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Halophilic bacteria of the Arabian–sea and their role in regulating salt concentration and electrical conductivity of saline media

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ABSTRACT: Microbes including bacteria play an important role in their habitat, some roles are known, and yet others are to be discovered. These bacteria are known to be present in several ecosystems and environments including glaciers and oceans on the earth. The bacteria present in the salty water of the ocean and sea are termed bacterial halophiles due to their ability to grow in saline environments and are important in the regulation of this environment of the sea. However, all the beneficial activities of these halophiles have not been reported yet. In the present investigation, we studied the bacterial halophiles of the Arabian Sea in India, at two locations. At least 3 distinct bacterial halophiles were present in Arabian Sea water at Mumbai, while 5 distinct bacterial halophiles were present in the same Arabian Sea water at Dahanu beach in Palghar district. Based on their salt tolerance limit, these were moderate halophiles (8 % salt tolerance) and extreme halophiles (25 % salt tolerance) and were observed to be of different colony morphology at these locations. These were found to have a beneficial activity *viz.* to increase the pH (*i.e.* decreased the acidity in the range of 0.36-0.82) and decrease in electrical conductivity (EC: in the range of 0.46 – 0.88) of its saline growth medium. These bacterial halophiles were tested for their ability in the rectification of saline soils under *in vitro* studies. It was apparent from the result that the isolates of bacterial halophiles were capable of lowering the EC of saline soil, although not to the level of the required EC (<4) of normal cultivable soil. Therefore, further studies are required on the use of halophiles to achieve a lower EC of <4 in saline soils to make these suitable for crop cultivation. The use of bacterial halophiles in salt-affected soils for their reclamations can be a new area in microbial research.

Keywords: Saline soils, Microbial reclamation, bacterial halophiles, prevalence-niches.

INTRODUCTION

Bacterial Halophiles are characterized/classified based on their salt requirement (Corral *et al*., 2020) for growth in hypersaline conditions and are classified into 4 groups *i.e.* non-halophiles (grow best at < 2 percent salt), slight halophiles (2 to 5 percent salt), moderate halophiles (5 to 20 percent salt) and extreme halophiles (20 to 30 percent salt). These bacterial halophiles are present in saline environments may be saline soils and salty lakes, and oceans/seas. The presence of extreme halophiles in seawater in Spain, Italy, and the Basque Lakes, British Columbia, Canada is reported by Martínez *et al*. (2022), Villanova *et al*. (2021); and Fox-Powell and Cockell (2018) respectively; in the hypersaline lake, Siwa oasis, Egypt by Elsaied *et al.* (2017); in Indonesia by Chasanah *et al*. (2020), and from Indian salterns by Rathakrishnan and Gopalan (2022).

Halophiles are involved in centuries-old processes such as the manufacturing of solar salt from seawater (Chasanah *et al*., 2020) and the production of traditional fermented food (Oren, 2010) and in the food industry (Sekar and Kim, 2020). Other possible uses of halophilic microorganisms are in the treatment of saline and hypersaline wastewater and the production of biofuel (Amoozegar *et al*., 2019), in the biological treatment of industrial process brine contaminated with aromatics (Mainka *et al.,* 2021), in biotechnology (Mohammadipanah *et al*., 2015), in pharmaceuticals, drug delivery, agriculture, biodegradable plastic production, metal recovery, biofuel energy generation, concrete crack repair and in other sectors (Vigneshwari *et al*., 2021., Datta and Bandopadhyay, 2022).

Rising soil salinity is a global concern for crop production as salinity affects all aspects of plant development. Halophiles mitigate salt stress and enhance plant growth (Datta and Bandopadhyay, 2022) and therefore can be used in the agriculture sector for the reclamation of saline soils. Soil salinity refers to dissolved salts such as sodium chloride, calcium chloride, and magnesium chloride and such soils are acidic in nature. Soil electrical conductivity (EC) is a measure of salts in soil. It is an excellent indicator of nutrient availability and loss, soil texture, and available water capacity. pH is a measure of acidity or basicity that can influence the availability of nutrients in the soil, while EC is a measure of the concentration of nutrients and other ions in the soil that can indicate the fertility of the soil. Halophilic bacteria thrive in the saline ecosystem and have a role to play. The beneficial activity of halophilic bacteria particularly its effect on regulating pH and EC in saline water/media is not yet studied. In the present investigation, we report these two beneficial activities of bacterial halophiles isolated from the Arabian sea water in Maharashtra state, India. The exploitation of these halophiles in bioremediation of saline conditions need to be exploited (Shivanand and Mugeraya, 2011).

MATERIALS AND METHODS

Sea water sampling for isolation of bacterial halophiles

The seawater samples were collected from the Arabian Sea at two different geographical locations *viz*. Dahanu - beach in Palghar district, and Juhu- beach in Mumbai, Maharashtra state, India. The seawater samples were collected in sterile glass bottles from the seawater at a 2-foot depth above the water surface on the sea.

Isolation of bacterial halophiles

1 ml of seawater sample was serially diluted with distilled sterile water to make a dilution of up to 10^{-7}. 1 ml aliquot of each dilution was transferred to a separate sterile petri plate and 15 ml of nutrient agar having 3 percent NaCl salt (to isolate slight halophilic bacteria), 8 percent NaCl salt (to isolate moderately halophilic bacteria) and 25 percent NaCl salt (to isolate extreme halophilic bacteria) was added to these individual plates. These plates were incubated at 37 ^{0}C temp (as halophilic prefer this temperature for their growth) for 3-7 days to observe the presence and growth of respective halophilic bacteria.

Confirmation and assessment of the halophilic nature of bacterial isolate

To confirm and assess the halophilic nature of bacterial isolates, the isolated bacterial halophiles were grown on NaCl salt-containing media (3 percent, 8 percent, and 25 percent respectively). A full growth of bacterial isolate on a respective salt concentration was considered as true halophilic at that concentration.

Growth performance of the halophilic bacterial isolates on basal saline media

The basal saline medium (peptone, 5.0 g; beef extract, 3.0 g; $CaCl_2$, 2.0 g; $MgSO_4.7H_2O$, 2.5 g; $NaHCO_3$, 5.0 g; KCl, 1.0 g; Agar, 20 g; distilled water, 1L and respective concentration of NaCl) having pH below 8.5 (actual pH 6.85) and EC above 4ds/m (actual EC 6.68) was formulated and prepared to select salinity preferring bacterial isolates. The basal saline medium (BSM) represented all the salts that are present in saline seawater and /or in saline, sodic, and saline-sodic soils. The BSM was inoculated with respective bacterial isolate, incubated at 37°C for 2-7 days and the growth was recorded.

Effect of the halophilic bacteria on pH and EC values of basal saline broth

The pH and EC values of the saline seawater are two important components regulating the saline environment in the sea. The role of halophilic bacteria in regulating these two components was tested under laboratory studies by employing basal saline broth.

The sterilized basal saline broth (45 ml) was inoculated with 2 ml of individual halophilic bacterial suspension (10^8 cfu /ml), and incubated at 37^{0}C on a rotary shaker (120 rpm) for 12 days. Initial (at 0 h) and final (after 12 days) pH and EC values of broth were measured to obtain the best halophilic isolates which increase the pH values while decreasing the EC values in the growth medium and to note their role in the regulation of these values.

Effect of the halophilic bacterial isolates on pH and EC values of saline soils under *in vitro* test

The saline soil for the laboratory studies was collected

from the salinity-affected fields of the Agricultural Research Station, Kasbe-Digras in Sangi district under the university jurisdiction. The saline soil (500 g) was filled in polythene bags and sterilized by a standard procedure, to which 10 ml suspension (10x 10^{10} cfu/ml) of each individual isolate was added. The bacterial suspension was thoroughly mixed with soil. The un-inoculated soil as a control was kept for comparison. The inoculated and un-inoculated soils were incubated at 37°C for 45 days. The inoculated material was turned at weekly intervals to ensure uniform growth of halophilic bacteria in the soil. After completion of the incubation period, the pH and EC values of the salinity-affected soils were measured by standard procedure. This study aimed to ascertain to use of halophilic bacteria in the reclamation of the saline soils.

RESULTS AND DISCUSSION

Halophilic bacteria of the Arabian Sea and their morphological characteristic

At least 3 bacterial types were present in Arabian sea water at Mumbai (Juhu beach location) which were moderately halophilic in salt tolerance. These bacterial isolates produced yellowish to yellowish-white bacterial colony growth, having either circular or irregular colony shape, with entire/ lobate colony margin and convex colony elevation.

The Arabian Sea water at Dahanu Beach (in the Palghar district of Maharashtra state) has at least 5 bacterial types. Four distinct bacterial types were moderately halophiles while 1 bacterial type was extreme halophile. These bacterial isolates produced Milky-white to yellowish bacteria colony growth, with entire/ lobate /undulate colony margin and convex colony elevation.

One bacterial type was common in both locations which produced a yellowish colony with irregular colony shape, with a lobate margin, and convex elevation, and was moderate halophile (Table 1).

The Arabian Sea water at the Dahanu location has a species of extremely halophilic bacteria that was not present in the Mumbai location. Most of the bacterial species isolates were yellowish in colony colour. Baxter (1960) reported that the yellow colour in the halophilic bacteria colony may be due to neurosporene-like pigment.

Several workers (Petyon *et al.,* 2004; Sánchez-Porro *et al*., 2003; Van Trappen *et al*., 2004; Ivanova *et al*., 2004; Prado *et al.*, 2006; Dodia *et al*., 2006; Park *et al*., 2006; Tiquia *et al*., 2007; Banciu *et al*., 2008; Kanekar, 2008; and Rohban *et al*., 2009) have isolated halophiles from various saline environments like the great salt-lake in Utah, Ownes lake in California, the dead sea and evaporation ponds, hypersaline environments in South Spain, Arctic-sea water, Antartic-water, in Amursky Bay (sea of Japan, Pacific Ocean), in lake Tebenquiche on the Atacama salten, Chile and hypersaline soda lake in southwestern Siberia etc.

Truper *et al.* (1991) identified these isolated halophilic bacteria covering a wide spectrum of bacterial genera representing Halomonas, Pseudomonas, Vibrio, and Actinopolyspora and a range of gram-positive rods and cocci, particularly Bacillus, Micrococcus and Salinococcus. Oren (2002) isolated a comparatively wide range of taxa of halophilic bacteria which include members of genera Haloferax, Halogeometricum, Holococcus, and Halobacterium. Dodai *et al*. (2006) isolated 8 different moderately halophilic alkaliphilic bacteria from a saline habitat, on the Gujrat coast in western India. Ramya *et al*. (2012) isolated moderately halophilic bacteria from saline water collected from Asian sea bass and reported it as Bacillus laterosporus. Donio *et al*. (2013) isolated Halomonas and *Bacillus sp*. from solar salt work in Thamaraikulam in kanyakumari district in Tamilnadu

Growth performance of halophilic bacterial isolates on artificial basal saline medium (pH 6.85 and EC 6.68)

Based on the growth performance of the halophilic bacterial isolates on artificial basal saline media, these were differentiated into 3 categories viz. isolates producing full growth, isolates producing scanty growth, and isolates producing no growth on this specific media (Table 2). Seven halophilic bacterial isolates produced full growth on basal saline media, while one halophilic isolate produced scanty growth, and another one produced no growth, suggesting their variable salt preference in artificial basal saline media (Shivanand and Mugeraya, 2011).

Though the isolate ASW-54 and ASW-58 have similar colony morphology and salt tolerance, these appeared to be different in their growth pattern and therefore may be of different bacterial species. Most of the halophilic bacterial isolates produced full growth on basal saline media.

Effect of halophilic bacteria on pH and EC values of basal saline broth

The effect of inoculated halophilic bacterial isolates on the pH and EC value of basal saline broth was recorded at 0 hours and after 12 days to record the changes in these values of salinity conferring parameter. The result (Table 3) indicated that all the halophilic test isolates increased the pH values of the saline broth while some isolates increased the EC values, and the other isolates decreased the EC values. The maximum increase in pH was 0.82 while the maximum decrease in EC value was 0.17dsm^{-1}.

As regards the EC values, some halophilic bacterial isolates increased the EC values, while others decreased the EC values in the saline broth. The bacterial isolates that reduced the EC values of saline broth were DBW-60

Table 1. Morphological characteristic of halophilic bacterial isolates from seawater of Arabian Sea at Mumbai and Dahanu-beach in Maharashtra state, India.

Source and Location	Morphological characteristics of halophilic bacterial isolates in saline water of specific locations in Maharashtra state					Na Salt tolerance limit (%)
	Isolate no.*	Colony colour	Colony Shape/form	Colony margin	Colony elevation	
Saline water of Arabian Sea Beach, Mumbai	ASW-54	yellowish	circular	entire	convex	8
	ASW-55	yellowish	irregular	lobate	convex	8
	ASW-56	Pale-Yellow	circular	entire	convex	8
	ASW-58	Yellowish	circular	entire	convex	8
	DBW-59	Milky white	circular	entire	convex	8
	DBW-60	yellowish	circular	lobate	convex	8
Saline water of Arabian Sea Beach, Dahanu	DBW-61	yellowish	circular	entire	convex	25
	DBW-62	yellowish	circular	undulate	convex	8
	DBW-66	yellowish	irregular	lobate	convex	8

* Halophilic bacterial isolates: ASW = Mumbai Seawater, DBW=Dahanu-beach Seawater.

Table 2. Growth performance of halophilic bacterium on artificial basal saline medium (pH below 8.0 (actual 6.85) and EC above 4.0 (actual 6.68).

Isolates produce full growth	Isolates produce scanty growth	Isolates produce no growth
ASW-54, ASW-55, ASW-56, DBW-59, DBW-60, DBW-62, DBW-66	DBW-61	ASW-58

Table 3. Effect of halophilic bacterial isolates on pH and EC values of saline broth.

Isolate no.	Effect of halophilic bacterial isolates					
	At 0 hrs		After 12 hours		Increase in pH (decrease in acidity)	Increase (+)/ Decrease (-) in EC
	pH*	EC*	pH*	EC*		
ASW-54	8.67	9.83	9.09	11.24	0.36	+1.41
ASW-55	8.79	10.43	9.16	11.87	0.37	+1.44
ASW-56	8.73	10.86	9.20	11.24	0.47	+0.38
ASW-58	8.69	10.07	9.13	11.53	0.44	+1.46
DBW-59	8.67	10.55	9.15	11.06	0.48	+0.51
DBW-60	8.46	10.56	9.19	10.10	0.73	-0.46
DBW-62	8.51	10.22	9.16	10.84	0.65	+0.62
DBW-66	8.33	10.51	9.15	10.43	0.82	-0.88

*Means of two replications.

and DBW-66. The decrease in EC values was in the range of 0.46 – 0.88. All the halophilic isolates increased the pH of the saline broth (i.e decreased the acidity of the broth) and the decrease was in the range of 0.36-0.82.

These results are of great significance in selecting the halophilic bacterial isolates that have the capacity to increase the pH (an increase of pH in acidic soil will rectify these soils to a neutral side) and to decrease the EC (decrease of EC in saline soils make these cultivable) of the saline medium.

Zhu *et al*. (2011) found the growth of halophilic strain to cause a significant increase in acidity of the medium containing $Ca_3 (PO_4)_2$. In about 4 days the acidity increased from pH 7.2 to 4.24. In our studies, our halophilic isolates were found to decrease the acidity *i.e.* increase the pH towards the neutral side.

The EC decreasing isolates viz. DBW-60 and DBW-66 were used to study their effect on EC of saline soil along with the other EC-decreasing halophilic bacterial isolates available in our laboratory (whose source of isolation was

Table 4. Effect of EC decreasing halophilic bacterial isolate on pH and EC values of saline soils (mean of three replications).

Treatment no.	Saline soil (pH 7.72 & EC 4.86) inoculated with bacterial isolates	Soil pH and EC values of saline soils after bacterial inoculation			
		pH at 25°C	EC (ds/m) at 25°C	Increase (+) in soil pH due to individual isolate	Increase (+)/ decrease (-) in soil EC due to individual isolate
T 1	MDS-11	7.90	4.60	+0.18 (2.33)	-0.26 (0.05)
T 2	ARS-13	7.90	4.70	+0.18 (2.33)	-0.16 (3.29)
T 3	SDS-2	7.88	4.21	+0.16 (2.07)	-0.65 (13.37)
T 4	DBS-37	7.98	5.29	+0.26 (3.37)	+0.43 (8.85)
T 5	DBW-64	7.94	5.25	+0.22 (2.85)	+0.39 (8.02)
T 6	LLS-34	7.98	5.10	+0.26 (3.37)	+0.24 (4.94)
T 7	ARS-14	8.02	4.76	+0.30 (3.87)	-0.10 (2.06)
T 8	DBW-60	8.08	4.64	+0.36 (4.66)	-0.22 (4.53)
T 9	DBW-66	8.02	5.30	+0.30 (3.87)	+0.44 (9.05)
T 10	DBS-40	7.81	4.00	+0.09 (1.17)	-0.86 (17.70)
T 11	Control	7.72	4.86		
SE ±		0.06	0.12		
CD@5%		0.16	0.35		
CD@1%		0.22	0.47		
CV		1.20	4.29		

Figures in parentheses indicate the percent value.

from saline soils) viz.SDS-2, MDS-11, ARS-13, ARS-14, LLS-34, DBS-37, DBS-40.

Effect of halophilic bacterial isolates on pH and EC values of saline soils

The saline soils were inoculated with the test bacterial isolates and incubated for 45 days to assess their effect on soil pH and EC. The results (Table 4) indicated that all the isolates increased the soil pH in saline soil and the maximum increase was 0.36. Some isolates increased the EC while some were capable of decreasing the EC of saline soil. The isolates capable of decreasing the EC (salinity) of the soil were MDS-11, ARS-13, SDS-2, ARS-14, and DBS-40. The maximum decrease in EC *i.e.* 0.86 dsm^{-1} was obtained by the isolate DBS-40.

It was apparent from the result that these halophilic bacterial isolates were capable of lowering the EC of saline soil, although not to the desirable level, the results signify the importance of halophilic or salt-loving bacteria in the reclamation of salt-affected lands to cultivable land with the use of halophilic bacteria. Al-Abed *et al*. (2004) studied the bioremediation of saline soils by halophiles at a laboratory scale and reported that these were efficient in reducing the EC values in the first week of application by almost 50 % for all the soil samples. After that, the reduction of EC was gradual and reached almost 99.9% after 104 days for some samples. Our halophilic bacterial isolates were found to reduce the EC but not to the extent of normalization of saline soils and requires further studies on the quantum of bacterial inoculum to be applied and frequency of application with the time interval of the application period to achieve the lower EC of <4 for making the saline soils as cultivable for the crop cultivation.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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