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Evaluation of Halophilic Actinomycete *Actinopolyspora* sp. for Osmolyte Production

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Abstract: Halophilic actinomycetes *Actinopolyspora* sp. was evaluated for osmolyte production. Different concentrations of NaCl were used in the medium for osmolyte synthesis and the growth started declining from 28th day onwards. Poor growth was noticed in the medium containing 15 and 25% NaCl concentrations. At 20% NaCl concentration, protein content of the strain was higher ($14 \mu\text{g mL}^{-1}$) on the 20th day and most of the halophilic proteins have been recorded to be higher in content during the late exponential period. SDS-PAGE profile showed a distinct band of protein with a molecular weight 92 kDa on 20% NaCl concentration.

Key words: Actinomycetes, *Actinopolyspora*, osmolyte, growth, protein profile

INTRODUCTION

Halophilic actinomycetes are having the capacity to balance osmotic pressure of the environment and resist the denaturing effects of salts by secreting osmolytes (compatible solutes) such as glycine and betaine (Oren, 1999). Compatible solutes, especially glycine, betaine and ectoines have gained considerable attention in recent years (Ventosa *et al.*, 1998) because they can act as stress protectants and stabilize the enzymes, nucleic acids and membrane and whole cells. Moderate halophiles can be used to remove phosphate from saline environments as a cheaper alternative to chemical approaches (Rodríguez Valera *et al.*, 1979).

New restriction endonuclease and other enzymes for molecular biological studies are present in halophilic microbes of hyper saline habitats which can be discovered and exploited (Ventosa *et al.*, 1998). Moreover, halophilic actinomycetes constitute an excellent model for the molecular study of the osmoregulatory mechanisms that permit them to grow over a wide range of salt concentrations. This aspect shows their possible application in agriculture to construct salt resistant plants (Ventosa *et al.*, 1998). Despite such importance, studies on halophilic actinomycetes are a few (Javor, 1989; Ventosa *et al.*, 1998; Sivakumar, 2001; Senthil Kumar, 2003). Therefore, the present work was undertaken to study the growth profile of the halophilic actinomycetes, *Actinopolyspora* sp. and separate osmolyte from it.

MATERIALS AND METHODS

Actinopolyspora sp. was isolated from the *Suaeda* (salt marsh) region of the Vellar estuary (Lat. $11^{\circ}29' \text{N}$; Long. $79^{\circ}46' \text{E}$), situated along the southeast coast of India. The strain was introduced into 250 mL Erlenmeyer flasks containing complex medium of Starch Casein Agar, supplemented with different NaCl concentrations viz., 15, 20 and 25%. The flasks were incubated in water bath cum shaker at 90 rpm at 37°C for 34 days. Growth of the strain was determined at consecutive days by

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measuring the turbidity at 595 nm using HITACHI 220UV-VIS spectrophotometer. The protein estimation was carried out using the method of Lowry *et al.* (1951) and expressed as $\mu\text{g mL}^{-1}$.

Pre and late exponential phases of cell extracts of the strain were obtained from starch casein broth containing different NaCl concentrations viz., 15, 20 and 25%. Cell suspensions were centrifuged for 10 min at 12000 rpm at 4°C. Cell pellets were resuspended in 3 M KCl with 50 mM Tris HCl buffer (pH 8). Debris was removed by high speed centrifugation for 10 min at 20,000 rpm at 4°C. Supernatants were separated and Trichloroacetic acid precipitation was performed with various NaCl concentrations (Torreblanca *et al.*, 1994). The precipitated protein was then washed and resuspended in 2 N NaOH and centrifuged to remove excess trace salts and positive ions (Torreblanca *et al.*, 1994). Remaining trichloro acetic acid was removed by the air drying for 30 min. Then, the protein was separated on SDS-PAGE. Bovine serum albumin (97.4 kDa), ovalbumin (68 kDa), phospho fructo kinase (43 kDa), carbonic anhydrase (29 kDa) and α -lactalbumin (14.3 kDa) were used as standard molecular weight markers.

RESULTS AND DISCUSSION

Growth of the strain was monitored for a period of thirty four days and the corresponding OD values were recorded (Fig. 1). The strain which was grown in the medium containing 20% NaCl concentration showed the maximum growth on the 24th day probably due to the synthesis of osmolytes and growth was constant up to the 28th day. Thereafter, growth started declining from 28th day onwards. Poor growth was noticed in the medium containing 15 and 25% NaCl concentrations which could be due to lack of osmolyte synthesis. At 20% NaCl concentration, protein content of the strain was higher ($14 \mu\text{g mL}^{-1}$) on the 20th day (Fig. 2) and most of the halophilic proteins have been recorded to be higher in content during the late exponential period (Karamanou and Katinakis, 1988). Similar observations have been made in *Halobacterium cutirubrum* by Choquet *et al.* (1986).

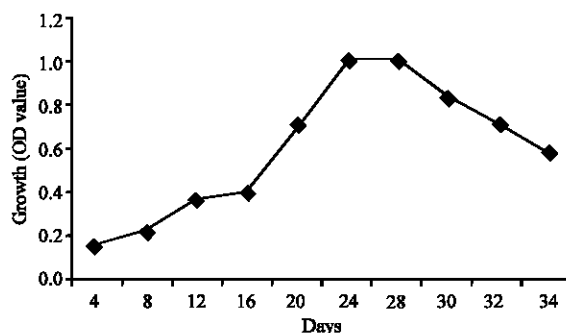


Fig. 1: Growth of *Actinopolyspora* sp. (in terms OD value at 595 nm) at 20% NaCl concentration

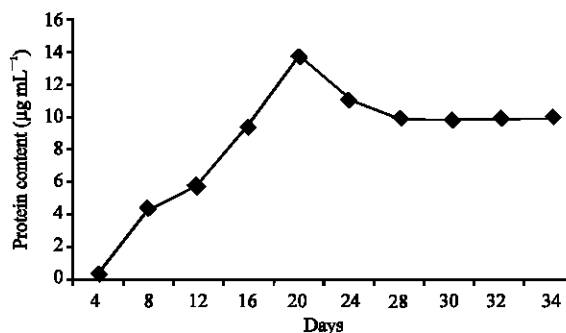


Fig. 2: Protein content of *Actinopolyspora* sp. at 20% NaCl concentration

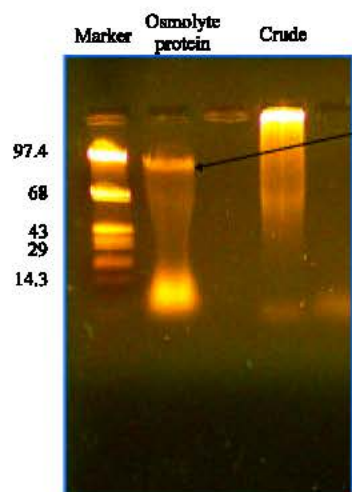


Fig. 3: SDS-PAGE profile of omolyte

Proteins were isolated by trichloro acetic acid method from the 20 to 24th day old cultures of the strain as the concentration of protein was found to be higher during this period. Protein samples of the strain with different concentrations of NaCl viz., 15, 20 and 25% were separated by SDS-PAGE. SDS-PAGE profile of the separation steps showed a distinct band of protein with a molecular weight 92 kDa (which is identical to the region of the marker carbonic anhydrase which also possesses the same molecular weight of 92 kDa) on 20% NaCl concentration (Fig. 3). No such bands were observed in either 15 or 25% NaCl concentrations. It is worth mentioning here that higher salt related protein profiles have been identified in the range between 15 to 39 KDa in *Halomonas elongate* (Mojica *et al.*, 1997).

The present results indicate that distinct protein band was found only in the 20% NaCl concentration marking the synthesis of osmolyte. Similarly, *Halobacterium salinarum* and *Haloarcula marismortui* showed marked increase of osmolyte with high NaCl concentrations during their optimum growth (Anton *et al.*, 2002). Oren (2002) have also made detailed investigations to show the similar strategy of adaptation by *Halobacterium salinarum* and *Haloarcula marismortui* to high salt by measuring their intracellular concentrations of NaCl and osmolytes during their optimal growth.

CONCLUSION

The results of the present study clearly show the presence of osmolyte in the strain. Further-in-depth research on the strain may yield novel compatible solutes of biotechnological importance.

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