



FUNGAL STRAIN SELECTION FOR PROTEINASES PRODUCTION AND THEIR POTENCIAL USE FOR BIOPETIDE GENERATION

Gloria A. Martínez, Arely Prado-Barragan, Cristóbal N. Aguilar,

Departamento de Investigación en Alimentos. Facultad de Ciencias Químicas. Universidad Autónoma de Coahuila. Blvd. Venustiano Carranza, s/n, esquina con LC. Salvador Gonzalez Lobo, Colonia Republica Oriente, C.P. 25, 000. Saltillo, Coahuila, México).
aly_0353@hotmail.com

Key words: Proteinases, submerged culture, bioactive peptides.

The proteases belong to a group of enzymes whose catalytic activity consist in hydrolyze the peptide bonds from macromolecules like proteins, these are distributed from large kind of source such animals, plants and microorganisms and being necessary for carry out so many critical functions in organism ranging from metabolism regulation, complement system, enzyme modification, apoptosis pathway, to nutrient digestion. Furthermore, a study of proteolytic enzymes is important due to their use in different forms of medical therapies, their importance as laboratory, clinical and industrial reagents. They have widely uses in several industrial processes like detergents, food, leather, pharmaceutical and leather [6].

The pharmaceutical industry search for new therapeutic molecules with reduced side effects, in recent years was established bioactive peptides derived from the protein hydrolysis possess certain bio-functionalities and serve therapeutic roles in body systems, this molecules may induce functionalities such as antioxidative, antimicrobial, antihypertensive, cytomodulatory and immunomodulatory effects in living system. Bioactive peptides may be produced from a protein enzymatic hydrolysis from microbial enzymes [2]. Generally, proteases produced from microorganism are partially inducible in nature and under culture conditions for this reason this study aimed to discover the protease production potential from fungal proteolytic strains for a potential use *in vitro* bioactive peptide production.

The fungal strains used was proved from Food Research Department, for the identification of fungal strains with an extracellular protease production a skim milk agar was prepared on petri dishes, after was inoculated with fungi spores and incubated at 30 °C and examined to find a hydrolysis halo around the inoculum zone. The strains considered positives was inoculated on a plentiful liquid casein medium on Erlenmeyer flasks for 36 hours and the proteolytic activity was determinate with the Kunitz [4] methodology for choose de strains with the best proteolytic potential and the right time for most elevated proteinases production. Then the enzymatic extract was filtrated and lyophilized for concentrated de enzymes.

From the eighteen fungal strains used in the skim milk agar, six strains results positive for an extracellular proteases production with a light area surrounded de inoculum, this strain corresponds to: *Aspergillus niger*, *Aspergillus oryzae*, *Blakeslea trispora*, *Fusarium sp.*, *Mucor sp.* y *Rhizomucor pusillus*. The highest tittle in proteolytic activity was for *Rhizomucor pusillus* and *Blakeslea trispora* with 35740.74 and 39120.37 U/L respectively at the 24 hours on the submerged fermentation; a completely randomized block design was applied and this results doesn't have any statically difference. The proteinase production from several fungal strains like *Aspergillus niger*, *Aspergillus oryzae* and *Fusarium sp.* was reported for so many different authors [7,1,5] and also the production from the order *Mucorales* fungi group proteases like *Mucor sp.*, *Blakeslea trispora* and *Rhizomucor pusillus* [3].

The extensive enzymatic production including the proteases in many different plant pathogen fungus is related with the material degradation during the infection and the biotransformation of materials in available nutrients for the organism, their production is dependent of medium characteristics. The highest tittles of extracellular proteinase from some fungal strains make's an interest alternative for industrial production.

1. Agrawal D., Patidar P., Banerjee T., Patil S. (2005). Alkaline protease production by soil isolate *Beauveria feline* under SSF condition and application to soy protein hydrolysis. *Process Biochemistry*, 40: 1131-1136.
2. Agyei D., Danquah M.K. (2011). Industrial-scale manufacturing of pharmaceutical-grade bioactive peptides. *Biothec. Advances*. 29:272-277
3. Alves M., De Campos M., Okada K., Ferreira I., Milanez A. (2005). Detection of an extracellular protease in *Mucor* species. *Rev Iberoam Micol.*, 200522: 114-117.
4. Kunitz M. (1946). Crystalline Soybean Trypsin Inhibitor. II General Properties. *J Gen Physiol*. 30: 291-310.
5. Pekkarinen A., Mannonen L., Jones B., Niku M. (2000). Production of Proteases by *Fusarium* Species Grown n Barley Grains and in Media Containing Cereal Proteins. *J.Cereal Science* 31: 253-261.
6. Rani K., Rana R., Datt S. (2012). Review on latest overview of proteases. *International Journal of Current Life Sciences* 2:12-18
7. Takahashi, K. (1995). Proteinase A from *Aspergillus niger*. *Methods in enzymology*, 10 th ed. Vol 248, p.146-155.