Isolation and molecular identification of halophilic protease producing actinomycete and evaluation of effective factors for maximal enzyme production

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Introduction: Proteases are one of the most applicable hydrolyzing enzymes especially in food and beverage industries. Isolation of thermophilic or halophilic proteases was aimed by many investigations. The present study was designed to screen soil samples for halophilic actinomycetes capable to produce protease and evaluation of factors affect on the enzyme production.

Methods: Twenty soil samples were collected from salty land around Kerman, Iran and aseptically transferred to biotechnology lab and allowed to dry. Ten grams of each soil was extracted using NaCl (0.9%) sterile solution and added to Casein Glycerol Agar (CGA) or nutrient agar medium containing skim milk (1%) and NaCl (10%) and incubated at 30◦C untill the halo zone of protease activity was formed. The ratio of halo zone/colony size of isolated actinomyctes was used as an index to select the most suitable strains. Morphological and biochemical tests as well as molecular identification using 16S rDNA technique were then applied for identification of the strain. Evaluation of chemical factors such as carbon sources, nitrogen sources and inorganic salts as well as physical factors such as temperature and pH on protease production of the selected strain was performed using one factor at a time approach.

Results: Seven protease-producing actinomycetes were isolated in preliminary studies among which one isolate (identified as *Nocardiopsis* sp.) was the most efficient one able to produce 650 U/mL protease after 5 days incubation in CG medium containing 10% NaCl. Evaluation of factors is now conducting to obtain the maximum protease production.

Conclusion: one halophilic actinomycete able to produce protease was isolated in the present study and evaluation of factors affect on the enzyme production is now performing.

Keywords: Protease; Actinomycete; Screening; 16S rDNA

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