ABSTRACT

Actinomycetes are Gram positive filamentous bacteria well known for the production of bioactive compounds. They also contribute to cycling of nutrient elements by producing extracellular hydrolytic enzymes. Amylase and cellulase are two important enzymes that are used in food, beverages, detergent, textiles, paper, pharmaceutical and biofuel industries. The demand for enzymes functional at high pH, temperature and salt concentrations is increasing day by day which emphasizes on the need of isolation of enzyme producing actinomycetes from extreme habitats. In order to suffice this demand a saline habitat of Rajasthan, Sambhar salt lake was investigated for its actinomycete population and the enzymes produced by them. This lake forms a unique ecosystem divided in to salterns that possesses wide range of salinities in its waters and is used for salt production. Water samples were collected from salterns in rainy, winter and summer season and analyzed for its pH and salinity content. Actinomycetes were isolated on actinomycetes isolation agar medium supplemented with NaCl. All the isolates were characterized on the basis of morphological features and biochemical tests whereas phylogenetic analyses were conducted for only a few isolates. These isolates were screened for their ability to produce extracellular amylase and cellulase enzymes by agar plate assays and the isolates that showed maximum enzyme productions were selected for optimization of culture conditions in submerged fermentation. A total of sixteen colonies of actinomycetes growing at wide range of salinity were isolated from water samples. They were proposed to be identified as members of genera *Streptomyces, Nocardiopsis, Pseudonocardia, Saccharopolyspora, Microbispora, Actinobacterium, Kokuria* and *Georgenia* on the basis of phenotypic and phylogenetic analysis. Thirteen isolates were found to produce both the enzymes as evident by hydrolysis observed on starch and CMC agar plates. The relative enzyme activity of all positive isolates was compared on media plates and the isolate SSL 6 (*Actinobacterium* sp.) and SSL 14 (*Nocardiopsis* sp.) producing maximum amylase and cellulase enzyme respectively were studied for culture conditions optimization. Maximum production of amylase by SSL 6 and CMCase by SSL 14 occurred during late log phase achieved after 96 h of incubation. Optimum temperature determined was 30 °C for both the enzymes. Optimum pH for amylase was 8.0 and for CMCase was 9.0
confirming the alkaline nature of isolates and hence the enzymes produced. Amylase was produced maximally at 9 % NaCl and CMCase at 12 % NaCl. Both the isolates were indigenous to Sambhar Salt Lake and this view was further strengthened by the optimum production of enzymes at high pH and salt concentrations. Starch and CMC were found to be best carbon sources for amylase and CMCase respectively which produced maximum amount of these enzymes. Soluble sugars like glucose repressed the production of enzymes indicating the carbon catabolite repression effect. Organic complex nitrogen sources, beef extract for amylase production and yeast extract for CMCase were found give higher enzyme productions in comparison to inorganic salts. The isolates were also studied for their antibacterial activity against selected Gram positive and Gram negative bacteria and surprisingly no antagonistic activity was shown by them. In a nut shell, this study projects Sambhar salt lake as a potential source of actinomycetes that can be exploited for commercial applications. Production of amylase and cellulase at an alkaline pH and high salt concentration would be useful for processes that operate under these conditions.

**Keywords:** Actinomycetes, Amylase, Antibacterial, Cellulase, Halophiles, ISP, Nocardiopsis, Phylogeny, Streptomyces, Sambhar Salt Lake.