Microbiological Studies on Sambhar Lake (Salt of Earth) Rajasthan, India.

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ABSTRACT

Sambhar Lake (Salt of Earth), the largest inland saline and alkaline lake of India is situated in the state of Rajasthan, and is referred in the epic Mahabharata. When full, it covers an area of 230 sq km. It lays some 60 km west of Jaipur, adjacent to the Salt Lake City. Flocks of flamingoes are attracted by the delicious spirulina algae that flourish when the water reaches a medium degree of salinity. There are many reports on the flora and fauna of this environment; however, there are very few reports on the isolation and characterization of extremely halophilic microorganisms, especially archaea from Sambhar Lake. We initiated bacteriological studies on this unique hypersaline and alkaline environment in 1984 under the guidance of Moiz F. Mullakhanbhai who along with Helge G. Larsen were the first to isolate a moderately halophilic bacterium (*Halobacterium volcanii*, now *Haloferax volcanii*).

Several strains of red, extremely haloalkaliphilic archaea, have been isolated and characterized from the brine and salt samples. All these strains possessed the diether core lipids, phosphatidylglycerol (PG), phosphatidylglycerophosphate (PGP), and bacteriorubins characteristic of halophilic archaea. Strains producing extremozymes (proteases, lipases, etc.) and bacteriocins have been isolated. Halophilic anoxygenic phototrophic sulfur oxidizing bacteria have also been cultivated. Data of our studies will be presented.

Keywords: Bacteriocins, Ectothiorhodospira, Halocins, Haloalkaliphilic archaea, Hypersaline environments, Natrialba, natronobacteria, soda lakes.

INTRODUCTION

Naturally occurring alkaline and hypersaline environments, popularly called soda lakes and soda deserts represent the most stable high pH environments on the Earth. The large amount of carbonate minerals causes the generation of pH values 9-11.5 (Jones *et al.* 1994). These environments are widely distributed around the world in the interior, inaccessible semi-arid or deserts areas. Some of the best studied soda lakes include the East African Rift Valley (Jones *et al.* 1994), Central Asian Soda Lakes (Zhilina and Zavarin 1994), and Mono Lake, California (Humayoun *et al.* 2003). Sambhar Lake, literally means *Salt of the Earth*, is the largest intermittent, saline and alkaline lake situated (Longitude 75°05’E Latitude 26°58’N) in the middle of a closed depression in the Aravalli schists in Rajasthan (Figure 1). It covers an area approximately 230 sq. km. and has been designated as Ramsar site, as this wetland serves as a wintering area for flamingoes and other migratory birds from northern Asia. The alkaliophilic members of the *Halobacteriaceae* vary from other members as they not only require high NaCl concentration but also high pH (between 8.5 and 11) and low Mg²⁺ concentrations (less than 10nM) for growth. We have earlier reported the chemical composition of brines from various kyars (salt ponds) and the isolation and characterization of extremely halophilic archaea from Sambhar Lake (Upasani and Desai, 1990, Upasani VN *et al.* 1994, Kamekura M *et al.* 1997). The taxonomy of the red extremely halophilic archaea is as described in Bergey’s Manual of Systematic Bacteriology, (2nd edition, volume 1, 2001). We summarize here our earlier findings and also report our studies on the cultivation of extremely halophilic phototrophs, and the isolation of bacteriocin producing *Natrialba* strain.

MATERIALS AND METHODS

Bacterial Strains and growth conditions

*Natrialba SSL1* (ATCC 43988) and SSL6 (ATCC 43987) were isolated from Sambhar Lake brines. *Natronobacterium gregoryi* (NCMB 2189) and *Natrialba magadii* (NCMB 2190) were provided as gift by Dr. H.N.M. Ross and Dr. W. D. Grant. These strains were grown on modified Brown (1963) medium as described by Tindall *et al.* (1984).

Screening for bacteriocinogenic activity

The strains *Natrialba* SSL1, SSL3, SSL4, SSL5 and SSL6 were screened for bacteriocinogenic activity against each other and the reference cultures mentioned above by spot inoculation and agar diffusion method. Modified Brown medium agar plates were spread with 0.2ml of log phase culture suspension and allowed to stand at room temperature...
for 1-2 hours. The plates were spot inoculated in the centre with the test culture and incubated at 37°C for 10-15 days. Plates were observed for zone of inhibition around the colony inoculated in the centre.

**Growth relationship**

Further studies on the inhibitory substance produced by *Natralba* SSL1 were carried out with *Natronobacterium gregoryi* as the sensitive indicator organism. Strain SSL1 was grown in 250ml of Brown medium broth in 1 liter Erlenmeyer flask as per conditions mentioned earlier. Samples were aseptically collected after 24, 36, 48, 60, 72, 96, 100, 110, 115 and 120 hours of incubation. The samples were centrifuged at 10,000 rpm at 4°C for 15 minutes. The supernatant was filtered through 0.4µm syringe filters and assayed for bacteriocinogenic activity as described by Rodriguez-Valera et al. (1982). The degree of inhibition was determined by measuring the size of zone of inhibition.

**Enrichment of Anoxygenic Phototrophs**

The red and purple pink brines and soil samples from Sambhar Lake were inoculated into two media as described by Imhoff and Truper (1977), and Imhoff *et al.* (1981) for the cultivation of extremely halophilic anoxygenic phototrophs from soda lakes. The tubes were incubated in sunlight, at 25 to 30°C for 7-10 days.

**RESULTS AND DISCUSSION**

Antagonistic interactions among halobacteria due to bacteriocin production have been described by Rodriguez-Valera *et al.* (1982) and Rdest and Strum (1987). We observed such interactions among extremely haloalkaliphilic archaea while isolating these organisms from Sambhar Lake brines by spread plate technique (Upasani and Desai, unpublished). During further screening it was revealed that one of our isolates *Natronobacterium* (now *Natralba*) SSL1 produced inhibitory substance against natronobacterial strains (Figure 2). All the strains tested showed equal degree of sensitivity to the inhibitory action (Table 1).

![Figure 2. Inhibition of Growth (a) *Natralba magadiii*, and (b) *Natralba* SSL6 by *Natralba* SSL1.](image)

Growth relationship studies showed highest bacteriocinogenic activity at the end of the exponential growth phase (96 - 100 hours). The inhibitory activity decreased gradually with after 100 hours growth.

These results show that the *Natralba* strain SSL1 produces a bacteriocin like substance which inhibits the growth of related haloalkaliphilic archaebacterial strains. Bacteriocins are bactericidal agents which are distinguished from other antimicrobial substances as they possess an essential protein moiety and a narrow spectrum of action against related organisms. Bacteriocins production in extremely halophilic archaea namely *Haloferax mediterranei* was first described by Rodriguez-Valera *et al.* (1982). Meseguer *et al.* (1986) termed these substances halocins and characterized a protein (halocin H4) of 28 kD from *Haloferax mediterranei*. Rdest U and Sturm M. (1987) purified and characterized a halocin Hal R1 produced by strain *Halobacterium* spp GN101. Their data indicated that Hal R1 is a peptide of Mr = 6.2 kD possibly complexed with a larger protein. Reeves (1972) has suggested that some bacteriocins may have originated from phages. Certain bacteriocins are plasmid determined (Stanier *et al.* 1976).

### Table 1. Screening for bacteriocin production in natronobacteria.

<table>
<thead>
<tr>
<th>Indicator strain used for spreading on medium</th>
<th>Strains tested for bacteriocin production</th>
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<tbody>
<tr>
<td>SSL1</td>
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<tr>
<td>SSL3</td>
<td>++</td>
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<tr>
<td>SSL4</td>
<td>++</td>
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<tr>
<td>SSL5</td>
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<td>SSL6</td>
<td>++</td>
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<tr>
<td><em>Natronobacterium gregoryi</em> (NCMB 2189)</td>
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<tr>
<td><em>Natralba magadii</em> (NCMB 2190)</td>
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A variety of colors are observed in the brines of Sambhar Lake as shown in the NASA satellite picture (Figure 1). This is due to mass bloom of halophilic and alkaliophilic algae, green and purple sulfur bacteria as well as the extremely haloalkaliphilic archaebacteria. These organisms serve as food for the migratory winter birds especially the flamingoes. We were successful in...
cultivating red, anoxygenic halophilic phototrophic purple bacteria belonging to the genus *Ectothiorhodospira* in the medium used for the cultivation of *Ectothiorhodospira vacoulata* (Imhoff *et al.* 1981). The cells are spiral or vibroid or rod shaped, and motile. They oxidize sulfide with extracellular deposition of elemental sulfur granules. These organisms thrive in the Sambhar Lake imparting purple pink color to the brine. *Ectothiorhodospira halochloris* has been isolated and identified from Wadi Natrun, Egypt by Imhoff and Truper (1977), *Ectothiorhodospira vacoulata* from Jordanian and Kenyan alkaline salt lakes by Imhoff *et al.* (1981), and *Ectothiorhodospira* spp. from Lake Magadii, Kenya (Grant *et al.* 1979).

Future studies are aimed at purification and characterization of the bacteriocins and other extracellular enzymes of biotechnological significance from SSL1, and characterization of the *Ectothiorhodospira* spp.

REFERENCES: