



## Characterisation of a carotenoid producing extremely halophilic archaeon *Halorubrum sodomense* MS5.1 isolated from a solar saltern in Tamil Nadu, South India

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### ABSTRACT

A carotenoid producing extremely halophilic archaeon designated MS5.1 was isolated out of brine samples from a crystalliser pond of a marine solar saltern in Kanyakumari District, Tamil Nadu, South India. The red pigmented, long rod shaped haloarchaeon was found to be able to grow at temperature range of 20-40°C, salt concentration of 10-35% and pH range of 6 to 9 with optimum conditions for growth being 28°C; 30% salt and pH 7. The archaeal cells were found to be Gram negative and got lysed when placed in distilled water. Analysis of 16S rDNA sequence revealed that the isolate is phylogenetically related to species of the genus *Halorubrum* under the family *Halobacteriaceae*, with close relationship to *Halorubrum sodomense*. Further analyses of the phenotypic and biochemical characteristics of the isolate confirmed the identity of the organism as *H. sodomense*. The gene sequence of the strain was deposited in the NCBI GenBank with Accession No. MW332265. Polar lipid characterisation of the strain by thin layer chromatography (TLC) identified the major polar lipids as Phosphatidylglycerol (PG), Phosphatidylglycerol phosphate methyl ester (PGP-Me), Diglycosyl archaeol (DGA) and Sulfated diglycosyl archaeol (S-DGA). The strain was further screened for antibiotic sensitivity and found insensitive to antibiotics that target peptidoglycan layer and found sensitive only to Nitrofurantoin and Rifampicin, which works by inhibiting nucleic acid synthesis. As halophilic archaea are known natural sources of carotenoids, an attempt was made to extract these pigments from the cells and analysed by UV-VIS spectrophotometry. Present study characterised the haloarchaeal strain *H. sodomense* MS5.1 isolated from a coastal solar saltern, optimised the growth conditions and the results clearly indicated that the strain is a potential source of carotenoids and halophilic enzymes.

Keywords: Carotenoid pigment, Haloarchaea, Halobacteriaceae, Manakudi salt pan, Polar lipids

### Introduction

Archaea are a group of atypical prokaryotic organisms originally called the archaeobacteria and later the archaea (Jarrel *et al.*, 1999). These are mostly distinct class of microbes with unique features, phylogenetically more similar to eukarya than bacteria. Unlike bacteria, archaeal membrane lacks peptidoglycan in their cell wall and have altered membrane lipid bonding (Ferokh and Nadia, 2016). Archaeal lipids have side chains comprising repeated units of isoprene and lack the fatty acids found in bacteria and eukaryotes. They are key players in the biogeochemical cycles in the ocean and many archaea are able to fix carbon from inorganic sources and thus influence greenhouse gas emission (Offre *et al.*, 2013).

Aerobic halophilic archaeobacteria *i.e.* haloarchaea are halophiles par excellence and are the main component of microbial biomass in hypersaline environments. Hypersaline environments such as soda lakes, salt lakes and solar salterns are the common sources for isolation of haloarchaea. Haloarchaea have also been isolated from salt fermented seafood (Roh *et al.*, 2007; Roh and Bae, 2009). Extremely halophilic archaea are well adapted to saturated NaCl concentrations and grow optimally at salt concentrations above 3.4-5.1 M or 20-30% NaCl (McGenity and Grant, 1995). Adaptation to such high salt containing environments has evolved unique properties in these microorganisms with considerable biotechnological potential. They have several novel molecular characteristics, particularly for their enzymes

that function at high salt levels (3-4 M NaCl), such as lipases, proteases, amylases and glucose dehydrogenase (Wanaporn Tapingkae *et al.*, 2010) that find potential application in salt based and detergent industries.

The halophiles have potential importance in the field of food preservation and leather industry (Elevi *et al.*, 2004). According to Akolkar *et al.* (2008; 2010), most strains of halophilic archaea are proteolytic in nature and can function under high salt concentrations that cause denaturation of other nonhalophilic proteins. These can be utilised for commercial salt-based applications like production of fish sauce and antifouling coatings (Aparna Singh and Anil Kumar Singh, 2018). Due to the presence of acid amino acids, proteins of haloarchaea possess highly negative surface charge which compensate for the extreme ionic conditions (Reed *et al.*, 2013). Halophiles produce a range of unique and stable biomolecules of practical application. The biodegradable polymer, polyhydroxyalkanoate (PHA), produced by many haloarchaea has great potential for use as alternative to non-degradable plastics (Don *et al.*, 2006). Gas vesicles produced by some halophilic archaea have been explored for their use as drug delivery vehicle. Haloarchaea have potential application in bioremediation as well as in food fermentation (Espinosa *et al.*, 2007; Akolkar *et al.*, 2010). Halophilic archaea are utilised for bioremediation in waste water treatment in textile industry, especially the first dye bath liquor from the dyeing process, which contains a high salt load (Margesin and Schinner, 2001).

Carotenoid pigments are especially prominent in hypersaline environments (Naziri *et al.*, 2014). Red and orange colour of hypersaline ecosystems are generally due to the presence of pigmented microorganisms primarily *Dunaliella* rich in  $\beta$ -carotene; Haloarchaea which produce mainly bacterioruberin as well as halophilic bacteria *viz.*, *Salinibacter ruber* producing the carotenoid salinixanthin (El-Banna *et al.*, 2012; Jehlicka *et al.*, 2013). Bacterioruberin in haloarchaea acts as cellular membrane reinforcement since it increases membrane rigidity and decreases water permeability (Lazrak *et al.*, 1988; Fang *et al.*, 2010). It protects the microorganism from DNA damaging agents namely ionising radiation and ultraviolet radiation (Oren, 2002; Singh and Gabani, 2011). Membrane proteins like bacteriorhodopsin is recognised commercially for use in artificial retina, holograms, photoelectric devices and optical computing (Hampp and Oesterhelt, 2008). Carotenoid production by eubacteria has been investigated extensively but reports on carotenoid profile of archaea are scarce (Takano *et al.*, 2006; Marshall *et al.*, 2007).

The above mentioned facts, along with the occurrence of novel and stable biomolecules in haloarchaea, indicate great potential for these microorganisms in the future. Characterisation of such extremely halophilic archaea from unexplored habitats is therefore needed to unravel their biotechnological prospective. Marine solar salterns are centres for production of common salt from seawater through the summer months. Archaea are generally encountered in many extreme environments and marine salterns are no exception. This paper presents data on the characterisation of an extremely halophilic archaeon isolated from a solar saltern in Tamil Nadu, South India and provide information regarding taxonomic position of this strain based on phenotypic, biochemical and molecular analyses; polar lipid composition and antibiotic sensitivity in addition to carotenoid production.

## Materials and methods

### *Isolation of haloarchaea and culture conditions*

Brine samples were collected from five different stations from a solar saltpan located in Manakudi (8°6'9.57"N; 77°29'22.35"E and 8°6'2.78"N; 77°29'21.29"E), Kanyakumari District along the southern coast of India (Fig. 1). Brine samples were analysed for pH using a pH meter (Cyber Scan PC 6500, Eutech Instruments, Singapore) and salinity was measured using a handheld refractometer (BS Eclipse, Bellingham+Stanley®, UK).

### *Growth medium and culture conditions*

The archaeal medium (AM) used for isolation of haloarchaea from the brine samples contained ( $\text{g l}^{-1}$ ): NaCl - 125;  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  - 160;  $\text{K}_2\text{SO}_4$  - 5;  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  - 1 g; Yeast extract - 1 g and 2% agar. pH of the medium was 7.2. The cultures were incubated at room temperature for 7-12 days. The selected colonies differing in shape, size and pigmentation were selected and pure cultures were obtained by repeated transfers of separated colonies on agar plates of the same medium. The isolated strains were routinely cultivated aerobically at  $28 \pm 2^\circ\text{C}$  in the agar medium and were preserved in double strength broth (without agar) with 50% glycerol at  $-80^\circ\text{C}$  and also in semisolid agar at room temperature using mineral oil overlay method (Hartsell, 1953). Out of these, a deeply red pigmented strain designated MS5.1, isolated out of brine sample from a crystalliser pond (8° 6'3.59"N; 77°29'21.55"E), which appeared to serve as a potential source of carotenoid pigment was selected for further characterisation and carotenoid estimation.

### *Morphology and optimisation of growth conditions of haloarchaeal strain MS5.1*

Actively growing culture of MS5.1 in the logarithmic phase from agar plates was suspended and washed in 30%

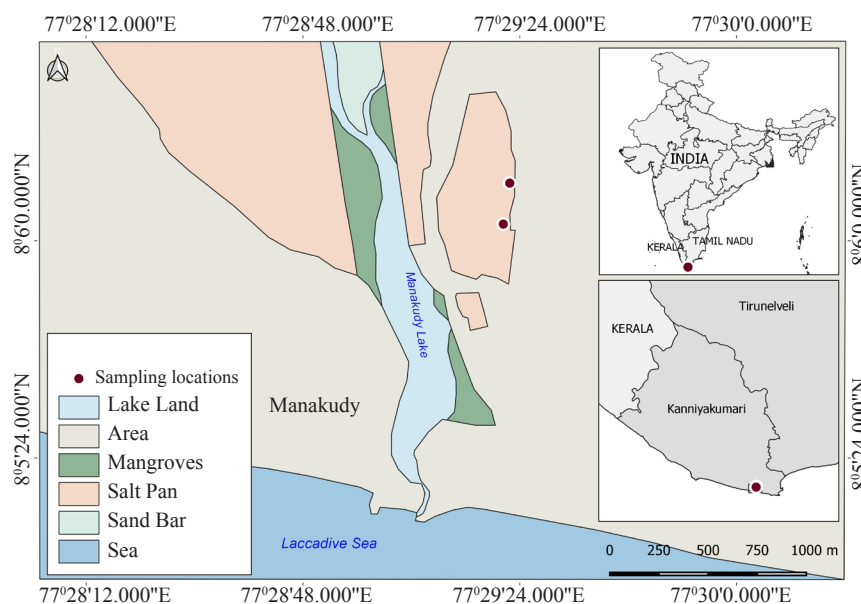


Fig. 1. Map showing sampling location

saline broth by centrifugation (2000 g; 5 min) in order to break up the culture which is usually slimy. Culture suspension (50  $\mu$ l) was then placed in the wells of a 96 well sterile tissue culture plate and observed under phase contrast objective (x400) of an inverted microscope (Nikon TS100, Japan) to study morphology as well as motility and photomicrographs were taken. Gram staining was performed as per the procedure described by Dussault (1955) after fixation of air-dried smears using 2% acetic acid for 5 min. We also attempted routine Gram staining protocol after washing the cells in phosphate buffered saline (PBS, pH 7.2), three times to remove the salt content. The cells were then pelleted by centrifugation at 2000 g for 5 min and then resuspended in PBS for preparation of smears. Air-dried smears were heat fixed and stained by the routine Gram staining protocol (Gram, 1884). The slides were observed under oil immersion objective (x1000) in a compound microscope (Leica LB2, Germany). The range of salt concentration that allows growth and the optimum salt concentration were established by cultivating the strain in liquid medium with various concentrations of NaCl (5, 10, 15, 20, 25, 30 and 35%). The cultures were incubated at 28°C for 7 days. Growth was also tested at various temperatures (4, 20, 24, 28, 35 and 40°C) and different pH levels (5, 6, 7, 8 and 9). Growth was assessed by counting the cell number using nephelometer (BD, Germany) after 7 days (end of log phase). Lysis of cells at low salt levels was ascertained by microscopic examination and also by reduction in turbidity.

#### *Phenotypic and biochemical characterisation*

Phenotypic and biochemical characterisation of the strain was performed as described by Elevi *et al.* (2004). Colony characteristics, pigmentation, motility and a series of biochemical tests were performed (Holding and Collee, 1971; West and Colwell, 1984). Production of enzymes that can hydrolyse casein, starch, tween 80 and gelatin was checked on agar plates containing casein, starch, tween 80 and gelatin as substrates respectively. All biochemical and physiological tests used the basal archaeal medium and for all the tests performed, incubation was carried out at 28 $\pm$ 2°C for a minimum period of 96 h.

#### *Molecular identification by 16SrRNA gene sequencing*

Total genomic DNA was isolated and 16S rRNA genes were amplified by PCR performed with archaeal specific primers. Genomic DNA was isolated by phenol chloroform isoamyl alcohol extraction protocol (Sambrook and Russel, 2006). Archaeal 16S rDNA were amplified by performing PCR with the primer pair A-21F (TTCCGGTTGATCCYGCCGGA) and A-958R (YCCGGCGTTGAMTCCAATT). PCR reaction mixture (25  $\mu$ l) comprised 10 pmol of each primer, 900 ng of DNA template and 23  $\mu$ l of 1xDream Taq PCR master mix (Thermo Fischer). PCR cycling profile comprised: Initial denaturation at 94°C for 5 min; 35 cycles of denaturation at 94°C for 60 s, annealing at 55°C for 60 s, elongation at 72°C for 75 s and final extension at 72°C for 10 min. The amplified products were separated on 1.5% agarose gel that was stained with ethidium bromide and were

visualised under UV excitation in a gel documentation system (Azure Biosystems, USA). The PCR products were then sequenced in both forward and reverse directions using the same set of primers in Applied Biosystems AB 3730 capillary sequencer at the sequencing facility (Agrigenome Pvt. Ltd., Kochi, India). The raw DNA sequences obtained were trimmed using BioEdit sequence alignment editor (ver. 7.0.5.2). Aligned sequences were entered in the GenBank BLAST search programme (Altschul *et al.*, 1990); the Ribosomal Database Project SIMILARITY\_RANK programme (Maidak *et al.*, 1997) and EzBioCloud 16S database (www.ezbiocloud.net) to obtain closely related phylogenetic sequences. Sequences were aligned by MUSCLE (Edgar, 2004) and phylogenetic tree was created with the neighbour-joining method (Saitou and Nei, 1987) using MEGA X package (Kumar *et al.*, 2018). Confidence in the branching pattern was assessed by analysis of 1000 bootstrap replicates (Felsenstien, 1985).

#### *Antibiotic sensitivity test*

Sensitivity to the antimicrobial agents was tested by spreading the culture suspension on solid medium (AM) as per Bauer *et al.* (1996). The isolates were tested using antibiotic discs (Hi-Media, Mumbai) impregnated with a set of 13 antimicrobial agents. We selected representative antibiotics from major classes of antibiotics *viz.*, Aminoglycosides (Amikacin, Kanamycin and Streptomycin), Macrolides (Erythromycin), Quinolones and Fluoroquinolones (Nalidixic acid), Glycopeptides and Diglycopeptides (Vancomycin), Rifamycins (Rifampicin), Penicillins (Penicillin-G, Amoxycylave and Carbanicillin), Tetracyclines (Tetracycline), Nitrofurans (Nitrofurantoin) and Chloramphenicol, for testing. The cultures were incubated at room temperature for 7 days. The sensitivity was noted on the basis of zone of growth inhibition.

#### *Polar lipid characterisation by thin layer chromatography (TLC)*

The culture was harvested at logarithmic phase by centrifugation (2000 *g* for 5 min) and subjected to polar lipid extraction with methanol/chloroform/0.3% NaCl (2:1:0.8 by vol.) (Bligh and Dyer, 1959; Card, 1973). Lipids were separated using silica gel TLC (Kieselgel 60 F254; Merck) in chloroform-acetic acid-methanol-water (85:22.5:10:4, by vol.). The plates after drying were sprayed with 5% ethanolic phosphomolybdic acid for total lipids and further characterised by spraying with ninhydrin (specific for amino groups), molybdenum blue (specific for phosphates),  $\alpha$ -naphthol (specific for sugars) and cresyl violet (for sulfolipid; Soto *et al.*, 2000).

#### *Carotenoid extraction and estimation*

Pigment extraction was done as per Cojoc *et al.* (2019). Biomass from 50 ml of culture was centrifuged at 7200 *g* for 15 min and washed with 30% saline broth twice by centrifugation. Pellet thus obtained was weighed and suspended in methanol (7.5 ml) and incubated at 60°C for 15 min in a water bath until visible pigment was extracted completely and then centrifuged at 2000 *g* for 15 min. Supernatant was scanned in the wave length region 400-800 nm using a UV-VIS spectrophotometer (Nanodrop One<sup>®</sup>, Thermo Scientific). Absorbance was measured at 490 nm and total carotenoid content was calculated using the formula (Davies, 1976):

$$\text{Total carotenoid content } (\mu\text{g g}^{-1}) = \frac{A \times V \text{ (ml)} \times 10^4}{A_{1\text{cm}}^{1\%} \times M \text{ (g)}}$$

where, A = Absorbance, V = Total extract volume, M = Sample mass,  $A_{1\text{cm}}^{1\%}$  =  $\beta$ -carotene absorption coefficient in petroleum ether = 2592.

## **Results and discussion**

#### *Isolation and identification of the haloarchaeon strain MS5.1*

The strain MS5.1 isolated from brine sample had a brick red pigmentation (Fig. 2) and the colonies on solid agar medium appeared smooth, entire, slightly raised, round (0.5 -1 mm in dia), shiny and mucoid. Under phase contrast objective, live cells appeared long rod shaped and motile. The cells were found to be long rods and Gram negative confirming absence of peptidoglycan in the cell wall (Fig. 3a, b).

The most important factors determining the growth of halophilic microbes is salt concentration. The strain

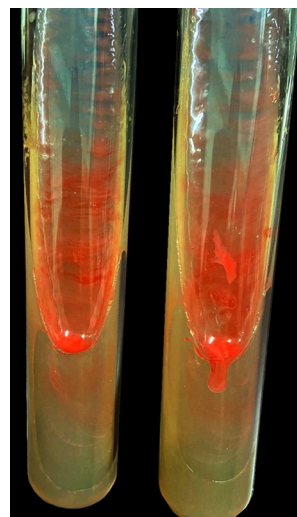


Fig. 2. Haloarchaeon strain MS5.1 growing on agar slants, having deep brick red pigmentation

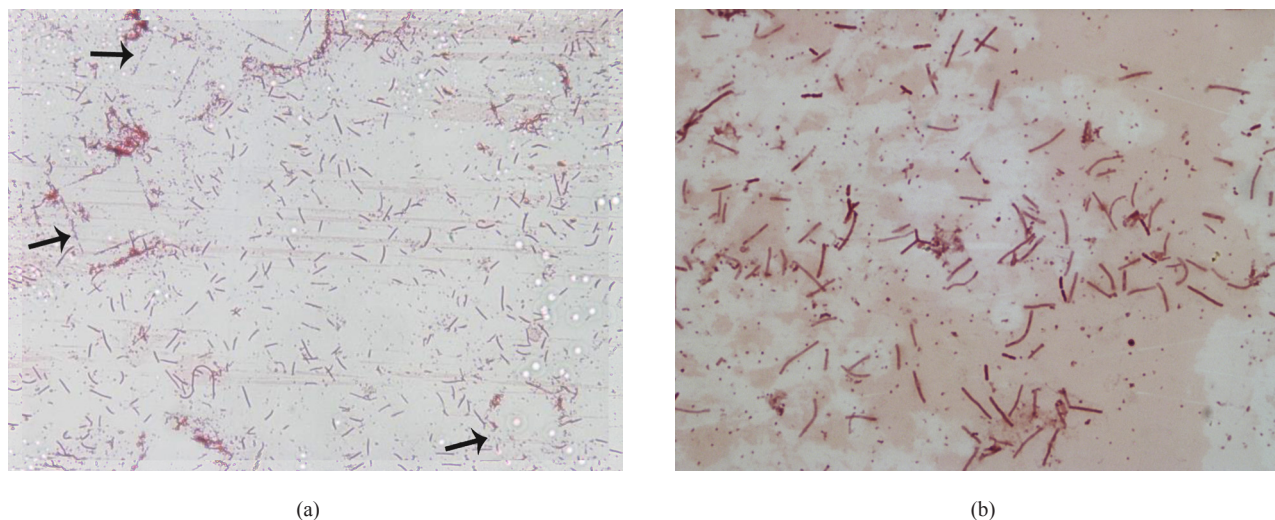


Fig. 3. Gram stained smear [(a) x400 and (b) x1000] of strain MS5.1 (Arrows indicate presence of salt crystals)

was found to be an extreme halophilic archaeon growing in high concentrations of NaCl ranging from 10 to 35% (w/v) with an optimum of 30%. The strain grows at temperature range of 20 to 40°C and pH range of 6 - 9, with optimum conditions being 28°C and pH of 7.0.

Many studies have reported difficulty in Gram staining of halobacterial isolates due to high salt concentration (Lochhead, 1934). Dussault (1955) introduced a modified technique by desalting the smear with 2% acetic acid for 5 min and therefore subsequent studies followed this protocol for Gram staining of haloarchaea too. However, in the present study, we obtained better results with the routine Gram staining protocol performed after washing the cells in PBS as compared to staining as per Dussault (1955). Washing the cells in PBS helped to remove the excess salt content and facilitated better visualisation of the Gram negative rods, though a few salt crystals were discernible in the stained smears (Fig. 3a).

The strain MS5.1 exhibited significant phenotypic and biochemical similarities with the type strain of *Halorubrum sodomense* (*H. sodomense* ATCC 33755). The biochemical characteristics of the strain MS5.1 in comparison with the type strain *H. sodomense* ATCC 33755 are given in Table 1. McGenity and Grant (1995) reported that *Halorubrum* cells are rods/irregular shaped, motile/non-motile, strictly aerobic and oxidase and catalase positive. Slight variations observed in the tests could be attributed to the strain variation owing to isolation from geographically distinct location. *H. sodomense* strain MS5.1 was found to hydrolyse starch, gelatin and Tween 80, which indicates that the strain is a potential source for halophilic enzymes.

Haloarchaea are classified under the family Halobacteriaceae in the order Halobacteriales of phylum Euryarchaeota. As per the List of Prokaryotic Names with Standing in Nomenclature (Euzéby, 1997), Halobacteriaceae presently comprises 40 genera. The genus *Halorubrum* proposed formally by McGenity and Grant (1995), at present includes 25 valid species. Species of the genus *Halorubrum* are ubiquitous in hypersaline environments especially in solar salterns (Hana Trigui *et al.*, 2011).

#### *Molecular characterisation and phylogenetic analysis*

Gel electrophoresis of the PCR product on agarose gel gave archaeal specific product (Fig. 4). Based on 16S rRNA gene sequence analysis and homology search using BLAST in the NCBI GenBank database as well as with the EZ Biocloud and RDP nucleotide sequence databases, the haloarchaeon strain MS5.1 was found to be a member of Halobacteriales order and more precisely *H. sodomense*. 16S rRNA gene sequence data of the isolate MS5.1 has been deposited in the NCBI GenBank nucleotide sequence database with the accession number MW332265. Fig. 5 depicts the phylogenetic tree constructed by neighbour joining method using MEGA X software.

#### *Antibiotic sensitivity*

*H. Sodomense* strain MS5.1 was found resistant to bactericidal antibiotics that target peptidoglycan confirming the absence of peptidoglycan in the cell wall. However significant inhibition was observed with Nitrofurantoin and Rifampicin, drugs which act by inhibition of nucleic acid (DNA/RNA) synthesis (Fig. 6; Table 2). Antibiotic sensitivity data is one of the baseline information for the characterisation of new isolates in the order Halobacteriales (Oren *et al.*, 1997).

Table 1. Biochemical characteristics of *H. sodomense* strain MS5.1 in comparison with the Type strain *H. sodomense* ATCC 33755

Characteristics	<i>H. sodomense</i> MS5.1	Type strain <i>H. sodomense</i> ATCC 33755
Cell shape	Rod	Rod
Motility	M	M
Pigmentation	Brick red	Red
Gram reaction	-	-
Minimum NaCl (% w/v) that supports growth	10	NK
Optimum NaCl (% w/v) required for growth	30	26
Range of NaCl (% w/v) that supports growth	10-35	14 – 26
Temperature range	20 - 40°C	20 - 50°C
Anaerobic growth on nitrate	-	NK
Catalase and oxidase	+	+
Production of enzymes which hydrolyse :		
Starch	+	+
Gelatin	+	-
Casein	-	NK
Tween 80	+	NK
Acid from carbohydrates :		
Dextrose	+	+
Mannose	-	-
Galactose	+	-
Fructose	+	+
D-Xylose	+	NK
Maltose	+	+
Sucrose	+	+
Lactose	+	-
Inulin	-	NK
Raffinose	+	NK
Trehalose	-	NK
Starch	+	+
Glycerol	+	+
Mannitol	+	NK
Sorbitol	-	NK
Decarboxylation of :		
Arginine	+	+
Lysine	+	+
Ornithine	-	NK
Histidine	+	NK
Indole production	-	-
ONPG	+	NK

M: Motile; + : Positive reaction; - : Negative reaction; NK : Not known

The characteristic pattern of sensitivity has found use in the isolation of archaea from a complex population of natural habitats (Elevi *et al.*, 2004). Antibigram data could be effectively used as strain markers which would help to exclude unwanted halophilic archaeobacterial strains from possibly mixed cultures.

#### *Polar lipids*

Polar lipid analysis helps to characterise halophilic archaea. Most of the representatives of the group contain the diphytanyl diether analogues of phosphatidylglycerol (PG) and the methyl ester of phosphatidyl glycerol phosphate (Me-PGP) (Litchfield *et al.*, 2001). TLC analysis of polar lipids is a useful and quick method for identification of haloarchaea, often to the genus level

(Ross *et al.*, 1981; Morth and Tindall, 1985; Litchfield *et al.*, 2000). Data on polar lipid content is essential while describing new taxa of Halobacteriaceae (Oren *et al.*, 1997). The strain MS5.1 was analysed for polar lipids by TLC and the results showed that polar lipids of the strain comprised C20, C20 derivatives of phosphatidylglycerol (PG), phosphatidylglycerol phosphate methyl ester (PGP-Me), diglycosyl archaeol (DGA) and sulphated diglycosyl archaeol (S-DGA) (Fig. 7). The polar lipid composition of the type strain *H. sodomense* ATCC 33755 isolated from the Dead Sea has been reported as C20, C20 derivatives of PG, PGP, PGS and a sulphated mannosyl glucosyl diether glycolipid (McGenity and Grant, 1995). The members of the genus *Halobacterium* possess PGS, glycolipids S-TGD (sulphated triglycosyl diether) and S-TeGD (sulphated

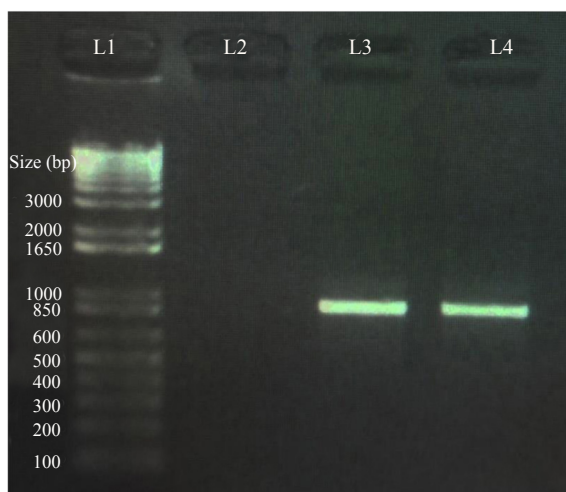


Fig. 4. Gel image of PCR product. L1: Ladder, L2: Control, L3 and L4: *H. sodomense* strain MS5.1

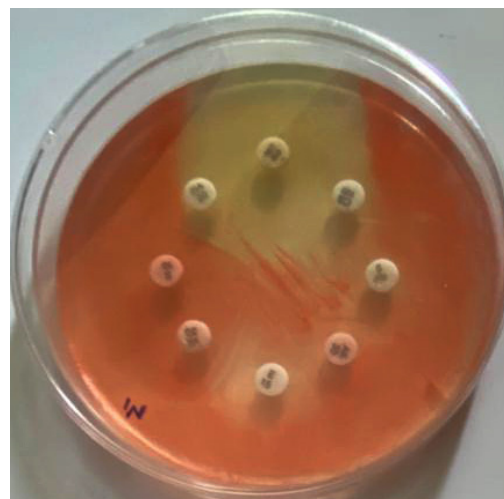


Fig. 6. Antibiotic susceptibility of strain MS5.1

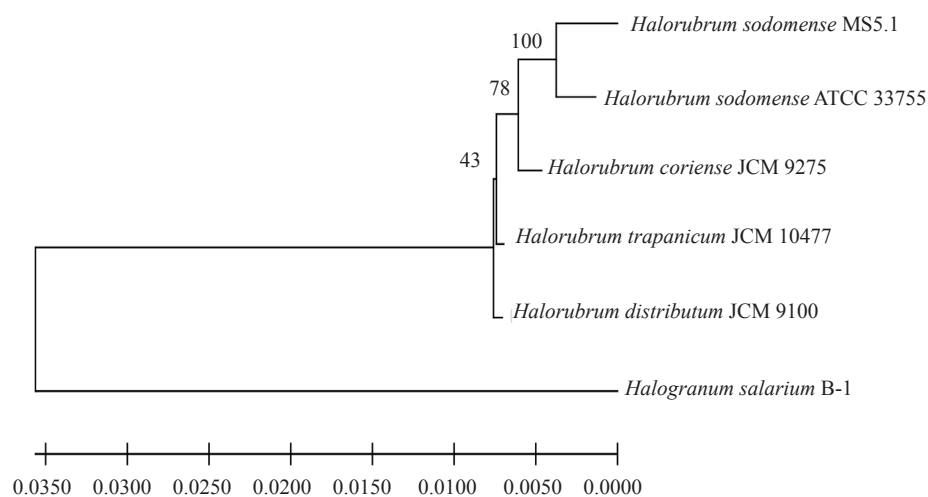
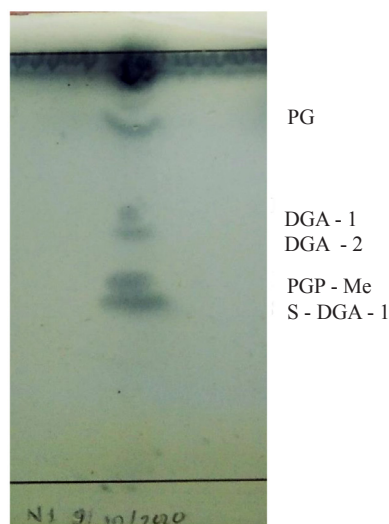


Fig. 5. Phylogenetic tree showing the position of *H. Sodomense* strain MS5.1, constructed with MEGA X. *Halogranum salarium* was used as an out group

Table 2. Antibiotic sensitivity of *H. sodomense* strain MS5.1

Antibiotics	Concentration ( $\mu\text{g}$ per disc)	Sensitivity and Zone of inhibition
Chloramphenicol	30	R
Tetracyclin	30	R
Amikacin	30	R
Erythromycin	15	R
Kanamycin	30	R
Amoxyclave	30	R
Nalidixic acid	30	R
Vancomycin	30	R
Rifampicin	5	S (15 mm)
Carbancillin	100	R
Streptomycin	10	R
Penicillin-G	10	R
Nitrofurantoin	300	S (40 mm)



PG: Phosphatidylglycerol; PGP-Me: Phosphatidylglycerol phosphate methyl ester; DGA: Diglycosyl archaeol; S-DGA: Sulfated diglycosyl archaeol

Fig. 7. Polar lipid composition of *H. sodomense* strain MS5.1 separated by TLC

tetraglycosyl diether) (Aparna Singh and Anil Kumar Singh, 2018). Archaeal cell membranes are composed of glycerol-ether lipids, consist of isoprenoid chains, 20 to 40 carbons in length and are usually saturated. The stability of archaeal lipids makes them useful biomarkers.

Archaea possess diphytanyl- or phytanyl-sesterterpanyl diether lipids that essentially differ from straight chain fatty acid glycerol ester lipids of bacteria and eukarya. Therefore, they can easily be separated from the ester-based lipids and furthermore, different genera of Halobacteriaceae contain different glycerol diether-based polar lipids and at least 12 different glycolipids have been described from haloarchaea (Litchfield and Oren, 2001). Several of these glycolipids are diagnostic for genera or even species. Besides, all representatives of the group have diphytanyl diether analogues of PG and Me-PGP. Presence/absence of diphytanyl diether equivalent of phosphatidylglycerol sulfate (PGS) is characteristic for certain genera under Halobacteriaceae. Hence, the spectrum of polar lipids extracted from hypersaline environments provides evidence on the types of halophilic archaea present in the community. Litchfield and Oren (2001) employed polar lipid analysis and pigments as experimental tool to gather information on diversity of microbial communities of solar salterns from various geographic locations.

#### Carotenoid production

Carotenoids are a group of terpenoids having antioxidant properties which are found in wide variety

of plants and animal kingdom (Sasidharan *et al.*, 2013). These are naturally occurring yellow to orange red pigments synthesised as hydrocarbons or their oxygenated derivatives by plants, bacteria, algae and fungi. Commercial interest has been developed in these molecules predominantly as natural colourants owing to their ability to confer colour. Many carotenoids have proved beneficial in prevention and treatment of certain cancers and in prevention of heart diseases by quenching free radicals (Laura *et al.*, 1970). Amongst many natural sources of carotenoids; red pigmented, salt resistant archaea have gained more importance due to possible recovery of carotenoids at low ionic strength solution and under non-aseptic condition (Asker and Ohta, 2002).

Significant carotenoid production (102.87  $\mu\text{g g}^{-1}$ ) by *H. sodomense* MS5.1 was recorded at pH 7, temperature 28°C and NaCl concentration of 30% after 7 days of incubation. Spectrophotometric analysis of pigment revealed that the type of pigment produced is of bacterioruberin group as it showed highest absorption at 490 nm. Results indicate that *H. sodomense* MS5.1 can be used as a potential source of carotenoid for food and pharmaceutical industries. The cell lysis occurs in lower NaCl concentration, so the process to get the carotenoid is a simple process, when archaea is concerned. The extraction could be done directly from the cells without any mechanical operation which is performed in case of plants and production of carotenoids from haloarchaea could be considered as an alternative commercial source for carotenoids (Naziri, *et al.*, 2014). The UV-VIS spectra of the carotenoids serve as a helpful source for their identification. Both the wavelengths of maximum absorption ( $\lambda_{\text{max}}$ ) and the shape of the spectra are specific for each carotenoid. Further investigations on Fourier-Transform Infrared (FTIR) and Raman spectroscopy analyses of the carotenoid extract from the strain is needed to delineate accurately the types of carotenoids produced by the strain. One of the main strategies used to minimise the cost and to increase the yield of carotenoid production by microorganisms is to optimise the culture conditions (Santos *et al.*, 2016). There are not many reports available on investigation on marine microorganisms as source of pigments mainly carotenoids. Nevertheless, based on numerous isolates sourced from marine ecosystems, it has been reported that 31.3% were yellow, 15.2% orange, 9.9% brown and 5.4% red or pink (Zobell and Feltham, 1934). Latest advances in molecular biology of carotenoids biosynthesis from organisms have provided information on a variety of genes that can be used as tools for a new approach of heterologous expression in different host organisms (Sasidharan *et al.*, 2013). By engineering of the microbial pathway enzyme, high quantity of carotenoids can be produced in industrial process from this strain.



Diversity studies of hypersaline areas around the world have indicated that *Halorubrum* is one of the dominant culturable members of haloarchaea (Kabilan *et al.*, 2012). They have the capacity to balance the osmotic pressure of the environment and resist the denaturing effects of salt. The present strain *H. sodomense* MS5.1 was selected at random by their brick red pigmentation. Colonies appearing in red shades is the indication of haloarchaeal presence. Manakudi saltpan is one of the major salterns in Tamil Nadu and the salt produced from this saltern is used for various industrial purposes. No information regarding the nature of the microbiota of this saltern is available so far. This work formed part of an attempt made to know the characteristics of this hypersaline environment and to study the archaeal community. We plan to further characterise the haloarchaeal strain to delineate the type of carotenoids produced and to optimise conditions for production of various halophilic enzymes produced by the strain.

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