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Full Length Research Paper

Physico-chemical analysis of saline soils of solar salterns and isolation of moderately halophilic bacteria for poly (3-hydroxybutyric acid) production

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Abstract

Multi-pond solar salterns spreaded over the coastal areas of Orissa and West Bengal, India, are commercially exploited for salt production. Soil samples of these salterns showed wide degree of variation in salinity (55.4 -172.5 ppt) along with high sulfate but low phosphate and nitrogen content and were differentiated as high, moderate and low salinity categories. Aerobic, heterotrophic bacterial population of these soils indicated predominance of Gram-negative motile rods, which developed white to cream colonies on Ventosa's agar medium. A total of 203 moderately halophilic bacteria capable of growing well between 5-15% NaCl were isolated and systematic screening revealed that 53, 45 and 72% of isolates from high, moderate and low saline soil categories respectively accumulated poly (3-hydroxybutyrate) [P(3HB)] ranging from 10-51% of cell dry weight during growth and appeared to be interesting candidates for large scale production of this biodegradable bioplastic.

Keywords: Multi-pond solar salterns, moderately halophilic bacteria, microbial activity, poly (3-hydroxybutyrate), thermoplastics.

INTRODUCTION

Solar salterns are hyper saline water bodies located along the sea-coast and are the main source of generating salt through the evaporation of seawater. They are generally composed of a system of shallow ponds with salinities ranging from of seawater to supersaturated brines. These elevated saline concentrations represent an extreme environmental conditions leading to the growth of only a few specialized group of microorganisms-the halophiles. These halophilic microbial communities (Oren, 2005, Pedros-Alio, 2005) are adapted to life at high salt concentrations and to the high osmotic pressure of their environment resulting from the high salinity. In addition, other factors like temperature, pH, oxygen, nutrient availability and solar radiations prevailing in such hypersaline environments also limit the growth of microorganisms.

Extremely halophilic Archaea, which grow optimally in media containing 2.5-5.0 M NaCl have been isolated and characterized from saltern crystallizer ponds by both culture-dependent (Rodriguez-Valera *et al.,* 1985; Oren, 2002) and culture-independent rRNA-based studies (Casamayor, 2000). Moderately halophiles, on the other hand with most representatives in the domain Bacteria are specialized to grow optimally in media containing 0.5-2.5 M NaCl (Kushner, 1978; Ventosa *et al.,* 1998). The potentials of the halophilic microorganisms for a variety of biotechnological applications have been emphasized (Margesin and Schinner, 2001).

In India, major salt-producing multi-pond solar saltern sites are located in coastal areas of Gujrat, Tamil Nadu and Andhra Pradesh, but are not uncommon along the coasts of Orissa and West Bengal. Very few studies have analyzed the physico-chemical (Dave and Deshai, 2006) and microbiological characteristics of soil samples collected from Indian marine salterns. The present study was carried out to evaluate physico-chemical and microbiological characteristics of saline soils of multi-pond solar salterns spreaded over the coastal areas of West Bengal and Orissa, India with a goal to exploit the potential of moderately halophilic bacteria for the production of poly(3-hydroxybutyric acid), the intracellular polymer that has been identified as an environment

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friendly alternative to thermoplastics.

MATERIALS AND METHODS

Collection of samples

Soil samples used during the present study were collected from multi-pond solar salterns spreaded over the coastal areas of Orissa (20º 0'N, 84º 0'E) and West Bengal (23º 0'N, 88º 0'E), India during the period of May-June after harvesting of salt. Soils collected in sterile polyethylene containers were brought to the laboratory and stored at 4°C until used for physico-chemical and microbial analysis.

Physico-chemical analysis of soil

Soil pH

The pH of the soil was measured following the method of Jackson (1973). Air dried, powdered soil was suspended in distilled water in the ratio of 1:2 and stirred with magnetic stirrer at 30ºC for 2 h. The pH of the suspension was measured using a Systronic pH meter.

Electrical conductivity

For determination of electrical conductivity (E_C), air dried soil (20 g) was suspended in distilled water (100 ml/500 ml Erlenmeyer flask) and agitated at 30ºC for 2 h. Electrical conductivity (ms/cm) of the soil suspension was measured using Universal Pocket Meter (Orbeco Analytical System Inc., Farmingdale, NY).

Soil salinity

Soil sample was suspended in distilled water in the ratio of 1:5 and agitated on a rotary shaker (120 rpm) for 1 h. Soil salinity (ppt) was measured using Universal Pocket Meter (Orbeco Analytical System Inc., Farmingdale, NY).

Estimation of phosphate

Estimation of available phosphate in the soil was made following the method of Watanabe and Olsen (1965). Air dried soil (2 g) was extracted with 40 ml of sodium bicarbonate extracting solution (0.5 N $NaHCO_3$, pH 8.5) for 30 min in a mechanical shaker and filtered through Whatman No. 1 filter paper. To 3.0 ml of filtered soil extract, 9.0 ml of deionized water and 3.0 ml of ascorbic/molybdate reagent was added and the optical density was measured at 882 nm using UV-visible Spectro Varian Carry 50 Conc. The available phosphate was calculated from the standard curve and expressed as mg phosphate/g of soil.

Estimation of sulfate

For the estimation of sulfate, 20 g of air dried soil was taken in a beaker and suspended in 100 ml of distilled water under continuous stirring at 30ºC for 2 h in a magnetic stirrer. The suspension was then centrifuged and filtered. Twenty ml of the clear filtrate was taken in a 100 ml volumetric flask and diluted to 100 ml. To this clear filtrate Orbeco sulfate tablet was added and mixed throughly. The optical density was measured at 528 nm using Orbeco Helliige Sulfate Analyst (model MNO 975MP). The sulfate content of the soil (mg/g) was calculated from the standard curve prepared in the same way.

Estimation of total organic carbon

Total organic carbon was measured following wet potassium dichromate digestion of soil and titrimetric analysis of unreacted dichromate (Nelson and Somers, 1975). Air-dried and ground soil (0.1 g) was digested at 150ºC for 30-60 min in a microkjeldahl flask in presence of 5.0 ml of 1.0 M potassium dichromate and 7.5 ml of concentrated sulphuric acid. The digest was diluted to 100 ml with double distilled water and titrated against 0.2 M ferrous ammonium sulphate using 0.3 ml of indicator. The indicator solution was prepared by dissolving 0.1 g N-phenylanthranilic acid and 0.1 g sodium carbonate were dissolved in 60 ml distilled water and volume was made up to 100 ml.

Total organic carbon (%) was calculated as:

$$\% \text{ organic carbon} = \frac{\text{Titre value (ml)} \times 0.2 \times 0.3}{\text{Weight of soil (0.1 g)}}$$

Estimation of total nitrogen

Total nitrogen was estimated by the microkjeldahl procedure of Keeney and Nelson (1982). In this procedure 0.2 g of dried and sieved (1.0 mm mesh) soil was taken in a 100 ml microkjeldahl flask along with 4.4 ml of digestion mixture. The digestion mixture contained 0.42 g selenium powder, 14.0 g lithium sulfate, 350 ml of 30% hydrogen peroxide and 420 ml concentrated sulphuric acid. Digestion was carried out at 180ºC for 8 h until a clear solution was obtained. The digest was cooled and volume was made to 50 ml with double distilled water.

The digest (50 ml) was transferred to distillation flask with 25 ml alkali solution (500 g sodium hydroxide, 25 g sodium thiosulfate and 1000 ml distilled water). Boric acid

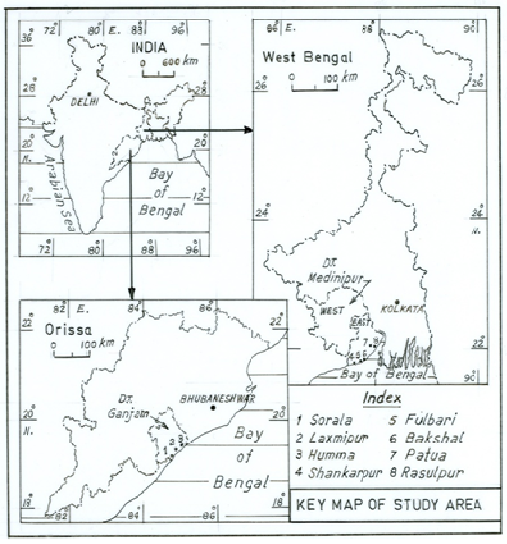

Figure 1. Map showing location of sampling areas in Orissa and West Bengal, India

(5 ml) indicator solution was taken in a graduated flask to collect 25 ml of distillate. The distillate was titrated against hydrochloric acid (0.01 N), the endpoint being indicated by a change in color from green to pink. From the volume of acid consumed, weight of soil and standard value of 1 ml of 0.01 M HCl (= 0.14 mg NH_4-N), the total nitrogen (%) was calculated following the equation:

$$\% \text{ total nitrogen} = \frac{\text{Titre value (ml)}}{50 \times \text{Weight of soil}}$$

Boric acid indicator solution: 20 g boric acid was dissolved in 600 ml distilled water. 20 ml of an indicator solution (prepared by dissolving 0.099 g bromocresol green and 0.066 g methyl red in 100 ml distilled water) was added to boric acid solution. 0.1 M sodium hydroxide was added to the mixture until a purple color appeared and volume was made to 1000 ml with distilled water.

Microbiological analysis

Moderately halophilic aerobic heterotrophic bacteria were isolated from soil samples following dilution and plating on Ventosa's agar medium (Ventosa, 1988) supplemented with different concentration of NaCl. Plates were incubated at 30-32°C for 4-6 days. Phenotypically distinguishable bacterial colonies were selected and further purified by dilution and streaking method on the same agar medium. The purified cultures were maintained on slopes of same agar medium and stored at 4°C. Microbial activity of the soil samples was estimated using the fluorescein diacetate method (FDA) of Schnurer and Rosswall (1982).

Screening for P(3HB) accumulation

For qualitative analysis of P(3HB) production, the bacterial isolates were grown in modified basal synthetic medium for 48-72 h at 30°C under continuous shaking. Cells were stained with aqueous Nile blue A solution at 55ºC for 10 min in a coplinjar and the excess stain was removed with water and 8% aqueous acetic acid. Intracellular accumulation of P(3HB) was examined under Leitz-Lux D Fluorescence Microscope with an I_2 filter which provides an excitation wave length of approximately 460 nm. P(3HB) containing cells appeared as orange patches against black background.

Quantitative assessment of P(3HB) was made from acetone dried cell mass harvested at different stages of growth using the method of Law and Slepecky (1961). The polymer was extracted from the cell mass with warm chloroform, evaporated to dryness and the dried mass was treated with concentrated H_2SO_4 in a boiling water bath for 10 min. The absorbance was recorded at 235 nm using a UV-VIS spectrophotometer (Jenway 6505) against a similarly prepared reagent blank. P(3HB) from Sigma (USA) was used as the standard.

Statistical analysis

Correlation of physio-chemical characters, mcrobial count and microbial activity of saline soil samples of different categories were determined by the Pearson correlation

matrix using statistical software, Minitab (version 13).

RESULTS

Physico-chemical analysis of soil

Sixteen soil samples were collected from crystallizer ponds of different multi-pond solar salterns immediately after the harvesting of salts. The sampling sites viz. Humma, Laxmipur, Sorala were distributed along the coastal areas of Ganjam district of Orissa, while Shankarpur, Bakshal, Fulbari, Patua and Rasalpur were located in East Medinipur of West Bengal, India (Fig.1). Soil samples were subjected to physico–chemical analysis following standard methods of APHA and the result are presented in Table 1.

The soils samples were slightly acidic to neutral and the pH ranged from 6.2-7.3. They showed wide degree of variability in terms of their salt concentration and were distinguished as high (126-172 ppt), moderate (77-92 ppt) and low (55-64 ppt) salinity categories. In general, the soils were characterized by high sulfate but low phosphate and nitrogen content but were moderate in terms of total organic carbon. Pearson correlation matrix of physico-chemical characteristics of saline soils of crystallizer ponds are shown in Table 2.

Microbiological analysis of soil

Microbial density of soil samples (c.f.u /g) was compared following dilution and plating method on Ventosa's agar medium supplemented with different concentration of NaCl. Results (Table 3) indicate that irrespective of salinity categories, bacterial population in 50% of soil samples was highest at 5% NaCl which decreased gradually with increase in NaCl concentration in the growth medium, while in rest of the soil samples it was highest in medium without NaCl and followed the same trend.

Microbial activity of the soils as estimated by fluorescein diacetate (FDA) method showed significant variation with respect to soil salinity categories and localities. In high salinity soils from Orissa, it ranged from 5.5-8.84 µg fluorescine/g dry soil/h, but was comparatively lower (4.5-7.5 µg fluorescine/g dry soil/h) in low saline soils of West Bengal. The total microbial count and microbial activity of the saline soils have been correlated by Pearson correlation matrix (Table 4).

A total of 203 aerobic, moderately halophilic bacterial cultures differing in phenotypic characteristics were isolated in pure form and maintained on Ventosa's agar medium with 10% NaCl. Majority of these bacterial isolates were Gram-negative, motile rods and developed white to cream coloured colonies on NaCl containing Ventosa's agar medium. A few of them were unable to grow on NaCl-free medium, but rest grew well between 5-15% NaCl and at temperature between 30-37 ^{o}C. All the isolates produced catalase, but do not produce any diffusible pigment. Production of extracellular polymeric substances was characteristic of some selected isolates irrespective of their Gram nature.

Screening for P(3HB) production

All 203 selected isolates were subjected to both qualitative and quantitative evaluation of intracellular P(3HB) following Nile blue A staining and chemical estimation respectively. Results as indicated in Fig. 2 revealed that out of 203 isolates, a total of 138 isolates including 56 (67.46%), 52 (61.9%) and 30 (83.33%) from high, moderate and low saline soils respectively showed different degrees of orange fluorescence characteristic of P(3HB) accumulation. However, these soil categories yielded about 53.0, 45.23 and 72.22% of their respective populations as confirmed P(3HB) producers following chemical estimation method of Law and Slepecky (1961).

Growth associated P(3HB) accumulation by these isolates after 48 and 72 h of incubation in modified basal synthetic medium containing 1% glucose showed a wide range of polymer accumulation efficiency, which ranges from 10-51% of cell dry weight (Table 5). The bacterial isolates (HMA 102, HMA 103, SUR 201) obtained from saltern soil samples of high salinity category of Orissa were found to be the most efficient in P(3HB) accumulation. The P(3HB) content of these isolates represented 51, 55 and 42% of cell dry weight. Isolate RAS 103 derived from soil of low salinity category of West Bengal was also not inferior in P(3HB) accumulation.

DISCUSSION

The soil samples derived from multi-pond solar salterns represented typical thalassic environment (Das Sarma and Arora, 2001) being derived by natural evaporation of sea water. Analysis of soil samples collected from selected saltern sites of Orissa and West Bengal, India (Fig. 1) during post-harvest period revealed that the physical parameters (pH, conductivity and salinity) of high saline soil category (Table 1) were positively correlated amongst themselves as well as with total nitrogen and carbon (Table 2). Similarly, sulfate and phosphate contents were negatively correlated with pH, conductivity, salinity, total nitrogen and organic carbon. However, the correlation coefficient of conductivity and pH with salinity and that of salinity with phosphate and total nitrogen is not statistically significant as seen from the significant values. Salinity and conductivity of moderately saline soils was positively correlated with most of the physical and chemical parameters but the correlation of

Table 1. Physico-chemical characteristics of soil samples of crystallizer ponds of multi-pond solar salterns

Soil salinity category	State	Locality	pH	Conductivity ms/cm	Salinity,	Total SO_4, %	Total PO_4, %	Total Kjeldahl N_2, %	Total organic C, %
High	Orissa	Humma 1	7.2 ± 0.17	45.0 ±2.12	172.5 ± 2.96	106.8± 2.54	0.11 ± 0.01	0.75 ± 0.07	0.55 ± 0.01
		Humma 2	7.4 ± 0.19	50.8 ± 2.54	167.5± 0.98	107.2 ± 0.56	0.12 ± 0.04	0.81 ± 0.01	0.54 ± 0.05
		Laxmipur 1	6.4 ± 0.11	35.8 ± 1.13	168.5± 3.95	117.7 ± 0.77	0.34 ± 0.03	0.08 ± 0.01	1.10 ± 0.02
		Laxmipur 2	6.5 ± 0.02	44.2 ± 0.70	171.5± 1.41	118.2 ± 0.35	0.34 ± 0.01	0.09 ± 0.04	1.11 ± 0.07
		Sorala 1	6.4 ± 0.07	31.9 ± 1.30	145.0± 2.40	131.5 ± 1.55	0.26 ± 0.07	0.04 ± 0.01	1.26 ± 0.02
		Sorala 2	6.5 ± 0.22	36.7 ± 1.66	126.0± 1.27	133.5 ± 0.84	0.30 ± 0.02	0.05 ± 0.07	1.28 ± 0.01
Moderate	Orissa	Humma 3	6.2 ± 0.21	36.1 ± 1.69	90.0± 2.12	134.5 ± 1.27	0.32 ± 0.02	0.04 ± 0.01	1.27 ± 0.02
		Sorala 3	6.3 ± 0.02	32.0 ± 0.42	86.2± 0.28	132.5± 1.06	0.28 ± 0.01	0.03 ± 0.02	1.26 ± 0.07
	West Bengal	Shankarpur 1	6.9 ± 0.11	123.5 ± 1.0	88.8± 1.31	102.8 ± 0.98	0.52 ± 0.05	0.46 ± 0.07	3.65 ± 0.02
		Shankarpur 2	7.0 ± 0.16	122.7 ± 1.0	92.4± 0.42	102.2 ± 3.53	0.53 ± 0.02	0.42 ± 0.03	3.93 ± 0.04
		Bakshal 1	6.7 ± 0.04	66.2 ± 0.98	84.0± 1.41	80.0 ± 0.70	0.46 ±0.02	0.28 ± 0.02	1.25 ± 0.01
		Bakshal 2	7.3 ± 0.02	93.9 ± 1.27	77.7± 0.70	20.2 ± 1.27	0.16 ± 0.07	0.66 ± 0.22	1.50 ± 0.01
Low	West Bengal	Fulbari 1	7.2 ± 0.05	88.5 ± 0.56	64.4± 1.69	11.2 ± 0.28	0.09 ± 0.04	0.17 ± 0.07	1.10 ± 0.07
		Fulbari 2	7.1 ± 0.02	85.5 ± 0.28	60.0± 1.69	10.5 ± 0.42	0.12 ± 0.07	0.16 ± 0.04	1.10 ± 0.08
		Patua 1	6.8 ± 0.01	22.0 ± 0.70	56.6± 1.27	83.5 ± 0.42	0.29 ± 0.06	0.18 ± 0.01	1.26 ± 0.03
		Rasalpur 1	6.6 ± 0.03	26.0 ± 0.42	55.4± 0.42	82.5 ± 1.41	0.33 ± 0.05	0.19 ± 0.02	1.27 ± 0.01

Physico-chemical characteristics of soil samples were done according to standard methods of American Public Health Association (APHA, 1992).Total organic carbon was estimated according to the method of Walkly and Black (1934).Values are average of triplicates ± standard error.

Table 2. Pearson correlation matrix of physico-chemical characters of saline soils from crystallizer ponds of multipond solar salterns

Soil salinity category	Parameters	pH	Conductivity	Salinity	Sulphate	Phosphate	Total Kjeldahl nitrogen	Total organic carbon
High	pH	1 (*)						
	Conductivity	0.816 (0.001)	1 (*)					
	Salinity	0.420 (0.174)	0.574 (0.051)	1 (*)				
	Sulphate	-0.768 (0.004)	-0.799 (0.002)	-0.866 (0.00)	1 (*)			
	Phosphate	-0.880 (0.00)	-0.589 (0.044)	-0.282 (0.374)	0.643 (0.024)	1 (*)		
	Total kjeldahl Nitrogen	0.962 (0.00)	0.810 (0.001)	0.502 (0.096)	-0.842 (0.001)	-0.929 (0.00)	1 (*)	
	Total organic carbon	0.962 (0.00)	0.810 (0.001)	0.502 (0.096)	-0.842 (0.001)	-0.929 (0.00)	1.00 (*)	1 (*)
Moderate	pH	1 (*)						
	Conductivity	0.724 (0.008)	1 (*)					
	Salinity	-0.511 (0.090)	0.115 (0.722)	1 (*)				
	Sulphate	-0.856 (0.00)	-0.408 (0.188)	0.806 (0.002)	1 (*)			
	Phosphate	-0.032 (0.922)	0.562 (0.057)	0.656 (0.021)	0.323 (0.306)	1 (*)		
	Total kjeldahl Nitrogen	-0.032 (0.922)	0.235 (0.462)	0.805 (0.002)	-0.897 (0.000)	-0.630 (0.028)	1 (*)	
	Total organic carbon	0.335 (0.288)	0.863 (0.000)	0.563 (0.056)	0.095 (0.769)	0.006 (-0.196)	-0.196 (0.542)	1 (*)
Low	pH	1 (*)						
	Conductivity	0.970 (0.00)	1 (*)					
	Salinity	0.907 (0.002)	0.860 (0.006)	1 (*)				
	Sulphate	-0.971 (0.000)	-0.998 (0.000)	-0.852 (0.007)	1 (*)			
	Phosphate	-0.993 (0.000)	-0.980 (0.000)	-0.911 (0.002)	0.983 (0.000)	1 (*)		
	Total kjeldahl Nitrogen	-0.727 (0.041)	-0.723 (0.043)	-0.696 (0.055)	0.751 (0.032)	0.032 (0.766)	1 (*)	
	Total organic carbon	-0.889 (0.003)	-0.909 (0.002)	-0.885 (0.003)	0.918 (0.001)	0.919 (0.001)	0.875 (0.004)	1 (*)

Values in parenthesis showed level of significance, * statistically significant.

Table 3. Microbiological analysis of soil samples of crystallizer ponds of multipond solar salterns

Soil salinity category	State	Locality	Total microbial count, c.f.u X 105 /g soil[a] NaCl concentration, % (w/v) in isolation medium 0	5	10	20	Microbial activity (µg fluorescene/g soil/h)[b]
High	Orissa	Humma 1	159.3±0.02	148.0±0.01	41.0±0.23	4.5±0.08	7.56±1.18
		Humma 2	136.3±0.02	118.3±0.01	63.0±0.23	3.5±0.08	7.40±1.18
		Laxmipur 1	75.6±0.01	165.6±1.0	93.3±1.1	2.5±0.08	5.5±0.01
		Laxmipur 2	92.0±0.02	65.0±0.02	35.3±1.0	4.0±0.06	6.5±0.09
		Sorala 1	14.3±0.01	196.0±0.06	112.3±0.02	0.96±0.07	8.84±1.12
		Sorala 2	12.3±1.0	191.6±0.03	92.6±0.04	1.4±0.06	6.8±0.08
Moderate	Orissa	Humma 3	137.0±1.0	55.0±0.03	17.3±0.04	0.96±0.06	9.0±0.08
		Sorala 3	75.0±0.01	40.0±0.02	94.0±0.01	33.0±0.05	4.5±0.06
	West Bengal	Shankarpur 1	110.0±0.08	188.0±0.08	92.0±0.03	25.6±0.02	4.70±1.4
		Shankarpur 2	85.5±0.02	128.5±0.04	52.5±0.02	8.4±0.01	7.85±1.2
		Bakshal 1	75.0±0.01	40.0±0.02	94.0±0.01	33.0±0.05	4.5±0.06
		Bakshal 2	90.0±0.03	112.0±0.01	85.0±0.02	12.5±0.06	4.95±0.02
Low	West Bengal	Fulbari 1	72.0±0.01	156.0±0.06	35.0±0.03	8.8±0.01	4.5±0.06
		Fulbari 2	73.0±0.05	154.0±0.08	36.6±0.04	9.2±0.02	4.9±0.07
		Patua 1	109.0±0.04	78.0±0.02	6.6±0.01	2.0±0.09	7.5±0.01
		Rasalpur 1	183.6±0.02	129.0±0.01	16.0±0.04	1.6±0.07	6.5±0.02

[a] Total microbial count was determined following dilution and plating on Ventosa's agar medium.
[b] Microbial activity was determined by fluorescein diacetate method (Schnurer and Rosswall, 1982. Values are average of triplicates ± standard error.

Table 4. Pearson correlation between total microbial counts and microbial activity of soil samples from crystallizer ponds of multipond solar salterns

Soil salinity category	Correlation value of total microbial count (c.f.u X 10^5 /g soil) and microbial activity of soil samples			
	NaCl (%, w/v) in isolation medium			
	0	5	10	20
High	-0.148 (0.646)	0.297 (0.349)	0.224 (0.484)	-0.202 (0.529)
Moderate	0.572 (0.052)	-0.139 (0.666)	-0.646 (0.023)	-0.660 (0.119)
Low	0.585 (0.128)	-0.915 (0.001)	-0.940 (0.001)	-0.899 (0.002)

Values in parenthesis showed level of significance.

Table 5. Growth and P(3HB) accumulation by some selected moderately halophilic bacteria isolated from saline soils of solar salterns.

Soil salinity category	Locality	Isolate No.	Incubation, h			
			48		72	
			Growth [a], g/l	P(3HB) [b], % CDW	Growth [a], g/l	P(3HB) [b], % CDW
High	Humma 1	HMA 102	1.18 ± 0.04	51.30 ± 1.0	0.99 ± 0.04	45.00 ± 1.65
		HMA 103	1.32 ± 0.06	55.33 ± 0.08	1.16 ± 0.07	55.00 ± 1.34
	Laxmipur 2	LAX 201	1.42 ± 0.01	19.78 ± 1.1	0.89 ± 0.04	15.66 ± 0.54
	Sorala 2	SUR 201	1.07 ± 0.01	42.50 ± 1.35	0.98 ± 0.01	40.00 ± 0.07
		SUR 203	1.41 ± 0.02	10.40 ± 1.5	1.53 ± 0.02	16.50 ± 1.1
Moderate	Shankarpur 1	SKP 101	0.96 ± 0.04	10.83 ± 0.06	1.03 ± 0.05	12.33 ± 1.25
		SKP 103	0.79 ± 0.08	24.66 ± 1.45	1.13 ± 0.01	30.33 ± 1.0
		SKP 201	0.80 ± 0.01	10.66 ± 1.12	0.87 ± 0.04	15.66 ± 1.1
	Bakshal 1	BAK 101	1.20 ± 0.02	19.33 ± 1.3	1.05 ± 0.01	23.66 ± 0.05
		BAK 103	0.78 ± 0.01	20.33 ± 0.02	1.29 ± 0.09	29.33 ± 0.08
		BAK 104	0.95 ± 0.01	17.33 ± 0.05	0.86 ± 0.03	15.00 ± 1.67
	Bakshal 2	BAK 201	0.87 ± 0.02	19.33 ± 0.06	1.16 ± 0.02	22.60 ± 1.62
		BAK 205	0.84 ± 0.02	10.66 ± 0.05	0.96 ± 0.01	13.33 ± 1.5
Low	Patua 1	PAT 101	1.20 ± 0.03	14.23 ± 1.12	1.54 ± 0.06	18.38 ± 0.09
		PAT 102	0.93 ± 0.02	16.65 ± 0.08	1.55 ± 0.02	18.00 ± 1.2
	Rasalpur 1	RAS 101	0.67 ± 0.04	17.99 ± 0.06	0.79 ± 0.01	20.80 ± 1.75
		RAS 102	1.11 ± 0.01	17.50 ± 0.09	1.30 ± 0.01	16.40 ± 0.08
		RAS 103	1.08 ± 0.03	40.33 ± 1.2	0.82 ± 0.03	38.80 ± 0.02

Isolates were grown in modified basal synthetic medium (Lillo and Valera, 1990) with 1% (w/v) glucose under continuous shaking (120 rpm) at 30 °C for 48-72 h.
[a] Growth was determined by cell dry weight after 48 and 72 h of incubation.
[b] P(3HB) was quantified following the method of Law and Slepecky (1961). Poor producers (<10%) are not shown in the table. Values are mean of triplicates ± standard error.

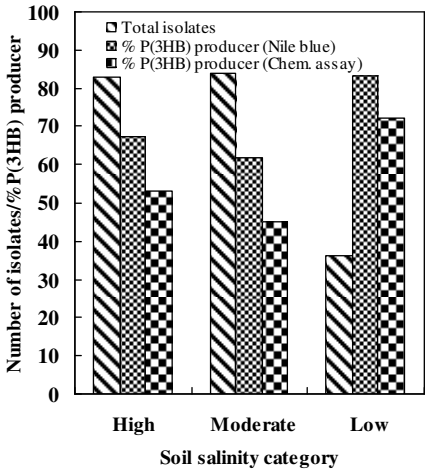

Figure 2. Screening of moderate halophilic bacterial isolates for P(3HB) production.

conductivity with rest of the characters except total carbon were statistically insignificant, while it was reverse with salinity.

In low saline soils, pH, conductivity and salinity were negatively correlated with rest of the four parameters, while sulfate, phosphate, nitrogen and carbon revealed positive correlation among themselves. In all the cases with the exception of correlation between salinity and phosphate with that of nitrogen, the correlation coefficients were statistically significant (Table 2). Dave and Deshai (2006) have analyzed the physico-chemical parameters like pH of composite samples collected from marine salterns located near Bhavnagar, Gujrat, India and indicated the presence of dissolved solids, salts and impurities in the form of hydroxide, carbonate and bicarbonates along with sodium chloride imparting alkaline nature of the habitat.

Results of microbial analysis (Table 3) indicate that salinity in addition to other soil properties influenced the density of moderately halophilic bacteria. It indicated the predominance of aerobic, heterotrophic, Gram-negative bacterial population similar to those reported by Quesada et al. (1985) and Ghozlan et al. (2006) from hypersaline environments. Measurement of in situ activities of heterotrophic halophilic bacteria from saline saltern ponds has been made based on the basis of thymidine incorporation. The present study indicated that correlation coefficient of the total microbial count of soil samples of high salinity category in media containing 5, 10 and 20 % NaCl with the microbial activity is not statistically significant, while the same for soils of moderate and low salinities were negatively correlated. However, the correlation values of all low saline soils and that of moderately saline soils at 10% NaCl were of high significance (Table 4). The optimum concentration of NaCl for growth of all the bacterial isolates obtained from solar saltern soil samples were found to be within 5-10% NaCl and were considered to be moderate halophiles as per the classification of halophilic microorganisms by Oren (2006).

All 203 moderately halophilic heterotrophic bacterial strains isolated from solar saltern soils were screened for the synthesis and intracellular accumulation of poly(3-hydroxybutyric acid) [P(3HB)] during growth under shake conditions in synthetic medium. The extent of P(3HB) production by these isolates was evaluated following both qualitative and quantitative methods. Qualitative analysis of P(3HB) accumulation following Nile blue A staining revealed that nearly 68 % of total isolates were capable of intracellular P(3HB) accumulation (Fig. 2). However, the frequency of occurrence of P(3HB) producers in low saline soils (83.33%) was more compared to moderate (61.9) and high saline soils (67.46). This might be due to consideration of lower number (4) of low salinity soils as well as bacterial isolates (36) derived from such sources as against comparatively higher number of isolates from moderate and high saline soils.

Further, these moderately halophilic bacterial isolates showed a wide degree of variation in their intracellular P(3HB) contents. Majority of them were weak to moderate producers of intracellular polymers (Table 5). Only 4 isolates namely HMA 102, HMA 103, SUR 201 from high saline soils and RAS 103 from low saline soil deserve special attention showing comparatively better polymer accumulating capability. The amount of P(3HB) accumulated by these isolates ranged from 40-55% of cell dry weight depending on the period of incubation and appeared to be promising for exploitation in the large scale production of this biodegradable polyester.

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