

Título: ISOLATION, IDENTIFICATION AND EVALUATION OF FIBRINOLYTIC PROTEASE PRODUCTION BY BACTERIAS ISOLATED FROM SOIL AND WATER IN THE AMAZON REGION

Autores: Lima, A.K. S.¹; Souza, T.C. ¹; Jesus, M.S.¹, Fernandes O.C.C¹.

Instituição: Instituto Leônidas e Maria Deane- ILMD/Fiocruz/Amazonas

ISOLAMENTO, IDENTIFICAÇÃO E INVESTIGAÇÃO DA ATIVIDADE PROTEOLÍTICA FIBRINOLÍTICA DE BACTÉRIAS ISOLADAS DE SOLO E ÁGUA NA REGIÃO AMAZÔNICA

7. Industrial Microbiology

7.3. Processes of Fermentations

Abstract

The fibrinolytic protease production by micro-organisms has been the target of research to serve as a cheaper and safer alternative in the treatment of cardiovascular diseases, especially thrombosis, one of the most frequent diseases of modern life. The aim of this study was to isolate, identify and evaluate the production of proteases with fibrinolytic action of 58 bacterias isolated from soil and water and after this to deposit them in the “*Coleção de Bactérias da Amazônia (CBAM)*”. The bacterial isolation from soil was performed using serial dilution technique and filter membrane for the isolation of bacteria from water. After this, a central inoculum from each sample was done in milk agar for evaluating protease production. The plates were incubated for 24 hours at 37 °C. The protease producers cultures were subjected to submerged fermentation in Manachini solution then were transferred 1 ml of cell suspension similar to that of 0.5 McFarland scale. The submerged fermentation was conducted in an orbital shaker for 24 h/150 rpm/ 37 ° C. The extract was separated from the biomass by vacuum filtration. On the recovered crude extract was made the quantification of proteolytic activity using casein as substrate. A proteolytic activity unit was defined as the amount of enzyme able to produce an increase in absorbance of 0.01 at 1 hour to 280 nm and it is expressed in U/ml. The crude extracts were inoculated into fibrin plate to determine the fibrinolytic action, being evaluated after 18 hours of incubation in BOD growth at 37 ° C. After investigation of the fibrinolytic potential of the bacterial cultures, the identification of species protease producer was performed to then be deposited in the CBAM. Colonies were identified by BBL Crystal commercial kit. In the protease tests, 71% of the bacterial cultures showed to be protease producer, especially the species *Bacillus cereus* CBAM 508 (138.33 U / ml). In determining the fibrinolytic action, 21% of the samples were positive with variation of 0.7 to 2.7 cm in halo size. *Pseudomonas aeruginosa* CBAM 0516 and *P. aeruginosa* CBAM 497 had a halo size of 2.7 and 2.5 cm, respectively. All the cultures/samples proteases producers (41) were deposited in the CBAM. This study shows the potential of Amazonian biodiversity for producing fibrinolytic protease from bacteria isolated from soil and water, moreover it helps to increase the amount of crops with potential biotechnological applications in the CBAM.

Palavras-chave: submerged fermentantion, fibrinolytic protease, Amazon

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