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Original Article

ISOLATION AND CHARACTERIZATION OF A MODERATE HALO-ALKALIPHILIC *PLANOCOCCUS. SP.* FROM SAMBHAR SALT LAKE

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Abstract: In an attempt to explore bacterial diversity in the water samples of Sambhar salt lake (26^0 57'01" N , 74^0 57'02" E), Jaipur Rajasthan, a Halo-alkliphilic, Gram-positive, obligate Aerobe, Coccoid strain was isolated and designated SL-17 . The isolate showed growth in salt concentration ranging between 1-15% and found to be motile. Convex, smooth, circular colonies with yellowish-orange pigmentation were formed by the strain. The strain was subjected to biochemical and molecular analysis to ascertain its exact taxonomic position. The 16s r-RNA gene of the isolate was amplified, and 1407 bp long amplicon was sent for sequencing. The phenotypic, Biochemical and genetic descriptions of the isolate are indicative of its relatedness to the genus Planococcus . It was found to show 99% similarity with P. plakortidis and P. maritimus. (note: The isolate has been submitted to gene bank with a accession no : KJ931667).

Key words:- *Planococcus, Halotolerant,* Sambhar Salt lake

INTRODUCTION

Salinity primarily affects all life forms existing in aquatic environments and modifies entire ecosystem. Hypersaline ecosystems are present on each continent and are primarily found in "arid and semi-arid regions [1]. Hypersaline habitats contain significant concentrations of sodium chloride or other salts. Occasionally salinity surpasses the ocean water (3.5%, i.e. 35 grams per litre). High Salinity is often combined with elevated pH and high temperature variation. Along with this, exposure to intense radiations makes them a inhospitable to most life forms. In such hypersaline environments only a few specialized halophilic microbial and crustacean species thrives [2].

Diversity, genetics and physiology of halophiles are trending topics in saline lake research. Research on halophilic microbes got accelerated due to the facts that, earliest microbes on earth are believed to have been halo-alkaliphiles, thus deep insight into salt/soda lakes and their organisms may give insight into the evolution of life [3]. Further a boom in saline environment research has been observed with the reports of hypersaline conditions on mars [4].

The ecology and diversity of Sambhar salt lake has been explored for its unique coloration and biotechnological potential and reports has established it as a treasure of prokaryotic groups of considerable phylogenetic diversity, including halo-alkaliphilic archea.

MATERIALS AND METHODS

Study Site: The source of sample water and brine is the salt lake famously known as Sambhar or

Shakambari Jheel located at 26° 55' 0" North, 75° 12' 0" East (Fig.1). The lake is often referred as playa owning to its area and size that varies with a maximum length of 22.5 Kms and a width ranging from 3.5 to 11.2 Km. The Sambhar lake basin has been divided into a larger western and smaller eastern sides by a 5.1 km long dam. Western part has natural origin and gets runoff from surrounding catchment area. However, eastern part is in the form of man-made evaporation ponds and is used for salt production.

Sample collection: Sambhar lake water and brine samples were collected in sterile bottles on monthly basis from Jhapok Dam area due to easy access. The water temperature and pH was measured at the sampling site and samples were carried to laboratory in cool boxes and subjected to microbial analysis.

Culture conditions, biochemical and molecular characterization: In order to isolate halophilic bacteria the 10 ml of water sample was added into a sterile Erlenmeyer flask (250 ml capacity) containing 150 ml halophilic broth supplemented 10% salt. The sample was subjected to enrichment for 5-7 days in rotary shaker incubator at 28-33°C. After enrichment, an aliquot of the culture (0·1 ml) was spread on to halophilic agar and incubated for 5-7 days at 33°C. Small convex yellowish-orange colonies appearing post incubation were observed for peculiar morphological features and a smear was stained with Gram's reagents following standard method [5]. Catalase test was performed by adding H_2O_2 to fresh culture slant (18-24hr incubation). Nitrate reduction, sugar fermentation, citrate utilization, urease and indol production tests were performed using standard procedures [6]. Hydrolytic exo-enzymes including Amylase, protease and lipase production was tested by plate assay technique [7].

Freshly cultured colonies were used for DNA extraction by phenol-chloroform method [8]. 16s r-RNA gene segments were amplified with the extracted DNA templates, employing, 18F and 1492R primers and optimized PCR conditions [9].

RESULT AND DISCUSSION

Sambhar and salt are synonymous in Jaipur, Rajasthan, as the lake water is used for production of Salt for ages. Amid white heaps of salt can be seen vivid colors ranging from pale green to bright red of the lake and evaporation ponds. The color variations are indicative of the salinity fluctuation of the lake. Microorganisms inhabiting sambhar change their hues as the salinity of the lake increases. The lake microbes are therefore highly specialized and are endowed with the ability to withstand wide osmotic changes.

In an attempt to study diversity of halophilic bacteria of Sambhar lake, a moderate halophilic strain was isolated. The isolated strain formed convex, smooth, circular colonies with yellowish –orange pigmentation (Fig. 2). Colonies developed from coccoid, gram positive, motile, aerobic cells, occurring as clumps. The strain grew between 1-15% salt concentration, with delayed growth above 10% and pH ranging from 6.83 to 11. It was positive for catalase and negative for oxidase, nitrate and urease. The strain utilized citrate and tested positive for exo-enzyme production, with positive results of Tween 80 and gelatin hydrolysis.

The amplified product was also sent to Xceleris, Ahmedabad for sequence analysis. Result of sequencing was aligned with other related species obtained from the GenBank database and blastn [10] was used to perform sequence similarity search. Sequences of closely related taxa were employed and aligned using the clustal_ x program [11] the alignment correction was done manually. Phylogenetic analysis was carried out by neighbour-joining method [12]. Phylogenetic exploration of the 16S rRNA gene sequence revealed that the isolate belongs to the phylum Firmicute and genus Planococcus and found related to the type strains of *P. plakortidis* and *P. maritimus* upto 99% (Fig.4).

The genus Planococcus was first proposed by Migula [13] belonging to family Planococcaceae within the pylum Firmicutes. Since then the genus has been amended twice by Nakagawa et al. [14] followed recently by Yoon et al. [15]. Amendments by Nakagawa et al. [14] included that cells are cocci, short rods or rods. Cells can be motile or non-motile. The DNA G+C content ranges between 39.0–51.2 mol %. Planococcus citreus, the type species, was proposed by Kocur et al. [16] and, subsequently, many species have been reported from saline environments. Planococcus stackebrandtii previously reported in this genus by Mayilraj et al. [17], has

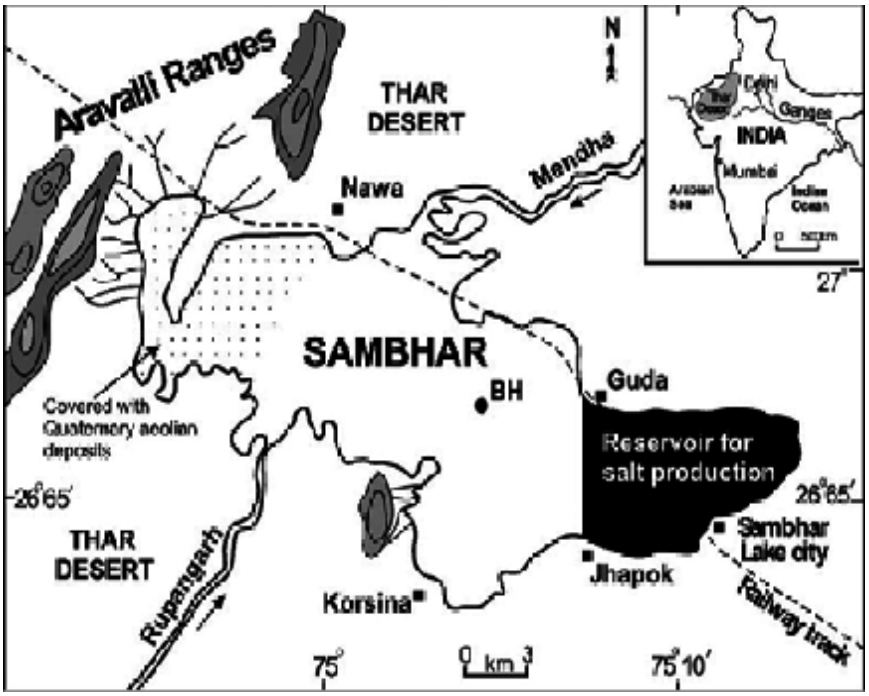

Fig. 1: Location map of Sambhar playa in the Thar Desert, and sample collection site, Jhapok (*Source : Sinha and Raymahasay [24]

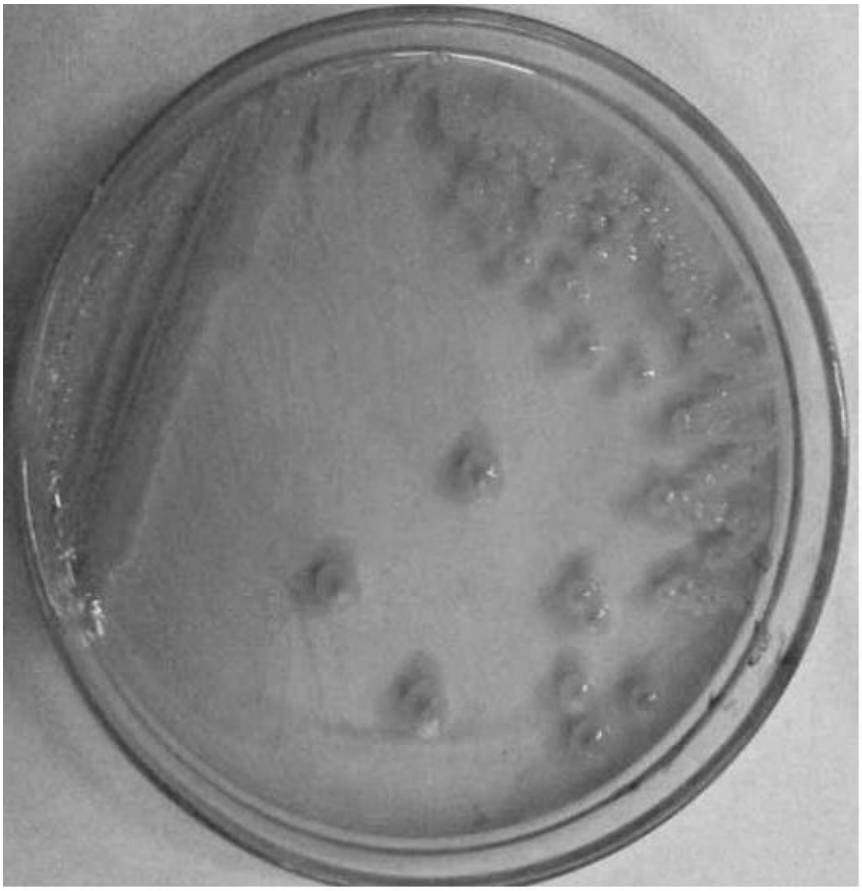

Fig. 2: Colony morphology of isolate SL-17 (*Planococcus Sp*.)

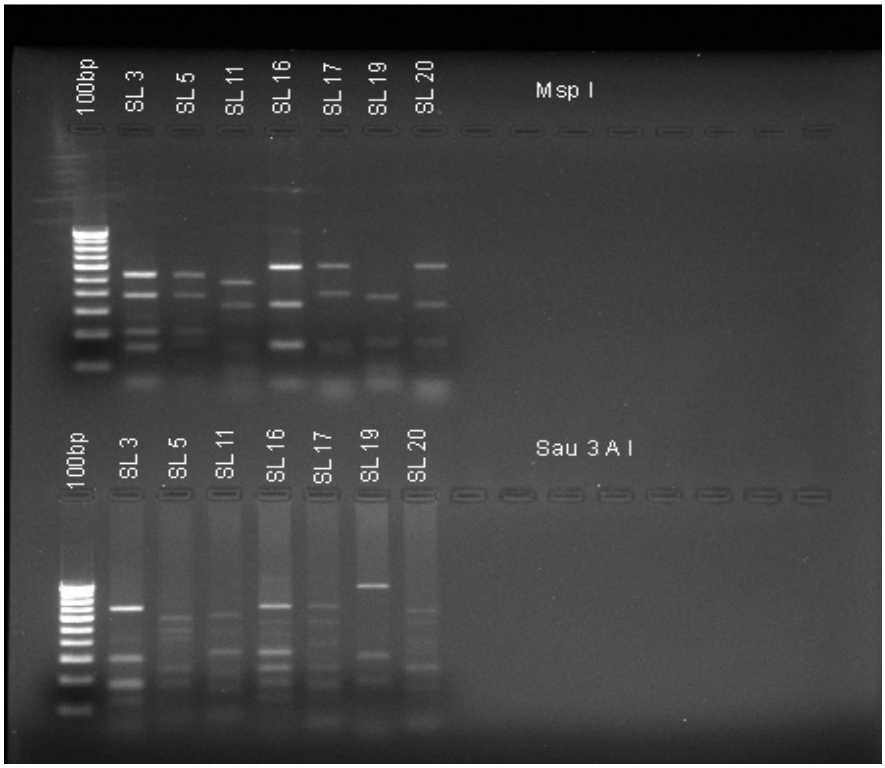

Fig. 3: Gel Image showing ARDRA using restriction enzymes MSP-1 and Sau 3A1. The isolated moderate halophilic strain was further subjected to DNA extraction. The 16s-rDNA was amplified and subjected to Amplified ribosomal DNA restriction analysis (ARDRA) using restriction enzymes, Msp I and Sau 3AI, to ascertain genetic diversity of the SL-17 from other isolates (Fig-3).

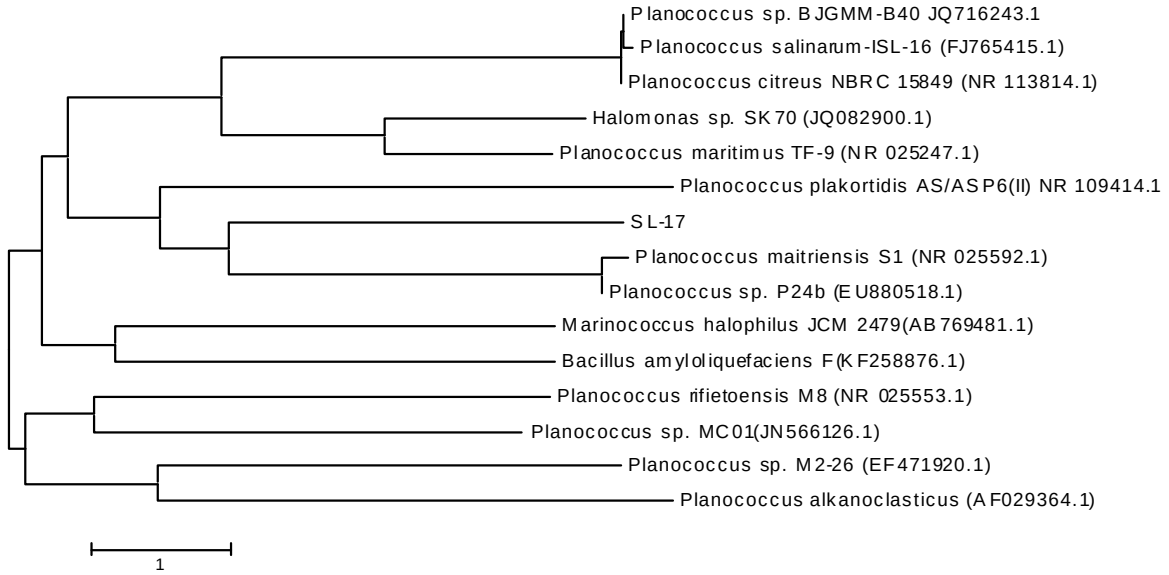

Fig. 4: Phylogenetic tree showing the relationships between strain Sl-17 and related bacteria based on 16S rRNA gene sequences alignment analysis. Bootstrap values from 1 000 replicates are included, the scale bar corresponds to 0.005-estimated nucleotide substitution per sequence.

been reclassified as *Planomicrobium stackebrandtii* by Jung et al. [18]. Number of species belonging to the genus reached 12, with the publication of Planococcus dechangensis by Wang et al. [19]. All members of the genus planococcus are characterized as aerobic coccoid bacteria showing positive Gram reaction and are non-endospore-forming. Cells containing C15:0 anteiso as the predominant fatty acid and the cell-wall peptidoglycan type of L-Lys-D-Glu [19,20].

All known species of *Planococcus* have shown considerable heterogeneity in habitat and salt tolerance and many have been isolated from slight to hyper-saline environments. The bacterium was also found associated with marine vertebrates and invertebrates [21]. Most of the halophiles have the capacity to survive in multiple stress conditions. According to Wang et al. [19] members of *planococcus* genus have the ability to grow at high salinity and low temperatures, therefore, several members of this genus have been isolated from cold and saline habitats in India. *Planococcus stackebrandtii* has been also isolated from cold desert of the Himalayas [17].

Industrial and bioremediation uses of exo-enzymes from halophilies are the hottest topics of scientific research nowadays. Vennila and Kannan [15] have reported hydrocarbon degradation and bioremediation of refinery effluent by *Planococcous halophiles*. Applicability of *Planococcus* sp. in bioremediation of tannery effluents has been cited by Behera et al. [22]. Desouky et al. [23] reported biodegradation of benzene, toluene, ethyl benzene and xylene by Halophilic *Planococcus* sp. (strain TS1).

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