES.