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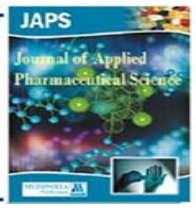

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Survey on Halophilic microbial diversity of Kovalam Saltpans in Kanyakumari District and its industrial applications

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ABSTRACT

Halophilic microorganisms are organisms that grow optimally in the presence of NaCl at least 0.2 M. The applications of halophilic bacteria include food and pharmaceutical industries, production of enzymes, polymers and various cosmetic products. The objective of the study was isolation and its characterization of potentially important microorganisms from saltpans. In this preliminary investigation, the total microbial counts were studied from the salt sample at three month intervals for one year. Totally 9 organisms were identified based on standard cultural, physiological and biochemical studies. These strains were subjected to screening of potential enzymes such as; amylase, protease, gelatinase, etc. The organisms *Natranobacterium sp-1*, *Staphylococcus epidermidis*, *Staphylococcus intermedius and Staphylococcus citreus* showed positive for amylase; *Halo bacillus salinus, Halococcus* salifodinae, *Staphylococcus epidermidis* and *Staphylococcus intermedius* showed positive for protease; *Halobacillus salinus, Halobacterium salinarum, Natranobacterium sp-1, Staphylococcus intermedius and Staphylococcus citreus* showed positive for gelatinase activity.

Key words: Microbial diversity**,** Halophilic bacteria, *Halococcus salifodinae*, Halophilic microorganisms.

INTRODUCTION

Biodiversity is the degree of variation of life forms within a given ecosystem, biome, or an entire planet. Biodiversity is a measure of the health of ecosystems. In terrestrial habitats, tropical regions are typically rich whereas Polar Regions support fewer species (Litchfield *et.al*. 2009). Microorganisms are distributed among three primary relatedness groups or domains: Archaea, Bacteria, and Eucarya. The general properties of representatives of the three domains indicate that the earliest life was based on inorganic nutrition and that photosynthesis and use of organic compounds for carbon and energy metabolism came comparatively later.

The microbial component is becomes an impossible task because It simply is not known or even well estimated how many species/genera there are of the microscopic algae, fungi, bacteria, and viruses. Thus the study of the microbial diversity and community characteristics of extreme environments such as hypersaline lakes and solar salt works is vital to beginning to estimate this major compartment of biodiversity. Concentrated salt solutions like salt or soda lakes, coastal lagoons or man-made salterns, inhabited by only a few forms of higher life, are dominated by prokaryotic microorganisms. There are two groups of bacteria involved in the microbial diversity in solar salterns. The first group is from the domain *Bacteria*. This group contains the bacteria most people are familiar with; those microbes that are responsible for the production of wine and beer, the production of yogurt and some cheeses and fermented meats, the production of antibiotics, waste water treatment, etc. as well as the being the principle agents of

many human and animal diseases. In this domain are some unusual *Bacteria* which can grow over very wide salinity ranges (*Halomonas)* or which seem to be a bridge (*Salinibacter*) between the *Bacteria* and the other domain of bacteria the *Archaea* (Litchfield *et.al*., 2009).Global salt deposits show that evaporation of marine salt water and the development of hypersaline habitats is an ongoing process for millions of years and providing ample time for the evolution of specialized halophilic Bacteria and Archaea. Halophiles, which require more than 0.5 M NaCl for optimal growth have developed two different basic mechanisms of osmoregulatory solute accumulation to cope with ionic strength and the considerable water stress (Reed *et al*., 1986). These mechanisms allow halophiles to proliferate in saturated salt solutions and to survive entrapment in salt rock.

Halophiles living in high-salt environment, due to a wide diversity of environmental adaptation, phylogenetic diversity, unique salt tolerance mechanisms and potential applications. Bacteria, and probably the fungi, are furthermore responsible for the degradation of organic matter and the conversion of organic nitrogen to ammonia which can be used by the algae and perhaps brine shrimp and brine flies so common to hypersaline environments.

MATERIALS AND METHODS

Collection of Samples

The samples from were collected from salt pan of Kovalam at four intervals for one year. All samples were collected aseptically in sterile 1 liter bottles and transferred to the lab for the further analysis.

Enumerations and Isolation of organisms

Samples were plated within 2 hours of collection and in duplicate using a modification of the spread-drop-plate method. An aliquot of 0.1 ml of each dilution from 10^{-1} to 10^{-6} was taken and spread evenly over the surface of modification of the R2A medium. R2A was modified by the addition of 20 g magnesium sulfate per liter. Each medium was prepared to contain 4, 10, 20, and 25% solar salt (w/v) and pH 7.3. They were incubated at 37°C and counted. Growth was scored according to the color of the colonies. (Fung and Kraft, 1968) These procedures were carried out for every three months (March to December, 2010) intervals for one year. Different colonies grown on media were selected and purified for further investigation.

Phenotypic characteristics and identification of isolates

The cultures were purified on the same salt concentration and medium from which they were isolated. All tests were performed at that salt concentration until the salt/temperature studies were performed. Standard bacteriological tests for extracellular enzyme production were performed for gelatinase, amylase, and proteinase. Selected cultures were tested for other biochemical characteristics such as indole, Methyl red, Voges proscauer, citrate, Urease, TSI and carbohydrate utilization. (Kannika Chookietwattana, 2003; Carol *et al*., 2006; Claus and Berkeley 1986; Grant *et al*., 2001**;** Kloos and Schleifer, 1986; Meyer, 1989; Palleroni, 1984; Schleifer, 1986)

RESULTS

Enumerations and Isolation of organisms

There are four samples were analyzed for this study at three months interval for one year (2010). The samples were analysed the microbial diversity. The samples collected from Kovalam Salt pans showed higher diversity in the march than other periods. It reveals that the low salt concentrations in the period due to the higher rain fall in the monsoon period resulted in the high density and diversity of halophilic bacteria. From the present study a total of 9 representative bacterial isolates were obtained from the soil dilution plate during the whole period of soil sample collection. The following number of halophilic bacterium isolates selected from this study is described in Table 1.

Table: 1 Enumeration of microorganisms

Sr. No.	Sample name	Dilution	No. of colonies
1	2010 March	10^{-1}	*
		10^{-2}	123
		10^{-3}	41
		10^{-4}	11
		10^{-5}	2
		10^{-6}	-
2	2010 June	10^{-1}	*
		10^{-2}	213
		10^{-3}	109
		10^{-4}	62
		10^{-5}	16
		10^{-6}	-
3	2010 Sep	10^{-1}	*
		10^{-2}	168
		10^{-3}	54
		10^{-4}	13
		10^{-5}	2
		10^{-6}	-
4	2010 Dec	10^{-1}	*
		10^{-2}	153
		10^{-3}	48
		10^{-4}	15
		10^{-5}	1
		10^{-6}	-

Phenotypic characteristics and identification of isolates

The colony morphology of all strains S1-S9 were oily, convex pale yellow; smooth, circular, raised, orange-yellow; Smooth, irregular red colour colonies; Circular. Slightly irregular, convex colonies; Reddish , circular raised colonies; Dirty white, Transparent, flat colonies; Slightly yellow, circular Transparent, flat colonies; Slightly yellow, circular Transparent, flat colonies and yellowish, circular Transparent, convex colonies respectively. The summarization of the number of halophilic bacterium isolates selected from this study is described in Table 2.

Table: 2 Characterization and identification of isolated organisms.

Characteristics	**S1**	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**
Optimum pH	7.0	7.0	7.0	7.0	8.5	7.0	7.0	7.0	7.0
Optimum T^0	37^0C	37^0C	30^0C	30^0C	37^0C	37^0C	37^0C	37^0C	37^0C
Growth at NaCl concentrations (%)									
5	+	+	-	-	+	-	+	+	+
10	+	+	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+
20	+	+	+	+	-	+	+	+	+
25	+	+	+	+	-	+	+	+	+
Pigments	+	+	+	-	+	-	-	+	+
Gram stain	Negative	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Positive
Morphology	Rod	Rod	Rod	cocci	Rods	Rods	cocci	cocci	cocci
Motility	+	+	-	-	+	-	-	-	-
Catalase	+	-	+	+	+	+	+	+	+
Oxidase	-	-	+	-	+	-	+	+	+
Indole	-	-	+	-	-	-	-	-	-
MR	+	-	-	-	-	-	-	-	-
VP	-	-	-	-	-	+	-	-	-
Citrate	+	-	-	-	-	+	-	-	-
Urease	-	-	-	-	-	-	-	-	-
Starch hydrolysis	-	-	-	-	+	-	+	+	+
Gelatin hydrolysis	-	+	+	-	+	-	-	+	+
Casein hydrolysis	-	+	-	+	-	-	+	+	+
Carbohydrate fermentation									
Glucose	+	+	-	+	+	+	+	+	+
Sucrose	+	+	-	-	+	-	+	+	+
Lactose	+	+	-	-	+	-	+	+	+
Arabinose	-	-	-	-	-	-	+	+	-
Maltose	+	+	-	-	-	-	-	-	-
Mannose	+	-	-	-	-	-	-	-	-

Different colonies grown on media were selected and purified for further investigation. A total of 24 colonies were selected for characterization and identification studies includes, 7 from March-2010, 6 from June-2010, 6 from September-2010 sample and 5 from December-2010 sample. Based on the cultural and phenotypic characteristics total of 9 strains were identified. The probably identified strains includes *Vibrio fischeri., Halobacillus salinus, Halobacterium salinarum, Halococcus salifodinae, Natranobacterium sp-1, Bacillus subtilis, Staphylococcus epidermidis, Staphylococcus intermedius and Staphylococcus citreus* from S1-S9 respectively

DISCUSSION

The results this study shows that the bacterial and archaeal communities of solar salterns are dynamic both spatially and temporally. Litchfield *et al.*(2009), summarized some of their work on the salterns in Bonaire, Netherlands Antilles where again there were major differences in the total numbers of bacteria and numbers of pigmented bacteria over time. The biodiversity noted in this study begs the question as to what useful microorganisms might be among these communities. Many of these bacteria contain potential enzyme production such as amylases, proteases, gelatinase, etc. The genus *Halomonas* are useful in drug deliverysystems and in photographic applications (Vreeland *et al.*, 1980). There are many ecologically and evolutionarily important microbes exist in salinas so further more study is needed to know that significant microbial biodiversity in the solar salterns.

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