



Research Paper

**ISOLATION AND CHARACTERIZATION OF MODERATE HALOPHILIC
Salinicoccus sp. PRODUCING EXTRACELLULAR HYDROLYTIC ENZYMES**

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Abstract

A moderate halophilic bacterium was isolated from the brine samples of Sambhar salt lake, Rajasthan (India). The isolate formed pigmented colonies and the bacterial cells are motile, Coccoid, Gram positive, strictly aerobic, catalase- and oxidase- positive, ferments carbohydrates, while failed to utilize citrate as sole carbon source. The isolate grew in presence of 2-20% (w/v) NaCl and at pH 7-11, with optimum growth at 12% (w/v) NaCl. Isolate showed extracellular enzymes such as Amylase, Protease and Gelatinase, but production of lipase was found to be negative. Genotypic analysis carried out by 16S rDNA amplification followed by phylogenetic analysis showed that the bacterium clustered with the members of the Genus *Salinicoccus*. The bacterial isolate is halophilic alkalitolerant with maximum similarity with Species *Salinicoccus roseus*. The Genebank Accession No of the isolate is KM461125.

Key words: Exo-enzymes, Halophiles, Molecular characterization, *Salinicoccus*.

INTRODUCTION

Moderately halophilic bacteria constitute the most versatile group of microorganisms that could be used as a source of salt-adapted enzymes. Adaptation to such high salt containing environments has evolved unique properties in these microorganisms with considerable biotechnological potential. These organisms may hold secrets, for the origin of life and unfold many questions about the stability of macromolecules, under extreme conditions. Moderate halophiles have advantage over extreme halophiles in that they do not have a strict salt requirement and grow in wide salt range.

Halophiles have mainly been isolated from saltern crystallizer ponds, the Dead Sea, solar lakes and hypersaline lakes. In Indian context, the halophilic diversity has been limited to the marine salterns near Bhavnagar, Lonar Lake and Peninsular coast. India consists of various saline environments including hypersaline lakes, ocean and salt pans, in which the microbial diversity has not been characterized yet; thus the potential of producing different hydrolytic enzymes and antibiotic resistant profile among them remains unknown.

Sambhar salt Lake (Raj) represents a unique environment yet it is clearly understudied in terms of its microbial ecology. Further, molecular characterization of the microbial biodiversity in Sambhar salt lake is still in its pioneer stage. Classical biological and microbiological studies have found only a limited variety of identifiable halophiles. The present study therefore focused on the isolation and molecular identification of moderate halophiles from Sambhar salt lake and screening for industrially important hydrolases (especially amylases, lipases and proteases).

MATERIAL AND METHODS

Collection of Samples

Sample collection site was selected by observing coloration of lake. The concentrated saline water called brine has reddish-orange color due to presence of halophiles. Such colored puddles of lake water around Sambhar, Joapok, Korsina, and Nawa were selected for study. Brine samples were collected in sterile bottles and were immediately transported to laboratory. Parameters like pH and temperature were tested with digital meters while Dissolved Oxygen was fixed on the spot.

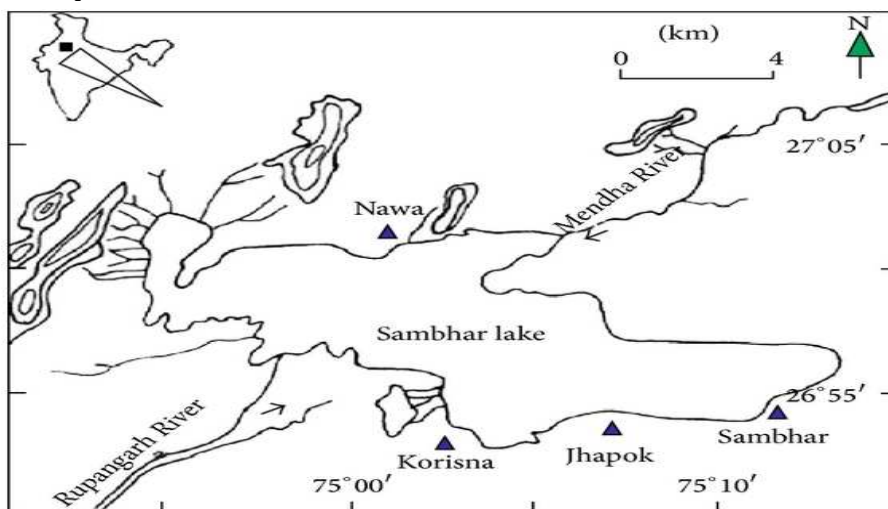


Plate 1: Sambhar lake Map Showing sampling sites

Enrichment Technique

Halophilic Broth (10%, 15%, 20% and 25% NaCl) was inoculated with 10 ml sample and incubated on shaker at 200 rpm for 3-7 days at 37°C to enrich aerobic halophiles. Post enrichment the broth was streaked on respective Halophilic agar plates and incubated for 3-5 days at the same temperature. Colonies growing well on 10-25% NaCl Concentration were selected for further investigation.

Characterization of isolated Halophiles

Morphological and Biochemical characterization was carried out on Halophilic Agar (10% NaCl). Colony shape, pigmentation, opacity and elevation were observed and Gram staining and 3% KOH test was performed. Biochemical investigations involved Oxidase-Catalase test, Citrate utilization test, Sugar fermentation test, Triple sugar iron (TSI) agar test, Nitrate reduction etc.

RESULTS AND DISCUSSION

Sambhar salt lake offers a Halophilic environment with salinity ranging from 0.34 to 168 ppt. Brine Samples collected for the study were very viscous, pink to red colored with penchant odors. Brine Samples were highly alkaline with pH ranging from 7- 9.89. The brine being high in salt and pH lacked life forms except a few protozoa and crustaceans. The various coloration of lake brine showed the presence of Halophilic bacterial strains. Enrichment and pour plate culture of lake brine led to the development of numerous colonies on Halophilic agar. One of the colonies with orange pigmentation was designated as SL-5 and subjected for detailed study. It showed opaque, glossy, convex surface with entire margin. The pigment production reduced at elevated salinity. The isolate SL-5 showed growth upto 20% NaCl, but the optimum salt concentration was 12% as the pigment production decreased post this level. It could tolerate a pH range of 7-11. The wide salinity and pH range in which the isolate grew make it evident that isolated bacterial strain is well adapted to halo-alkaline habitat.

The cells of the isolate were coccoid and gram positive and tested positive for catalase and oxidase. Isolate SL-5 also produced extracellular hydrolases such as Amylase, Protease and

Gelatinase. A nearly complete 16s r-RNA gene sequence was determined by isolating DNA from bacterial cells. In a phylogenetic dendrogram based on 16S r-RNA gene sequence analysis, isolate SL-5 was affiliated to the *Salinicoccus* clade, showing 99 % 16S rRNA gene sequence similarity to the recognized species of the genus *Salinicoccus*, in which *Salinicoccus roseus* strain NIOT-bflm-S12 was the nearest neighbour followed by *Salinicoccus roseus* strain SB15 .

TABLE 1 16s r-RNA Sequence of Isolate SL-5

Isolate	16s r-RNA sequence of SL-5	% Similarity	Accession No.
SL-5	CTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACT CCTACGGGAGGC AGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAAC GCCGCGTGAG TGAAGAAGGGTTTCGGCTCGTAAACTCTGTTGTCAGGGAAGAACG CCGACGGGAGTAA CTGCCCCGTGGGTGACGGTACCTGACCAGAAAGCCACGGCTAACTA CGTGCCAGCAGCC GCGGTAATACGTAGGTGGCAAGCGTTATCCGGAATTATTGGGCGTA AAGCGCGCGTAG GCGGTTTCGTTAAGTCTGATGTGAAAGCCCCGGCTCAACCCGGGGA GGGTCATTGGAAA CTGGCGGACTTGAGTGCAGAAGAGGAGAGTGGAAATCCATGTGTA GCGGTGAAATGCG CAGAGATATGGAGGAACACCAGTGGCGAAGGCGGCTCTCTGGTCTG CAACTGACGCTG AGGTGCGAAAGCGTGGGGATCAAACAGGATTAGATACCCTGGTAG TCCACGCCGTAAA CGATGAGTGCTAAGTGTTAGGGGGTTTCCGCCCCTTAGTGCTGCAG CTAACGCATTAAGC ACTCCGCCTGGGGAGTACGGCCGCAAGGTTGAAACTCAAAGGAATT GACGGGGACCCG CACAAGCGGTGGAGCATGTGGTTTAATTCTGAAGCAACGCGAAGAAC CTTACCAAATC TTGACATCCTCTGACCACCCTGGAGACAGGGTTTCCCTTCGGGGCA GAGTGACAGGTG GTGCATGGTTGTCGTCAGCTCGTGCTGAGATGTTGGGTAAAGTC CCGCAACGAGCGC AACCCTTATCATTAGTTGCCAGCATTAGTTGGGCACTCTAATGAG ACTGCCGGTGACA AACCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCCATTATG ATTTGGGCTACA CACGTGCTACAATGGACAGGTTACAAAGGGCAGCTAAGCCGCGAGG CCAAGCGAATCCC ATAAAACTGTTCTCAGTTCGGATTGGAGTCTGCAACTCGACTCCAT GAAGCTGGAATCGC TAGTAATCGTGGATCAGAATGCCACGGTGAATACGTTCCCGGGTCT GTACACACCGCCC GTCACACCACGAAAGTCTGGTAACACCTGAAGCCGGTGGGCCAACC TTTATGGAGGCAGC CGTCGAAGGTGGGACCGA-1189 Bp	99% Salinicoccus roseus strain NIOT- bflm-S12	KM4611 25

The genus *Salinicoccus* was proposed by Ventosa *et al.* to accommodate a moderately halophilic, Gram positive coccus, *Salinicoccus roseus*. Microbes belonging to the genus *Salinicoccus* have generally been found in salty environments, such as salt mines, salted fish and fermented seafood. *S. roseus*, *S. hispanicus*, *S. alkaliphilus*, *S. salsiriae*, *S. jeotgali*, *S. luteus*, *S. siamensis*, *S. kunmingensis*, *S. carnicancri*, *S. iranensis*, *S. albus*, *S. halodurans*, *S. sesuvii*, *Salinicoccus qingdaonensis* are the members of Genus *Salinicoccus*. Ventosa *et al.*, (1990) described L-Lys-Gly5 as major amino acid constituents of the cell wall for the genus *Salinicoccus*, with MK-6 as the predominant respiratory quinone while Zhang *et al.* reported iso-C15 : 0 and anteiso-C15 : 0 as major cellular fatty acids from that of species of this genus. The genomic DNA G+C content of the species in this genus lie within the range of 46–51 mol%.

In Indian context moderate halophiles belonging to the genera *Salinicoccus* were reported from coastal solar salterns of Gujarat, Orissa, and West Bengal by Biswas and Paul. *Salinicoccus roseus* was reported as major agent causing spoilage of salt cured fish. *Salinicoccus Sp.* belongs to the phylum Firmicutes that includes low GC containing gram positive bacteria. Earlier Sahay *et al.* also isolated 93 haloalkaline bacteria from Sambhar lake and categorized them into 32 groups, with each group representing a different taxa belonging to 3 phyla (Firmicutes, Proteobacteria and Actinobacteria).

Kumar *et al.* reported significant diversity among halophiles from saline habitats of India. According to them halophilic isolates are not only diverse in phylogeny but also in their enzyme characteristics. Halophiles are an excellent source of such enzymes which are not only salt tolerant, but also may be active at high temperature and pH values. Isolate SL-5 also showed extracellular hydrolases activity and produced salt stable Amylase, Protease and Gelatinase. Annapurna *et al.* (2012) earlier reported protease producing moderate Halophilic *Bacillus subtilis* from Sambhar lake, however *Salinicoccus sp.* JAS4 producing Amylase, Protease, Inulinase and Gelatinase was reported from west coast of Karnataka, India by Jayachandra *et al.*, (2012). Most of the industrial processes are carried out under specific physicochemical conditions which may not be definitely adjusted to the optimal points required for the activity of the available enzymes; thus enzymes from Halophiles that exhibit optimal activities at various ranges of salt concentration, pH and temperature, would be of great importance (Rohban, *et al.*, 2008).

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