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ISOLATION AND SCREENING OF PROTEASE ENZYME PRODUCING HALOPHILES FROM DIFFERENT SAMPLES

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ABSTRACT

Halophilic microorganisms were isolated from samples like saltern soil, sea soil, and salted fish collected from Tuticorin, Chennai and Coimbatore respectively. The isolates were studied for their Gram reaction and colony morphology. Among 9 isolates, 4 isolates showed positive result for protease enzyme production. Halophilic proteases have wide application in processing of food, leather and detergents.

Key words : Halophilic, Saltern, Protease, Screening.

INTRODUCTION

Extremophiles, the microbes dwelling in unusual habitats can potentially serve in a variety of industrial applications. As a result of adaptation to extreme environments, extremophiles have evolved unique properties which can be of biotechnological and commercial significance (Margesin *et al* 2001). Halophilic microorganisms are adapted to conditions of high salinity and require a certain concentrations of sodium chloride for their optimum growth. They have been isolated from various saline environments such as salt lakes, salterns, solar salts and sub surface salt formation. Research on hydrolytic enzymes from halophilic microorganisms was pioneered by Norberg and Hofsten at the end of 1960s (Norberg & Hofsten 1969). Halophilic proteases have wide applications in the processing of food, leather and detergents(Lanyi 1974).

MATERIALS AND METHODS

Isolation of halophiles: The samples collected for the study were saltern soil sample from Tutucorin, sea soil sample from Chennai and salted fish from Coimbatore. One gram of the collected sample was weighed and suspended in 100 ml of distilled water. The contents were serially diluted up to 10. From each dilution 0.1 ml was taken and inoculated on sterile Sehgal and Gibbons media (pH 7.2)

with different concentrations of sodium chloride (3,4,5 M) using spread plate technique(Sehgal & Gibbons 1960).Inoculated plates were incubated at 37°C for 7-10 days.

Identification of halophiles: After the incubation period, colonies were isolated and their Gram reaction was determined.The colony morphology of the isolates were also studied.

Screening of halophilic protease: All the bacterial isolates were subcultured in Sehgal and Gibbons medium with appropriate sodium chloride concentration. They were tested for proteolytic activity on Sehgal and Gibbons medium containing 0.8% sterile skimmed milk powder and suitable sodium chloride concentration (3, 4,5 M) for each isolate.The cultures were incubated at 30°C for 3-5 days. A positive reaction for proteolytic test was indicated by the clear zone of skim milk hydrolysis around the colony. Width of the clear zone was considered to be directly related to amount of protease enzyme produced.

RESULTS

From the samples, 9 strains were isolated and among them, four isolates showed protease activity. Colony morphology and pigment production varied among the four isolates (Table 1). Gram reaction of the four isolates were also studied (Table 1). The zone diameter of proteolysis was measured and from the zone diameter, isolates with maximum protease production was identified. Isolate H20:2 was found to have highest protease activity among the four isolates. (Table 1)

Table 1 Gram reaction and cultural morphology of the isolates

Isolates	Gram reaction	Cultural Morphology	Zone of hydrolysis (mm)
H15:1	Positive cocci	Thick raised,dry,opaque colonies showing yellow pigmentation	11
H15:2	Positive cocci	Dry regular transluscent round colonies showing orange pigmentation.	10
H20:1	Positive cocci	Round regular transluscent colonies showing creamy pigmentation	12
H20:2	Positive cocci	Thick raised,opaque moist colonies showing yellow pigmentation.	14

Extremely halophilic bacteria may be considered as models for biological salt tolerance. These organisms have evolved in saline environments and are able to overcome the deleterious effects of salt upto saturating concentrations

(Kanlayakrit & Bovornreunjro 2001-2002). Many haloarchaea secrete proteolytic enzyme which enable the degradation of proteins and peptides in the natural environment. Several of these serine proteases have been purified and characterized. These include neutrophilc haloarchaea including strains of *Halobacterium salinarum* (*Halobacterium halobium*) (Norberg & Hofsten 1969; Izotova *et al* 1983;Schmitt *et al* 1990; Ryu *et al* 1994).

Moderate halophiles and halotolerant bacteria are used in the production of a wide range of salty foods such as Thai fish sauce and soy sauce (Thongthai & Sontinanalert 1991). They have been isolated from pickling brines (Lefevre & Round 1919), oil field, production brines and leather curing vats (Vreeland 1993). The enzymes from extremely halophilic bacteria represent a fascinating example of adaptation. These enzymes perform their function *in vivo* and *in vitro* at 3 - 4.5 M sodium chloride, losing activity when exposed to low salt concentrations (Lanyi 1974).

From the above study, the isolates produced protease enzyme. Out of 9 isolates, 4 produced protease enzyme. The result of the above investigation revealed that halophilic microorganisms are a rich source of protease enzyme active at high salt concentrations. The results offer a future study for the purification and application of the enzyme in various industries.

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