Title: Exploration and production of heterologous protease encoded by deep marine bacteria .

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Abstract:

Proteases represent 40% of the volume of industrially commercialized enzymes. They can be applied in many fields, such as the production of food goods, detergents, drugs, leather and even the bioconversion of organic sub products. With the intent of enhancing the efficiency of such industrial processes, unique enzymes (more active, tolerant and substratum specific) are prospected from extreme environments. In this context, our work hypothesis in that deep-sea bacteria can present proteases that are active in mild temperatures (20-35°C) and are more specific to act upon proteins of marine source. Therefore, such characteristics can imply in higher hydrolytic efficiency as well as the energy demand for the hydrolyses of fish goods. In this project, out of 100 protease genes, 23 endopeptidic genes were selected, being the criteria for the selection of the final project three unique proteases that will be cloned and expressed. The selection was based upon the criteria that the genes must be complete, must translate into a protein with psichrophilous characteristics, and have a small size – smaller enzymes are more easily expressed. The percentage of identity with proteins that have been described, has also been considered as well as the presence of families with complete domains, we are focusing on new proteins. These three genes (two of them from *Bacillus stratosphericus* and the other from *Marinobacter excellens*) have been previously isolated (at 3.600 to 5.000m deep, in the South Atlantic Ocean) from the complete genomes of these microorganisms that were sequenced by our group. Protease genes were identified and screened using bioinformatics tools (*CLC Genomics Workbench*) for the selection of protease genes, trimming of the data and the assembly of the contigs (*Denovo assembly*), gene prediction (*ORF finder*), translation to protein and gene annotation (Blastp). Once the target was established, primers adequate to the specific times and temperatures of each gene were designed (using the restriction enzyme XhoI C|TCGAG; SalI G|TCGAC), and were used to ampify the genes (PCR). Afterwards, a gel electrophoresis test (Agarose 1%) was used to confirm amplification. Then the genes were used for cloning in the expression vector (pBADMycHisB) and introduced into chemically competent *E. coli* cells, plasmid extraction. The 3 L of produced proteases were then purified with chromatography of affinity to immobilized metal and characterised (optimum activity pH and temperature) with a stained substratum (Azocasein). Finally, the performances of the enzymes were evaluated/compared to a commercial product (Protamex), being used for such the optimum activity conditions identified for each enzyme. As a product we hope to obtain a partial characterization of new proteases, enabling possible patent deposits.

Keywords: bioprospecting; marine biotechnology; recombinant enzyme.

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