Hindawi Publishing Corporation
Chinese Journal of Biology
Volume 2014, Article ID 205731, 12 pages
http://dx.doi.org/10.1155/2014/205731

Research Article

Production of Extracellular Polymeric Substances by Halophilic Bacteria of Solar Salterns

Jhuma Biswas and A. K. Paul

Microbiology Laboratory, Department of Botany, University of Calcutta, 35 Ballygunge Circular Road, Kolkata 700 019, India

Correspondence should be addressed to A. K. Paul; amalk_paul@yahoo.co.in

Received 13 May 2014; Accepted 15 July 2014; Published 6 August 2014

Academic Editor: Yinguang Chen

Moderately halophilic aerobic bacteria were isolated from 31 soil and 18 water samples collected from multipond solar salterns of Gujarat, Orissa, and West Bengal, India. A total of 587 bacterial isolates with distinct morphological features were obtained from these samples following dilution and plating on MH agar medium supplemented with NaCl. The isolates were screened for growth associated extracellular polymeric substances (EPS) production in MY medium under batch culture. In all, 20 isolates were selected as potent ones producing more than 1 g/L of EPS. These EPS producing isolates were characterized in detail for their morphological, physiological, and biochemical features and tentatively identified as members belonging to the genera *Halomonas*, *Salinicoccus*, *Bacillus*, *Aidingimonas*, *Alteromonas*, and *Chromohalobacter*. Apart from EPS production, these isolates also hold promise towards the production of various biomolecules of industrial importance.

1. Introduction

Multipond solar salterns used for industrial production of marine salts by evaporation of sea water represent hypersaline environments which are popular habitats for studying halophilic bacteria and have great potential towards industrial and biotechnological applications [1, 2]. The diversity of halophilic bacteria so far isolated and characterized is categorized into four different classes according to NaCl requirement for their growth and includes slight halophiles, moderate halophiles, extreme halophiles, and border line halophiles. The halotolerant bacteria on the other hand do not require NaCl for their growth but can tolerate a high salinity [3–5]. Halophilic diversity of solar salterns has been studied quite extensively across the globe and reviewed by several authors [6–8]. However, only very few studies have been made on the halophilic bacterial community in coastal solar salterns of India [9, 10], which deserves special attention for exploration and commercial exploitation of these microbial resources.

Extracellular polymeric substances (EPS) are one of the industrially important compounds produced by a wide variety of marine microorganisms. Due to growing biotechnological interest [11], production of bacterial EPS has become an attractive field of research. EPS is used as thickeners, emulsifiers, and suspending agents in food, pharmaceuticals, and petroleum industries. They are also used as adhesives in detergents, textiles, papers, paints, and beverages industries. Moreover, EPS are used as metal removers and bioabsorbers in oil recovery, mining, and petroleum industries [12].

During the course of extensive search for new strains producing extracellular polymeric substances (EPS) in their natural hypersaline environments, large numbers of halophilic bacteria and archaea have been established as EPS producers. Most of them belong to the genera *Haloferax*, *Haloarcula*, *Halococcus*, *Natronococcus*, and *Halobacterium* [13, 14]. Nevertheless, the common halophilic EPS producing bacteria belong to the genus *Halomonas*, most importantly *H. maura* [8], *H. eurihalina* [15], *H. ventosae*, and *H. anticariensis* [16]. Exopolysaccharides synthesized by *Halomonas* strains unusually have high sulphate content and a significant amount of uronic acids determining their good gelifying properties. Moreover, recent reports have

also established that halophilic EPS producers belong to the gamma-proteobacteria (*Idiomarina* and *Alteromonas*) as well as alpha-proteobacteria (*Salipiger mucosus* and *Palleronia marisminoris*) [17, 18].

This study is focused on the isolation of halophilic bacteria from water and soil samples of some selected solar salterns located in the states of Gujarat, Orissa, and West Bengal, India, and evaluation of their EPS production efficiency under laboratory conditions. Attempts have also been made on the tentative identification of some potent EPS producing halophilic isolates based on their morphological and physiobiochemical features.

2. Materials and Methods

2.1. Collection of Samples. Soil and water samples from multipond solar salterns situated along the coast of Gujarat, Orissa, and West Bengal, India, were collected in sterile polypropylene containers and stored at 4°C until used for isolation of halophilic bacteria. A total of 12 soil and 7 water samples were collected from Jogrinar (23°13′ N and 69°59′51″ E), Khari Rohar (23°4′16″ N and 70°9′37″ E), Kandla (22°59′ N and 70°13′ E), and Albert Victor Port (21°0′58″ N and 71°32′54″ E) of Gujarat. Similarly 10 soil and 5 water samples were collected from Surala (19°84′73″ N and 84°65′ E) and Humma (19°26′ N and 85°5′ E), the two major solar salterns of Orissa. In West Bengal, the sampling sites were located at Dadanpatrabar (22°26′ N and 87°20′ E) and Baksal (22°1′ N and 87°67′ E) of East Midnapur, and a total of 9 soil and 6 water samples were collected.

2.2. Isolation of Halophilic Bacteria. Aerobic, heterotrophic, and halophilic bacteria of soil and water samples were isolated by serial dilution and plating on MH agar medium [19] supplemented with different concentrations of NaCl. The medium contained (g/L) yeast extract, 10; protease peptone, 5; glucose, 1; NaCl, 100; $MgCl_2$ $6H_2O$, 7; $MgSO_4$ $7H_2O$, 9.6; $CaCl_2$ $2H_2O$, 0.36; KCl, 2; $NaHCO_3$, 0.06; and NaBr, 0.026 (pH 7.2). The plates were incubated at 37°C for 3 to 5 days, and bacterial colonies with distinct morphology were isolated in pure form and maintained on slopes of the same medium. Total bacterial counts were expressed as colony forming units (cfu)/mL and /g of water and soil, respectively.

2.3. Screening of Halophilic Bacteria for EPS Production. To evaluate the EPS production capability, the bacterial isolates were grown in MY medium [20] supplemented with 5% NaCl for 12 days at 32°C under continuous shaking (120 rpm). The medium contained (g/L) NaCl, 50; $MgCl_2$ $6H_2O$, 9; $MgSO_4$ $7H_2O$, 13; $CaCl_2$ $2H_2O$, 0.2; KCl, 1.3; $NaHCO_3$, 0.05; NaBr, 0.15; $FeCl_3$ $6H_2O$, 0.005; glucose, 10; yeast extract, 3; malt extract, 3; and protease peptone, 5 (pH 7.2). The EPS from the growing culture was isolated using the method as described by Quesada et al. [15]. The culture was centrifuged (at 10,000 ×g for 10 min), the EPS in the supernatant was precipitated with chilled ethanol, and recovered by centrifugation (12000 ×g for 10 min) and washed with chilled 70% ethanol. The washed precipitate was collected by centrifugation, dissolved in known volume of distilled water, and used for quantification and chemical analysis.

2.4. Chemical Analysis of EPS. Total carbohydrate content of the EPS was estimated following the method of Dubois et al. [21]. To 1 mL of dissolved EPS sample, 0.5 mL of 5% phenol and 3.5 mL of concentrated sulfuric acid were added and incubated at 30–40°C for 10–20 minutes in hot water bath. Absorbance was read at 490 nm, and the amount of carbohydrate was determined from the calibration curve prepared using glucose as standard.

Protein content of the EPS was determined following the Folin phenol method of Lowry et al. [22]. To 1 mL of EPS sample, 5 mL of alkaline solution and 0.5 mL of Folin phenol reagent were added and incubated for 30 minutes at room temperature in dark. The absorbance was measured at 670 nm, and the concentration was read from the calibration curve prepared by using bovine serum albumin (BSA) as the standard.

2.5. Characterization and Identification of Selected Bacterial Isolates. The selected bacterial isolates were characterized morphologically and physiobiochemically following standard microbiological methods as described by Gerhardt et al. [23]. To determine the antibiotic sensitivity pattern of these isolates, the antibiotic impregnated discs (Himedia, 6 mm dia.) were placed on MH agar plates seeded with respective bacterial isolates. The plates were incubated for 24 h at 32°C, and diameter of inhibition zones was measured to the nearest mm. Production of acids from sugars by the bacterial isolates was tested on phenol red medium supplemented with 1% carbon source. Characteristics of the bacterial isolates were compared with those described in *Bergey's Manual of Systematic Bacteriology* [24] and that of Mata et al. [25] for determination of taxonomic identity.

3. Results

3.1. Isolation of Halophilic Bacteria. A total of 31 soil and 18 water samples collected from 8 different sites spread over the states of Gujarat, Orissa, and West Bengal, India, were analyzed for the aerobic, heterotrophic, and halophilic bacteria following dilution and plating on MH agar medium supplemented with 5, 10, and 15% NaCl. The total bacterial population of both soil and water samples as determined by colony forming units varied considerably and declined gradually irrespective of sampling sites with increasing NaCl concentration in the isolation medium (Tables 1 and 2).

A total of 587 halophilic and halotolerant, heterotrophic, and aerobic bacterial isolates were obtained in pure form. The majority of these isolates (410) were derived from soil samples (Table 3), while 177 were obtained from water samples (Table 4). Most of the isolates were Gram-negative, motile, aerobic

Table 1: Bacterial population of soil samples collected from solar salterns of Gujarat, Orissa, and West Bengal, India.

State	Sampling sites	Number of samples	Bacterial count (cfu/g of soil $\times 10^2$) NaCl (%) in medium		
			5	10	15
Gujarat	Jogrinar	4	0.74–86.6	0.21–36.6	0.11–4
	Kandla Port	3	0.86–7.35	1.21–2.11	0.08–0
	Khari Rohar	3	2.39–7.68	0.12–17.8	0.47–9.66
	Victor Port	2	1.73–2.83	0.40–0	—
Orissa	Humma	5	0.13–0.61	0.25–0.55	—
	Surala	5	1–3.66	0.07–0.23	—
West Bengal	Baksal	4	2–3.90	0.20–0.40	0.20–0
	Dadanpatrabar	5	2.7–7.9	0.07–0.60	0.07–0.30

Table 2: Bacterial population of water samples collected from solar salterns of Gujarat, Orissa, and West Bengal, India.

States	Sampling sites	Number of samples	Bacterial count (cfu/mL of water $\times 10^2$) NaCl (%) in medium		
			5	10	15
Gujarat	Jogrinar	2	0.28–5.56	1.72–0	0.40–0
	Kandla Port	2	0.53–5.40	0.16–1.1	0.66–0
	Khari Rohar	1	0.23–0	—	—
	Victor Port	2	12.6–190	—	—
Orissa	Humma	3	0.23–2.3	—	—
	Surala	2	0.27–1.20	0.83–0	—
West Bengal	Baksal	3	1.10–1.36	0.13–0.6	0.10–0.37
	Dadanpatrabar	3	0.5–1.90	0.07–0.50	0.1-0.2

rods and produced white to cream colored, circular, smooth-edged colonies on MH agar. In general, the isolates were capable of tolerating wide range of temperature and pH for their growth.

3.2. Screening for EPS Production. EPS producing ability of these isolates was examined in batch culture using MY medium supplemented with 5% NaCl, and the soluble EPS content of each of the isolates was evaluated in terms of its carbohydrate content as determined by Dubois method [21]. Among the 410 soil isolates, majority (184) produced soluble EPS ranging from 0.5 to 0.7 g/L in terms of their carbohydrate content, while only 15 isolates produced more than 1 g/L of EPS (Table 5). On the contrary, among the 177 bacterial strains isolated from saline water samples, only 5 produced soluble EPS accounting for more than 1 g/L of carbohydrate. However, majority (77) of them produced 0.5–0.7 g/L of soluble EPS (Table 6).

In all, 20 potent isolates producing >1 g/L of EPS were further allowed to grow in MY medium under continuous shaking (120 rpm), and the soluble EPS produced after 8 days of growth was estimated following the method as described above. Kinetics of growth and EPS production revealed that EPS production by these isolates increased with biomass formation (Figure 1), and a few of the selected isolates such as SUR202, SUR307, SUR310, JW307, and JS904 appeared to be promising with an EPS yield of 1.68–1.85 g/L.

3.3. Characterization and Identification of Selected Bacterial Isolates. Morphological and physiological studies revealed (Table 7) that these halophilic bacteria (with the exception of isolate SUR303) formed cream colored smooth colonies on MH agar medium. Most of them were Gram-negative, motile rods; only three isolates were Gram-positive; one of them (isolate SUS303) was coccus, while the isolate KW203 was the only endospore former. The isolates were capable of tolerating 15–20% NaCl in the medium and a pH of 5–11. Optimum growth was observed at a temperature of 32–37°C, but all were able to tolerate a temperature as high as 40°C.

Analysis of biochemical characters (Table 8) showed that the majority of these halophilic isolates gave negative response to MR-VP tests and failed to produce extracellular enzymes like amylase, cellulase, pectinase, inulinase, gelatinase, lipase, caseinase, xylanase, and urease. None of these isolates, however, were capable of producing H_2S, lysine-, arginine-, and ornithine decarboxylase.

Carbon source utilization pattern (Table 9) of these halophilic isolates varied considerably. All the isolates were able to utilize ribose, fructose, mannitol, salicin, cellobiose, acetate, benzoate, and succinate, and three of them (JS803,

Table 3: Bacterial isolates obtained from soil samples of solar salterns of Gujarat, Orissa, and West Bengal, India.

States	Sampling sites	Number of samples	Number of bacterial isolates NaCl (%) in medium		
			5	10	15
Gujarat	Jogrinar	4	44	23	4
	Kandla Port	3	19	12	4
	Khari Rohar	3	46	16	6
	Victor Port	2	20	5	—
Orissa	Humma	5	48	14	—
	Surala	5	31	31	2
West Bengal	Baksal	4	19	8	3
	Dadanpatrabar	5	29	16	10
Total		31	256	125	29

Table 4: Bacterial isolates obtained from water samples of solar salterns of Gujarat, Orissa, and West Bengal, India.

States	Sampling sites	Number of samples	Number of bacterial isolates NaCl (%) in medium		
			5	10	15
Gujarat	Jogrinar	2	21	14	5
	Kandla Port	2	19	5	2
	Khari Rohar	1	1	3	—
	Victor Port	2	26	—	1
Orissa	Humma	3	16	—	—
	Surala	2	12	8	3
West Bengal	Baksal	3	9	3	4
	Dadanpatrabar	3	17	4	4
Total		18	121	37	19

SUR301, and SUR307) appeared to be versatile in utilizing all 30 carbon sources. Fermentation pattern of these strains varied remarkably; the majority were unable to ferment most of the carbon sources. On the contrary, fructose, sorbitol, and benzoate were fermented by most of the isolates (Table 9).

Sensitivity of these isolates to 22 different antibiotics was tested by disc-diffusion method (Table 10), and the antibiotic resistance index (ARI) was determined (Figure 2). The majority of the isolates were sensitive to chloramphenicol (30 μg), gentamycin (10 μg), and norfloxacin (10 μg) followed by ampicillin (10 μg). Resistance to vancomycin (30 μg) followed by trimethoprim (30 μg) was predominant amongst the tested halophiles. As judged by the ARI values, the isolate SUR302 was the most resistant one (ARI = 0.65) followed by SUR301, KW203, and KW1805, while the lowest ARI (0.23) was indicated by the isolate JW307, which was most sensitive to the tested antibiotics.

The morphological, physiological, and biochemical characters including the carbon source utilization and fermentation patterns along with antibiotic susceptibility were analyzed and compared with the phenotypic characters of halophilic bacterial genera [5, 18] so far reported. According to the phenotypic and biochemical characteristics, 70% of the selected isolates (14) were tentatively identified as members of the genus *Halomonas*. The only Gram-positive coccus (isolate SUR303) and the rod shaped endospore forming isolate (isolate KW203) were assigned to *Salinicoccus* and *Bacillus*, respectively. Two of the isolates (KS1805 and SUR301) were placed in the genus *Chromohalobacter*, while the remaining two (isolates JS504 and JS904) were included under *Alteromonas* and *Aidingimonas*, respectively.

4. Discussion

Halophiles have mainly been isolated from wide diversity of environments such as saltern crystallizer ponds, the Dead Sea, solar lakes, and hypersaline lakes [26]. Culture dependent diversity studies on halophiles have been made from Tunisian solar saltern [27, 28], Tuzkoy salt mine [29], Sereflikochisar Salt Lake [30], Kaldirim and Kayacik of Tuz Lake [31], Turkey, Howz Soltan Lake, Iran [32], and hypersaline environments in South Spain [33–36]. In the Indian context, the halophilic diversity studies have been restricted mainly to the marine salterns of Bhavnagar [37–40], Lonar Lake [40], and Peninsular Coast [41, 42]. The present study reports

Table 5: Screening of halophilic bacterial isolates derived from soil samples for the production of extracellular polymeric substances.

States	Sampling sites	Number of isolates	Extracellular polymeric substances (g/L)			
			>0.50	0.51–0.70	0.71–1.0	>1.0
Gujarat	Jogrinar	71	18	42	7	4
	Kandla Port	35	13	13	7	2
	Khari Rohar	68	26	31	11	—
	Victor Port	25	20	3	2	—
Orissa	Humma	62	22	37	3	—
	Surala	64	15	25	15	9
West Bengal	Baksal	30	14	12	4	—
	Dadanpatrabar	55	32	21	2	—
Total		410	160	184	51	15

Table 6: Screening of halophilic bacterial isolates derived from water samples for the production of extracellular polymeric substances.

States	Sampling sites	Number of isolates	Extracellular polymeric substances (g/L)			
			>0.50	0.51–0.70	0.71–1.0	>1.0
Gujarat	Jogrinar	40	26	9	3	2
	Kandla Port	26	13	11	1	1
	Khari Rohar	4	1	3	—	—
	Victor Port	27	7	17	1	2
Orissa	Humma	16	4	11	1	—
	Surala	23	8	6	9	—
West Bengal	Baksal	16	7	6	3	—
	Dadanpatrabar	25	8	14	3	—
Total		177	74	77	21	5

the distribution of halophilic and halotolerant bacterial communities in the inland multipond solar salterns spread over the coasts of India. Halophilic bacterial communities of 31 soil and 18 water samples from 8 different sites were analyzed by dilution and plating method (Tables 1 and 2) and provide information regarding the availability and diversity of halophilic bacteria in the solar saltern ponds. Colony forming units of soil and water samples revealed that soil samples hold more viable bacterial counts than water samples. Raghavan and Furtado [41] studied the occurrence of extremely halophilic archaea in sediments from the continental shelf of west coast of India and reported the presence of relatively low average counts ($7–5 \times 10^3$) of extreme halophiles in offshore sediments in contrast to the very high counts (10^5–10^9) of marine eubacteria. Similar study by Joshi et al. [40] reported that the total numbers of microorganisms in the soil and water samples were 10^2–10^6 cfu/g and 10^2–10^4 cfu/mL, respectively.

A total of 587 halophilic and halotolerant bacterial strains were isolated showing different degrees of NaCl tolerance (Tables 3 and 4) and supported the observations of Quesada et al. [35] and Ventosa et al. [34]. However, during the present study we were unable to isolate extreme halophiles, which have frequently been identified as the dominant phylotypes in hypersaline environments along with solar salterns of India [39, 41]. Furthermore, soil samples showed more bacterial diversity than water ones, which is in accordance with the observations of Joshi et al. [40].

During the course of screening of the moderately halophilic isolates for EPS production (Tables 5 and 6), only 20 isolates appeared to be promising with significant yield of EPS (1.0–1.85 g/L) (Figure 1). Joshi and coworkers [40] similarly screened 86 halophilic bacteria from Lonar Lake and reported *Halomonas campisalis* and *Vagococcus carniphilus* as potent EPS producers. Similarly, Nanjani and Soni [43] also isolated 73 halotolerant and halophilic bacteria from soil samples of Veraval and Dwarka; 23 of them produced EPS ranging from 0.2 to 10.60 g/L. In addition reports on EPS production by moderately halophilic bacteria of the genus *Halomonas* are not uncommon [8, 15–17].

Attempts have been made to determine the taxonomic identity of all 20 promising EPS producing isolates following detailed physiobiochemical characterization (Tables 7–9) and comparison with *Bergey's Manual of Systematic Bacteriology* [24] and those of Mata et al. [25]. The majority (14 isolates) of them were Gram-negative, nonsporulating rods and capable of growing in 2.5–20% NaCl similar to those of *Halomonas* as reported by Quesada et al. [36], Ghozlan et al. [44], and Mata et al. [25]. However, the Gram-positive endospore forming isolate KW203 was assigned to the genus *Bacillus*.

Table 7: Morphological and physiological characteristics of the selected EPS producing halophilic bacteria isolated from solar salterns of India.

Character	Bacterial isolates									
	JS504	JS802	JS803	JS904	JW302	JW307	KS1101	KS1805	KW203	VW301
Morphological characters										
Colony morphology	Cream, circular	White, circular	Cream, circular	Cream, circular	Transparent, circular	Cream, circular	Cream, circular	White, circular	White, circular	Cream, circular
Gram nature	–	+	–	–	–	–	–	–	+	–
Cell shape	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Single, chains	Single, chains	Single, chains	Single	Single, clumped	Single, chains
Motility	+	+	+	+	+	+	–	–	+	+
Pigmentation	–	–	–	–	–	–	–	–	–	–
Endospore formation	–	–	–	–	–	–	–	–	+	–
Physiological characters										
pH range for growth	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11
pH optimum for growth	11	9	8	9	11	11	11	8	8	9
Temp. range for growth (°C)	27–40	27–40	27–40	27–40	27–40	27–40	22–40	27–40	27–40	22–40
Temp. optimum for growth (°C)	32	37	37	32	32	32	32	32	37	32
NaCl range for growth (%)	0–15	0–20	0–15	0–20	0–15	0–20	0–20	2.5–15	2.5–15	0–20
NaCl optimum for growth (%)	5	10	10	5	10	5	5	7.5	10	5
Growth on King's B medium	+	+	+	+	+	+	+	+	+	+
Growth on McConky agar	–	–	–	–	–	–	–	–	–	–
Tentative identity	*Alteromonas*	*Halomonas*	*Halomonas*	*Aidingimonas*	*Halomonas*	*Halomonas*	*Halomonas*	*Chromohalobacter*	*Bacillus*	*Halomonas*
Character	Bacterial isolates									
	VW402	SUR108	SUR301	SUR302	SUR303	SUR307	SUR309	SUR310	SUR202	SUR201
Morphological characters										
Colony morphology	Cream, circular	Cream, circular	White, gummy	Transparent, circular	Flat, reddish-pink	Cream, circular	Cream, circular	Cream, circular	Cream, circular	Cream, circular
Gram nature	–	–	–	–	+	–	–	–	–	–
Cell shape	Rod	Rod	Rod	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Cell arrangement	Single	Single	Single	Single	Single, clumped	Single	Single	Single	Single	Single
Motility	+	–	–	+	–	+	–	+	–	–
Pigmentation	–	–	–	–	Pink	–	–	–	–	–
Endospore formation	–	–	–	–	–	–	–	–	–	–
Physiological characters										
pH range for growth	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11	5–11
pH optimum for growth	9	7	7	7	11	7	7	7	7	9
Temp. range for growth (°C)	27–40	27–40	27–40	32–45	32–45	22–40	32–40	22–45	22–45	32–45
Temp. optimum for growth (°C)	32	32	37	32	37	27	32	27	27	32
NaCl range for growth (%)	0–15	0–15	2.5–15	2.5–15	2.5–15	0–20	0–20	0–20	0–20	0–15
NaCl optimum for growth (%)	5	2.5	5	7.5	5	2.5	7.5	2.5	5	7.5
Growth on King's B medium	+	+	+	+	+	+	+	+	+	+
Growth on McConky agar	–	–	–	–	–	–	–	–	–	–
Tentative identity	*Halomonas*	*Halomonas*	*Chromohalobacter*	*Halomonas*	*Salinicoccus*	*Halomonas*	*Halomonas*	*Halomonas*	*Halomonas*	*Halomonas*

Table 8: Biochemical characteristics of the selected EPS producing halophilic bacteria isolated from solar salterns of India.

Character	Bacterial isolates																			
	JS 504	JS 802	JS 803	JS 904	JW 302	JW 307	KS 1101	KS 1805	KW 203	VW 301	VW 402	SUR 108	SUR 301	SUR 302	SUR 303	SUR 307	SUR 309	SUR 310	SUR 202	SUR 201
Biochemical characters																				
Voges-Proskauer test	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Citrate utilization	+	−	−	−	−	+	−	+	−	+	+	+	+	−	−	+	+	+	+	+
Methyl red test	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−
Production of																				
Glutaminase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Asparaginase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+
Amylase	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Cellulase	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Pectinase	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Inulinase	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Gelatinase	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Lipase	+	−	−	−	−	−	−	+	+	−	−	−	+	−	−	−	−	−	−	−
Xylanase	+	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−
Caseinase	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−
Urease	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nitrate reductase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
H_2S	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Lysine decarboxylase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Arginine decarboxylase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Ornithine decarboxylase	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Indole	−	−	−	−	−	−	−	+	−	−	−	−	+	−	+	−	−	−	−	−
Phenylalanine deaminase	+	+	+	−	+	−	+	+	−	+	−	+	+	+	+	+	+	+	−	+
Tryptophan deaminase	+	+	+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	−

Table 9: Carbon source utilization and fermentation of the selected EPS producing halophilic bacteria isolated from samples of solar salterns of India.

Carbon source	Bacterial isolates																																							
	JS 504		JS 802		JS 803		JS 904		JW 302		JW 307		KS 1101		KS 1805		KW 203		VW 301		VW 402		SUR 108		SUR 301		SUR 302		SUR 303		SUR 307		SUR 309		SUR 310		SUR 202		SUR 201	
	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F	U	F
Glucose	+	+	+	+	+	−	−	+	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	−	−	−	−
Galactose	+	−	+	−	+	−	−	+	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	+	−	−	+	−	+	+	+	−	+	−	+	−	+	−
Arabinose	−	−	−	−	+	−	−	−	−	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	−	+	+	−	+	−	+	−	−	−	+	−	+	−
Mannose	+	−	+	−	+	−	−	+	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	+	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−
Rhamnose	+	−	+	−	+	−	+	−	−	−	−	−	−	−	+	+	+	−	−	−	+	−	+	−	+	+	−	+	+	−	+	−	−	−	−	−	−	−	+	−
Trehalose	+	−	+	−	+	−	−	+	−	+	−	−	−	−	+	−	+	+	−	−	−	−	+	−	+	−	−	+	+	+	+	−	−	−	−	−	−	−	+	−
Lactose	−	−	−	−	+	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	+	−	+	+	−	−	+	−	−	−	−	−	+	−	+	−
Sucrose	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	+	+	−	+	−	−	−	+	−	+	+	+	−	+	−	+	−	+	−	−	−	+	−
Xylose	−	−	−	−	+	−	+	−	−	−	+	−	+	+	−	+	−	−	+	−	+	−	+	−	+	−	−	+	−	+	+	+	−	−	−	−	+	−	−	−
Ribose	+	−	+	+	+	+	+	+	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	+	−	+	−
Fructose	+	−	+	+	+	−	+	+	+	−	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	−	−	−	−	+	−	−	+	−	+	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	+	−	+	−	+	+	−	−	−	−	−	+	−	+	−
Glycine	−	−	−	+	+	−	+	−	−	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	+	−	−	−	−	+	−	−
Glycerol	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	−	+	+	+	+	−	+	−	+	−	+	−	+	−
Adonitol	+	−	+	−	+	−	+	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	+	−	+	+	+	−	+	−	+	−	−	−	−	−	−	−
Salicin	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	+	−
Meso-inositol	+	−	−	−	+	−	+	−	+	−	+	−	+	+	+	−	+	+	+	−	+	−	−	−	+	−	−	+	+	−	+	−	+	−	+	−	+	−	+	−
Mannitol	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−
Sorbitol	+	+	+	+	+	−	−	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	+	−	+	+	+	−
Raffinose	−	−	+	+	+	+	−	−	+	−	+	−	+	−	+	−	+	+	−	−	−	−	−	−	−	−	+	+	+	+	+	−	+	−	+	−	+	−	+	−
Cellobiose	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	−
Melibiose	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Na-acetate	+	−	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	+	−
Na-citrate	+	−	+	+	+	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Na-fumarate	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	−	+	+	−	+	−	+	−	+	−	+	−	+	−
Na-formate	+	−	+	−	+	−	+	−	+	−	−	−	+	−	+	−	+	−	−	−	+	−	−	−	+	−	+	+	−	−	+	−	−	−	−	−	−	−	−	−
Na-benzoate	+	−	+	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	+	+	+	+	+	+	+
Na-malate	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−
Na-propionate	+	−	+	−	+	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	−	+	+	−	+	−	+	−	+	−	+	−	+	−
Na-succinate	+	−	+	−	+	−	+	+	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	−	+	+	+	+	+	−	+	−	+	−	+	−	+	−

U: utilization; F: fermentation.

Table 10: Antibiotic sensitivity pattern of the selected EPS producing halophilic bacteria isolated from solar salterns of India.

Antibiotics	Bacterial isolates																			
	JS 504	JS 802	JS 803	JS 904	JW 302	JW 307	KS 1101	KS 1805	KW 203	VW 301	VW 402	SUR 108	SUR 301	SUR 302	SUR 303	SUR 307	SUR 309	SUR 310	SUR 202	SUR 201
Ampicillin (10 μg)	S	S	R	S	S	S	S	R	I	S	S	S	R	S	S	S	S	S	S	S
Amoxicillin (30 μg)	S	S	R	R	S	S	S	R	S	S	S	S	R	R	S	I	R	S	S	S
Bacitracin (10 μg)	S	S	R	S	S	S	R	R	S	I	R	R	R	R	S	I	R	R	R	R
Ciprofloxacin (30 μg)	I	I	I	R	I	I	I	I	R	I	I	I	I	I	R	S	R	I	I	I
Chlortetracycline (30 μg)	S	I	S	S	I	S	R	R	S	S	S	S	R	R	S	S	I	I	I	S
Chloramphenicol (10 μg)	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	S	R	S	S	S
Erythromycin (15 μg)	S	S	I	S	S	I	S	I	I	I	I	I	I	S	R	R	I	I	S	S
Fusidic acid (30 μg)	I	R	R	S	S	I	R	R	S	R	I	R	R	R	S	I	R	I	I	R
Gentamycin (10 μg)	S	S	S	S	S	S	S	S	R	S	S	S	S	S	S	I	S	S	S	S
Kanamycin (30 μg)	I	I	S	R	S	I	I	S	R	I	I	R	I	R	R	R	I	R	I	R
Methicillin (5 μg)	S	S	R	S	I	R	R	R	R	R	R	R	R	R	S	R	R	R	R	R
Neomycin (30 μg)	S	S	I	R	I	I	R	I	I	I	R	I	I	R	R	R	I	I	R	R
Norfloxacin (30 μg)	S	S	R	S	S	S	S	S	I	S	S	S	S	S	S	S	S	S	S	S
Netillin (10 μg)	R	S	S	I	R	S	S	S	R	R	R	S	S	R	R	R	R	R	R	R
Nalidixic acid (30 μg)	R	R	S	R	R	I	I	R	R	I	I	R	R	R	R	S	R	R	R	R
Novobiosin (30 μg)	I	R	R	S	R	S	S	R	S	S	S	I	R	S	S	S	I	S	S	S
Polymixin B (50 μg)	R	R	I	R	I	I	R	R	R	R	I	I	R	R	R	I	I	I	I	S
Penicillin G (10 μg)	S	S	R	I	S	I	R	R	R	I	I	S	R	R	S	I	I	I	I	S
Streptomycin (10 μg)	S	S	S	R	S	R	R	S	R	R	R	I	S	R	S	S	R	I	I	R
Tetracycline (30 μg)	R	R	R	R	R	I	R	R	S	R	I	R	R	R	R	S	R	R	R	I
Trimethoprime (30 μg)	S	S	S	S	I	S	S	I	S	S	S	S	I	R	R	S	S	S	S	S
Vancomycin (30 μg)	R	R	R	S	R	R	R	R	S	R	R	R	R	R	S	R	R	R	R	R

S = Sensitive, R = Resistant, I = Intermediate.

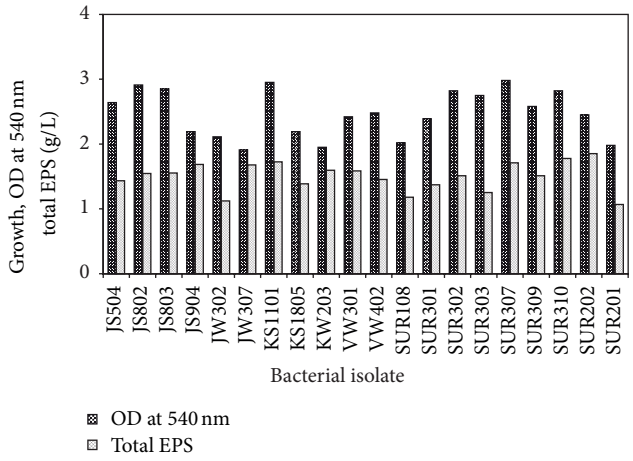

Figure 1: Screening of moderately halophilic bacteria isolated from soil and water samples of solar salterns for production of extracellular polymeric substances.

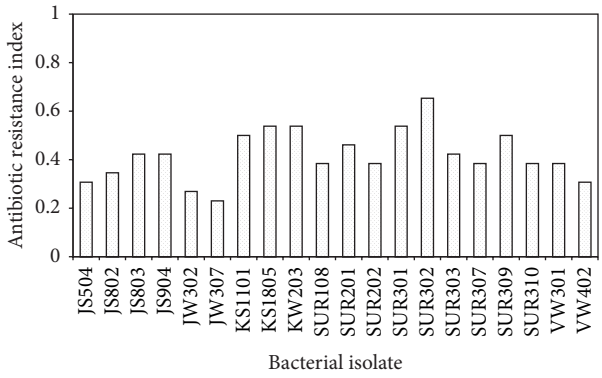

Figure 2: Antibiotic resistance index of the selected EPS producing halophilic bacteria isolated from soil and water samples of solar salterns of India.

The isolates SUR301 and KS 1805 were tentatively identified as *Chromohalobacter* based on the study by Arahal et al. [45] and bear striking similarity with those of *Chromohalobacter* sp. isolated from hypersaline soil sample of Triveni Sangam, Gujarat [38]. The physiological, morphological, and biochemical characteristics of isolate SUR303 were consistent with the features of the genus *Salinicoccus* [10], while isolates JS904 and JS504 were tentatively assigned to *Aidingimonas* and *Alteromonas*, respectively.

Finally, sensitivity of these isolates to antibiotics (Table 10 and Figure 2) also corroborates the findings of Mata et al. [25] and Hedi et al. [28].

5. Conclusion

It may be emphasized that study of these moderately halophilic bacteria from coastal hypersaline solar salterns of India with special attempt on screening for EPS production has led to the discovery of wide variety of halophilic species. Although attention has been focused on the production of EPS by these halophilic bacterial isolates, their physiobiochemical features indicate that they may equally hold potential towards production of various biomolecules of industrial interest.

Conflict of Interests

It is declared by the authors that there is no conflict of interests regarding the publication of this paper.

Acknowledgment

This study was financially supported by grants from University Grants Commission, India, (Sanction no. F.14-2(SC)/2008 (SA-III), 31 March, 2009) under the Scheme of Rajiv Gandhi National Fellowship.

References

[1] E. A. Galinski and B. J. Tindall, "Biotechnological prospects for halophiles and halotolerant microorganisms," in *Molecular Biology and Biotechnology of Extremophiles*, R. D. Herbert and R. J. Sharp, Eds., Blackie, London, UK, 1992.

[2] R. Margesin and F. Schinner, "Potential of halotolerant and halophilic microorganisms for biotechnology," *Extremophiles*, vol. 5, no. 2, pp. 73–83, 2001.

[3] F. Rodriguez Valera, F. Ruiz Berraquero, and A. Ramos Cormenzana, "Characteristics of the heterotrophic bacterial populations in hypersaline environments of different salt concentrations," *Microbial Ecology*, vol. 7, no. 3, pp. 235–243, 1981.

[4] F. Rodriguez-Valera, "Characteristics and microbial ecology of hypersaline environments," in *Halophilic Bacteria*, F. Rodriguez-Valera, Ed., vol. 1, pp. 3–30, CRC Press, Boca Raton, Fla, USA, 1988.

[5] D. J. Kushner and M. Kamekura, "Physiology of halophilic eubacteria," in *Halophilic Bacteria*, F. Rodriguez-Valera, Ed., vol. 1, pp. 109–138, CRC press, Boca Raton, Fla, USA, 1988.

[6] M. A. Amoozegar, F. Malekzadeh, and K. A. Malik, "Production of amylase by newly isolated moderate halophile, *Halobacillus* sp. strain MA-2," *Journal of Microbiological Methods*, vol. 52, no. 3, pp. 353–359, 2003.

[7] C. O. Jeon, J. Lim, J. Lee et al., "*Lentibacillus salarius* sp. nov., isolated from saline sediment in China, and emended description of the genus *Lentibacillus*," *International Journal of Systematic and Evolutionary Microbiology*, vol. 55, no. 3, pp. 1339–1343, 2005.

[8] S. Bouchotroch, E. Quesada, A. Del Moral, I. Llamas, and V. Béjar, "*Halomonas maura* sp. nov., a novel moderately halophilic, exopolysaccharide-producing bacterium," *International Journal of Systematic and Evolutionary Microbiology*, vol. 51, no. 5, pp. 1625–1632, 2001.

[9] A. Biswas, A. Patra, and A. Paul, "Production of poly-3-hydroxyalkanoic acids by a moderately halophilic bacterium, *Halomonas marina* HMA 103 isolated from solar saltern of Orissa, India," *Acta Microbiologica et Immunologica Hungarica*, vol. 56, no. 2, pp. 125–143, 2009.

[10] S. Y. Jayachandra, S. Anil Kumar, D. P. Merley, and M. B. Sulochana, "Isolation and characterization of extreme halophilic bacterium *Salinicoccus* sp. JAS4 producing extracellular hydrolytic enzymes," *Recent Research in Science and Technology*, vol. 4, no. 4, pp. 46–49, 2012.

[11] I. W. Sutherland, "Novel and established applications of microbial polysaccharides," *Trends in Biotechnology*, vol. 16, no. 1, pp. 41–46, 1998.

[12] M. W. Mittelman and G. G. Geesey, "Copper-binding characteristics of exopolymers from a freshwater-sediment bacterium," *Applied and Environmental Microbiology*, vol. 49, no. 4, pp. 846–851, 1985.

[13] J. Anton, I. Meseguer, and F. Rodriguez-Valera, "Production of an extracellular polysaccharide by *Haloferax mediterranei*," *Applied Enviromental Microbiology*, vol. 54, no. 10, pp. 2381–2386, 1988.

[14] H. Parolis, L. A. S. Parolis, I. F. Boán et al., "The structure of the exopolysaccharide produced by the halophilic Archaeon *Haloferax mediterranei* strain R4 (ATCC 33500)," *Carbohydrate Research*, vol. 295, pp. 147–156, 1996.

[15] E. Quesada, V. Bejar, and C. Calvo, "Exopolysaccharide production by Volcaniella eurihalina," *Experientia*, vol. 49, no. 12, pp. 1037–1041, 1993.

[16] J. A. Mata, V. Béjar, I. Llamas et al., "Exopolysaccharides produced by the recently described halophilic bacteria *Halomonas ventosae* and *Halomonas anticariensis*," *Research in Microbiology*, vol. 157, no. 9, pp. 827–835, 2006.

[17] I. Llamas, J. A. Mata, R. Tallon et al., "Characterization of the exopolysaccharide produced by Salipiger mucosus A3^T, a halophilic species belonging to the Alphaproteobacteria, isolated on the Spanish Mediterranean seaboard," *Marine Drugs*, vol. 8, no. 8, pp. 2240–2251, 2010.

[18] F. Martínez-Checa, E. Quesada, J. Martínez-Cánovas, I. Llamas, and V. Béjar, "*Palleronia marisminoris* gen. nov., sp. nov., a moderately halophilic, exopolysaccharide-producing bacterium belonging to the 'Alphaproteobacteria', isolated from a saline soil," *International Journal of Systematic and Evolutionary Microbiology*, vol. 55, no. 6, pp. 2525–2530, 2005.

[19] A. Ventosa, M. T. Garcia, M. Kamekura, H. Onishi, and F. Ruiz-Berraquero, "*Bacillus halophilus* sp. nov., a moderately halophilic *Bacillus* species," *Systematic and Applied Microbiology*, vol. 12, no. 2, pp. 162–166, 1989.

[20] R. A. Moraine and P. Rogovin, "Kinetics of polysaccharide B 1459 fermentation," *Biotechnology and Bioengineering*, vol. 8, no. 4, pp. 511–524, 1966.

[21] M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, "Colorimetric method for determination of sugars and related substances," *Analytical Chemistry*, vol. 28, no. 3, pp. 350–356, 1956.

[22] O. H. Lowry, N. J. Rosenbrough, A. L. Farr, and R. J. Randall, "Protein measurement with the Folin phenol reagent.," *The Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[23] P. Gerhardt, R. G. E. Murray, W. A. Wood, and N. R. Krieg, *Methods for General and Molecular Bacteriology*, American Society for Microbiology, Washington, DC, USA, 1994.

[24] G. M. Garrity, J. A. Bell, and T. G. Lilburn, "Taxonomic outline of the procaryotes," in *Bergey's Manual of Systematic Bacteriolog*, D. R. Boone and R. W. Castenholz, Eds., Springer, New York, NY, USA, 2nd edition, 2003.

[25] J. A. Mata, J. Martínez-Cánovas, E. Quesada, and V. Béjar, "A detailed phenotypic characterisation of the type strains of Halomonas species," *Systematic and Applied Microbiology*, vol. 25, no. 3, pp. 360–375, 2002.

[26] A. Oren, "Molecular ecology of extremely halophilic archaea and bacteria," *FEMS Microbiology Ecology*, vol. 39, no. 1, pp. 1–7, 2002.

[27] H. Baati, R. Amdouni, N. Gharsallah, A. Sghir, and E. Ammar, "Isolation and characterization of moderately halophilic bacteria from tunisian solar saltern," *Current Microbiology*, vol. 60, no. 3, pp. 157–161, 2010.

[28] A. Hedi, N. Sadfi, M. Fardeau et al., "Studies on the biodiversity of halophilic microorganisms isolated from El-Djerid salt lake (Tunisia) under aerobic conditions," *International Journal of Microbiology*, vol. 2009, Article ID 731786, 17 pages, 2009.

[29] M. Birbir, A. Ogan, B. Calli, and B. Mertoglu, "Enzyme characteristics of extremely halophilic archaeal community in Tuzkoy Salt Mine, Turkey," *World Journal of Microbiology and Biotechnology*, vol. 20, no. 6, pp. 613–621, 2004.

[30] M. Birbir and C. Sesal, "Extremely halophilic bacterial communities in Sereflikochisar Salt Lake in Turkey," *Turkish Journal of Biology*, vol. 27, no. 7, pp. 7–22, 2003.

[31] M. Birbir, B. Calli, B. Mertoglu et al., "Extremely halophilic Archaea from Tuz Lake, Turkey, and the adjacent Kaldirim and Kayacik salterns," *World Journal of Microbiology and Biotechnology*, vol. 23, no. 3, pp. 309–316, 2007.

[32] R. Rohban, M. A. Amoozegar, and A. Ventosa, "Screening and isolation of halophilic bacteria producing extracellular hydrolyses from Howz Soltan Lake, Iran," *Journal of Industrial Microbiology and Biotechnology*, vol. 36, no. 3, pp. 333–340, 2009.

[33] M. J. Garabito, M. C. Márquez, and A. Ventosa, "Halotolerant *Bacillus* diversity in hypersaline environments," *Canadian Journal of Microbiology*, vol. 44, no. 2, pp. 95–102, 1998.

[34] A. Ventosa, A. Ramos-Cormenzana, and M. Kocur, "Moderately halophilic gram-positive cocci from hypersaline environments," *Systematic and Applied Microbiology*, vol. 4, no. 4, pp. 564–570, 1983.

[35] E. Quesada, A. Ventosa, F. Rodriguez-Valera, and A. R. Cormenzana, "Types and properties of some bacteria isolated from hypersaline soils," *Journal of Applied Bacteriology*, vol. 53, no. 2, pp. 155–161, 1982.

[36] E. Quesada, V. Bejar, M. J. Valderrama, A. Ventosa, and A. R. Ramos Cormenzana, "Isolation and characterization of moderately halophilic nonmotile rods from different saline habitats.," *Microbiologia*, vol. 1, no. 1-2, pp. 89–96, 1985.

[37] S. R. Dave and H. B. Desai, "Microbial diversity at marine salterns near Bhavnagar, Gujarat, India," *Current Science*, vol. 60, no. 4, pp. 497–500, 2006.

[38] S. Kumar, R. Karan, S. Kapoor, S. P. Singh, and S. K. Khare, "Screening and isolation of halophilic bacteria producing industrially important enzymes," *Brazilian Journal of Microbiology*, vol. 43, no. 4, pp. 1595–1603, 2012.

[39] B. P. Dave and A. Soni, "Diversity of halophilic archaea at salt pans around Bhavnagar coast, Gujarat," *Proceedings of the National Academy of Sciences India Section B: Biological Sciences*, vol. 83, no. 2, pp. 225–232, 2013.

[40] A. A. Joshi, P. P. Kanekar, A. S. Kelkar et al., "Cultivable bacterial diversity of alkaline Lonar lake, India," *Microbial Ecology*, vol. 55, no. 2, pp. 163–172, 2008.

[41] T. M. Raghavan and I. Furtado, "Occurrence of extremely halophilic Archaea in sediments from the continental shelf of west coast of India," *Current Science*, vol. 86, no. 8, pp. 1065–1067, 2004.

[42] S. Vijayanand, J. Hemapriya, J. Selvin, and S. Kiran, "Biodiversity of extremely halophilic bacterial strains isolated from solar salterns of Tuticorin, Tamilnadu, India," *International Journal of Water Resources and Environmental Sciences*, vol. 1, no. 1, pp. 1–7, 2012.

[43] S. G. Nanjani and H. P. Soni, "Isolation and characterization of extremely halotolerant and halophilic organisms from Dwarka and Veraval," *Journal of Pharmacy and Biological Sciences*, vol. 2, no. 2, pp. 20–25, 2012.

[44] H. Ghozlan, H. Deif, R. A. Kandil, and S. Sabry, "Biodiversity of moderately halophilic bacteria in hypersaline habitats in Egypt," *Journal of General and Applied Microbiology*, vol. 52, no. 2, pp. 63–72, 2006.

[45] D. R. Arahal, M. T. García, W. Ludwig, K. H. Schleifer, and A. Ventosa, "Transfer of *Halomonas canadensis* and *Halomonas israelensis* to the genus *Chromohalobacter* as *Chromohalobacter canadensis* comb. nov. and *Chromohalobacter israelensis* comb. nov," *International Journal of Systematic and Evolutionary Microbiology*, vol. 51, no. 4, pp. 1443–1448, 2001.