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Characterization of an extremely halotolerant *Staphylococcus arlettae* HPSSN35C isolated from Dwarka Beach, India

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An extremely halotolerant bacterium designated as HPSSN35C was isolated from saline soil of Dwarka beach, India. It exhibited growth over a wide range of NaCl in medium varying from 0 to 6 M. The isolate produced peach–pink pigment above ~1.3 M NaCl. The culture was characterized using biochemical tests, bioMerieux Staph identification kit, API ID32 Staph system, and Biolog. Due to slow growth and extreme salt tolerance no ID was obtained in Biolog. Antibiotic sensitivity to various antibiotics was tested. Phenotypic characterization showed that it belonged to the novobiocin resistant staphylococci group. Analysis of 16S rRNA gene sequence comparison of 1452 base pairs showed that isolate is closely related to *Staphylococcus saprophyticus* group with close relationship to *Staphylococcus arlettae* (99% similarity). The halotolerant *S. arlettae* described in literature till date have been reported to tolerate 4.5 M NaCl and produce white to yellow pigment. The present study reports for the first time extremely halotolerant *S. arlettae* exhibiting growth up to ~6 M NaCl and producing peach–pink pigment above ~1.3 M NaCl.

Keywords: Halotolerant / Staphylococci / Saline soil

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Introduction

Extremophiles are bizarre microorganisms that can grow and thrive in extreme environments like high or low temperature, high or low pH, high salinity, high metal concentrations, very low nutrient content, very low water activity, high radiation, high pressure, and low oxygen tension which were formerly considered too hostile to support life [1]. Microorganisms requiring more than 0.5 M salt for their optimum growth are referred to as halophiles and organisms, which do not exhibit obligate requirement for high salt in the medium and can grow in absence of salt as well are designated as halotolerant [2, 3]. The halotolerant microbes are further classified on the basis of their growth response to varying salt concentration in medium as: slightly tolerant (growing upto 6–8% w/v NaCl), moderately tolerant (able to grow even upto 18–20% w/v NaCl) and extremely tolerant (able to grow over a wide range of NaCl upto saturation concentration) [4, 5].

Two mechanisms have been proposed to explain the survival of microbes at extremely high salt concentration, viz.: (i) salt-in-cytoplasm strategy – organisms following this strategy accumulate high salt in the cytoplasm and proteome in such organisms is highly acidic which would get denatured if exposed to low salt. Such organisms would fail to grow in low salt environments and are obligate halophiles; (ii) organic-solute-in strategy – in this case, accumulation of high salt concentration in the cytoplasm is prevented either by its expulsion and/or by synthesizing uncharged highly water soluble organic solutes. The proteome of such organisms remains largely unmodified and they exhibit growth over a wide range of salt concentration. The diverse strategies employed by halophilic and halotolerant organisms for survival and growth in high saline conditions led to evolution of unique enzymes and metabolites, which may be exploited for biotechnological applications [6]. For instance, the compatible solutes produced by halotolerant organisms are used as stabilizers of biomolecules (enzymes, DNA, membranes) and whole cells in hyper saline and low water activity environments. For example trehalose is useful as a

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cryoprotectant for the freeze drying of biomolecules [2]. The halophilic/halotolerant microorganisms producing biosurfactants may be useful in accelerated remediation of oil polluted saline environments, e.g., lychenisin A, produced by thermo and halotolerant *Bacillus licheniformis* BAS50 exhibit lowering of surface tension even at NaCl concentration upto 30% w/v [2, 7].

Exopolysaccharides (EPS) obtained from these organisms are preferably suitable for microbially enhanced oil recovery (MEOR) applications, where these polymers act as emulsifiers and mobility controllers. The sulfated EPS produced by halotolerant cyanobacteria, *Anabaena* sp. ATCC33047 [8] and *Anabaena* sp. ACTT51142 [2, 9] have been demonstrated as potential emulsifiers of crude oil. The *Halomonas* species (*H. maura*, *H. eurihalina*) produce large amounts of an extracellular polyanionic polysaccharides with significant emulsifying properties that also exhibit a pseudoplastic behavior [10, 11]. The *H. maura* exopolysaccharide ("mauran") has also been shown to be an immuno-modulator [12–14].

These organisms have been shown to produce a wide array of hydrolytic enzymes including proteases, amylases, xylanases, cellulases, lipases, DNases, etc. The extremophilicity make these enzymes suitable for applications in production of fermented food and food supplements, in animal feed, laundry detergents, textile industries, in environmental clean-up, etc. [2, 15, 16].

In this study, we describe isolation and characterization of an extremely halotolerant bacterium identified and designated as *Staphylococcus arlettae* HPSSN35C from saline soil of Dwarka beach, Gujarat, India. To the best of our knowledge, this is the first report of an *S. arlettae* exhibiting growth even at ~6 M NaCl. Previously *S. arlettae* isolates exhibiting growth over a 0.06–4.5 M NaCl have been reported by other researchers [17, 18]. The applications of these isolates have been demonstrated in treatment of azo dye containing textile effluents [19], bioremediation of hexavalent chromium containing wastewaters [20, 21] and as plant growth promoting bacteria [21, 22].

Materials and methods

Materials

All chemicals used in this study were of analytical grade and purchased from Hi-Media (Mumbai, India) or Merck (India).

Sample collection

The saline soil samples were collected from four different sites of Gujarat including Dwarka beach. Dwarka is located at the geographical coordinates of 22.23°N and 68.97°E. The collection of samples was done in November 2010. These were collected in sterile bags and preserved in refrigerator in laboratory till further analysis.

Enrichment and isolation

Enrichment cultures and isolation procedures to recover moderately to extremely halotolerant and halophilic microorganism were performed in medium containing the following in g/100 ml: KH_2PO_4, 1; peptone, 0.5; yeast extract, 0.5; glucose, 5.0; and NaCl varying from 0.5 to 35. The pH was adjusted to 7–7.2 before autoclaving. The enrichment and isolation was done using 100 ml of medium in 250 ml Erlenmeyer flasks at 30 ± 0.2 °C at static conditions. Upon enrichment, the organisms were isolated on solidified medium with aforementioned composition amended with 3% w/v agar as solidifying agent. The morphologically different colonies were subcultured and ascertained for purity. The pure cultures of bacterial isolates were then repeatedly sub-cultured on agar slopes of the medium with composition same as isolation medium and preserved at 4 °C.

Characterization and identification of isolate

Seventeen isolates were obtained upon enrichment, of which only one showed characteristic peach–pink pigmentation and extremely high salt tolerance and it was thus selected for further studies.

Salt tolerance. The 10 ml liquid media with varying NaCl (0–35% w/v) in sugar tubes were inoculated with 10^6 cells of HPSSN35C culture and incubated at 30 ± 0.2 °C for 11 days. On 11th day of incubation, the growth was measured by reading absorbance spectrophotometrically (Systronics Make, India) at 540 nm.

Biochemical tests. The ability of HPSSN35C isolate to ferment various sugars (maltose, mannitol, sucrose, glucose, fructose, galactose, lactose, and xylose) was investigated in aerobic as well as anaerobic conditions. The biochemical tests such as MR (methyl red), VP (Voges Prausker), catalase, and hydrolysis of starch, gelatin, casein, and tributyrin were determined. All the biochemical tests were performed following the procedures described in Bergey's Manual of Systematic Bacteriology [23].

Identification of isolate HPSSN35C. The isolate HPSSN35C was identified using, Microxpress Staph Identification kit (Tulip Diagnostics, India), API ID32 Staph identification system (bioMerieux, SA), Microlog bacterial identification system employing GP2 plates supplied by the manufacturer (Biolog, Inc., USA) and on the basis of 16S rRNA gene sequence. The sequencing of 16S rRNA gene was done by Oscimum Biosolutions (Hyderabad, India). The 16S rRNA

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gene sequence was analyzed using BLAST-n tool at NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) [24]. The phylogenetic tree was constructed with distance matrices and the neighbor joining method with MEGA version 4.0 [25].

Sensitivity to antibiotics. The sensitivity of the isolate towards various antibiotics was determined using Icosa-GI plus (Hi-media) biodisc on media with 15% w/v NaCl. The biodisc was placed on the solidified media seeded with $\sim 10^7$ cfu of HPSSN35C culture. The plate was then incubated at 33 ± 0.2 °C [26].

Results

The soil from coastal region of Dwarka in Gujarat, India was collected and its pH and electrical conductivity were found to be 8.8 and 0.06 mS cm^{-1}, respectively. A total of 17 isolates were obtained from this soil [27]. An isolate showing peach–pink pigmentation and tolerating upto 35% w/v NaCl was selected for further studies. This isolate was found to be Gram positive cocci in clusters and tetrads and produced round, convex, and small (~1 mm diameter) colonies on solidified medium.

Salt tolerance

The organism showed growth at all salt concentrations from 0.5 to 35% w/v. There was gradual increase in growth with increasing salt concentration. The maximum growth was observed at 10% w/v salt concentration. A slight reduction in growth was observed at 15 and 20% w/v salt concentration. With the further increase in salt concentration up to 35% w/v, very significant decrease in the growth was observed (Fig. 1).

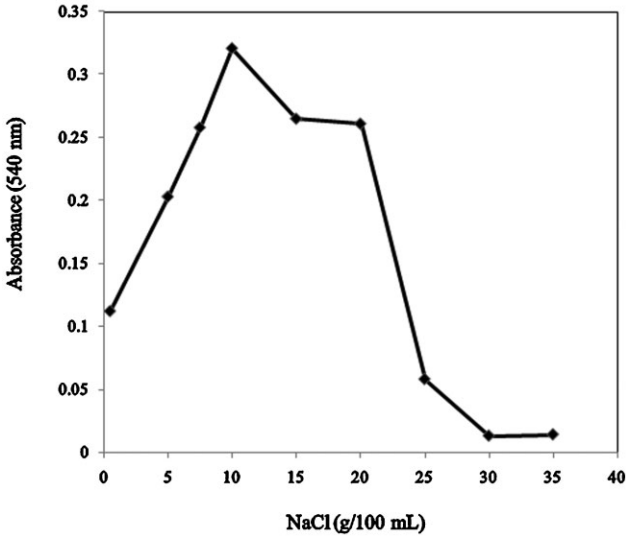

Figure 1. Salt tolerance of *S. arlettae*.

The culture exhibited peach–pink pigment production on solid media containing NaCl above 7.5 g/100 ml. The production of pigment was found to increase with increase in NaCl concentration upto 20% w/v. Further, increase in salt concentration above 20% w/v did not influence the intensity of pigment (Table 1). Although at extremely high salt concentration very scanty growth was observed in liquid media but on solid media good growth was observed from above 20% salt concentration till saturation (Table 1).

Biochemical tests

All sugars (glucose, lactose, maltose, mannitol, galactose, fructose, and sucrose) except xylose were fermented with production of acid, under aerobic conditions. The culture was found to produce catalase and did not exhibit growth under anaerobic condition. It exhibited hydrolysis of starch but did not hydrolyze gelatin, casein, and tributyrin (Table 2).

Identification of isolate HPSSN35C

The bacterial isolate was subjected to characterization by three different phenotypic bacterial identifications systems. The Microxpress Staph system did not yield clear identity of the isolate HPSSN35C. However, the isolate exhibited positive test for phosphatase and ONPG while negative test for VP, urease production, and arginine utilization. It fermented all sugars tested using kit with acid production except raffinose (Table 3).

When API ID32 Staph system was employed, the bacterial isolate HPSSN35C exhibited negative test for sodium pyruvate, 2-naphthyl phosphate, potassium nitrate, 4-nitrophenyl-βD-glucuronide, 2-naphtyl-βD-galactopyranoside, arginine β-napthylamide, ornithine, pyroglutamic acid-β napthylamide, and urea utilization. The culture fermented all sugars tested in kit with acid production except ribose. The isolate was found to be resistant to antibiotic novobiocin. As per results of API ID32 Staph system it showed 99% similarity with *Staphylococcus gallinarum* and 100% similarity with *Staphylococcus lentus* (Table 4).

When the culture was analyzed using GP2 plates of Biolog, no identity could be generated as the culture exhibited very scanty growth and exhibited utilization of very few carbon sources (D-fucose, L-fucose, D-glucose-6-PO_4, D-fructose-6-PO_4, D-aspartic acid, glycyl-L-proline, galacturonic acid, D-glucuronic acid, glucoronamide, and acetoacetic acid) even upon prolonged incubation (96 h).

The 1452 nucleotide long 16S rRNA gene sequence of the isolate HPSSN35C was found to be 99% identical to 16S rRNA sequence of *S. arlettae*. The alignment of the sequence with sequences available from GenBank

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Table 1. Effect of salt concentration on growth and pigmentation of *S. arlettae* HPSSN35C.

	Incubation time (days)					
	1		5		11	
NaCl (% w/v)	Growth	Pigmentation	Growth	Pigmentation	Growth	Pigmentation
0.5	+	−	+	−	+	−
5	+	−	+	−	+	−
7.5	+	−	+	+	+	+
10	+	−	++	+	++	++
15	+	−	++	++	+++	++
20	−	−	++	++	++++	+++
25	−	−	++	++	++++	+++
30	−	−	++	++	++++	+++
35	−	−	++	++	++++	+++

−, no growth/no pigmentation; +, visible growth or pigmentation; ++++, maximum growth or pigmentation.

database was carried out to construct a phylogenetic tree for this bacterium. The sequence has been submitted to NCBI GenBank and accession number is JQ609344 (Fig. 2).

Antibiotic sensitivity

The antibiotic sensitivity was performed in media containing 15% w/v NaCl as at low salt concentration very faint growth was obtained which obscured the results. The isolate was sensitive to all the antibiotics tested including novobiocin (Table 5). The *S. arlettae* has been described in literature to be novobiocin resistant and in order to consider any organism sensitive to novobiocin, it should exhibit a zone of inhibition larger than 16 mm diameter around a 5 mcg novobiocin disc [28]. The resistance to novobiocin as observed using API ID32 Staph system may be due to significantly lower concentration of antibiotic (1.6 mcg) employed in the test kit.

Comparison of closely related *Staphylococcus* strains to *S. arlettae* HPSSN35C

All the three *S. arlettae* strains were oxidase and urease negative while catalase and phosphatase positive (Table 6).

Table 2. Biochemical characteristics of *S. arlettae* HPSSN35C.

Test	Result
Sugar fermentation	
Glucose	+
Galactose	+
Mannitol	+
Xylose	−
Sucrose	+
Lactose	+
Fructose	+
Methyl Red test	−
Casein hydrolysis	−
Tributyrin hydrolysis	−
Gelatin hydrolysis	−
Catalase	+

+, positive test; −, negative test.

). The sugar fermentation profile of *S. arlettae* HPSSN35C was found to be significantly different from other related staphylococci. The variation in sugar fermentation profile of these staphylococci thus may be used as a tool for differentiating different staphylococcal strains and species. The isolated strain HPSSN35C exhibited growth over a wide range of NaCl (0–6 M), which is also remarkably higher than salt tolerance reported for other staphylococci. The other point which comes out of comparison is that out of these six organisms only *S. arlettae* HPSSN35C was sensitive to the antibiotic novobiocin.

Discussion

The present study describes the features of extremely halotolerant bacterium isolated from saline soil. The soil habitat is inherently inhomogeneous and it can be expected that a wide range of salinities might be present in a particular saline soil [32]. The initial enrichment and isolation experiment yielded 17 isolates from the sample. It was remarkable that one of the isolate showed salt

Table 3. Results of biomerieux Staph identification system.

Sr. no.	Test	Result
1	Phosphatase	+
2	VP	−
3	ONPG	+
4	Arginine	−
5	Urease	−
6	Arabinose	+
7	Lactose	+
8	Trehalose	+
9	Sucrose	+
10	Raffinose	−
11	Mannitol	+
12	Maltose	+

+, positive test; −, negative test.

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Table 4. Results of API test.

Tests	Active ingredients	QTY (mg cup^{-1})	Interpretation		Result
			Positive	Negative	
GLU	D-glucose	0.56	Red	Yellow	+ve
FRU	D-fructose	0.56	Red-orange	Yellow-orange	+ve
MNE	D-mannose	0.56			+ve
MAL	D-maltose	0.56			+ve
LAC	D-lactose	0.56			+ve
TRE	D-trehalose	0.56			+ve
MAN	D-mannitol	0.56			+ve
RAF	D-raffinose	0.56			+ve
RIB	D-ribose	0.56			−ve
CEL	D-cellobiose	0.56			+ve
βGUR	4-nitrophenyl-βD-glucuronide	0.0158	Colorless	Yellow	−ve
NIT	Potassium nitrate	0.054	Colorless	Pink-purple	−ve
PAL	2-naphthyl phosphate	0.0123	Colorless Pale purple Pale orange	Purple	−ve
VP	Sodium pyruvate	0.475	Colorless	Pink-red	−ve
ARA	L-arabinose	0.56	Red	Yellow	+ve
NOVO	Novobiocin	0.0016	Red-orange	Yellow-orange	+ve
TUR	D-turanose	0.56			+ve
NAG	*N*-acetyl-glucosamine	0.56			+ve
PyrA	Pyroglutamic acid-β napthylamide	0.0128	Colorless	Yellow	−ve
URE	Urea	1.12	Yellow	Orange Red-violet	-ve
ADH	L-arginine	0.76	Yellow	Orange-red	+ve
ODC	L-ornithine	0.76			−ve
ArgA	L-arginine-β-napthylamide	0.0172	Colorless Pale orange	Orange	−ve
ESC	Esculin Ferric citrate	0.224 0.032	Colorless-pale grey	Brown-black	+ve
β-GAL	2-Naphtyl-βD-galactopyranoside	0.0364	Colorless Pale purple Pale orange	Purple	−ve

tolerance upto 35% w/v salt in the growth medium and was identified as *Staphylococcus arlettae* based on nucleotide sequence of 16S rRNA gene. Three different bacterial identification systems were used to identify this isolate on the basis of biochemical characteristics, but these systems (Microxpress Staph identification kit and Biolog) either failed to identify or gave conflicting identity (bioMerieux, API ID32). The inability of these systems to identify the isolate HPSSN35C may be attributed to requirement of high salt for growth of this culture, while these systems have been designed to work at physiological salt concentration. The most significant finding of the study was that the isolate was able to tolerate wide range of salt concentration from 0.5 to 35% w/v. In liquid media at both the extremes, i.e., at very low (0.5%) and very high salt concentration (30 and 35%) growth was adversely affected. However, on solid media deviation was observed in growth response at high salt concentration. Although at extremely high salt concentration scanty growth was observed in liquid media but on solid media good growth was observed even above 20% w/v salt till saturation. The range of the salt concentration allowing the growth of the organism isolated far exceeded the salinity of the soil from which they were isolated. Similar results were obtained in a study of

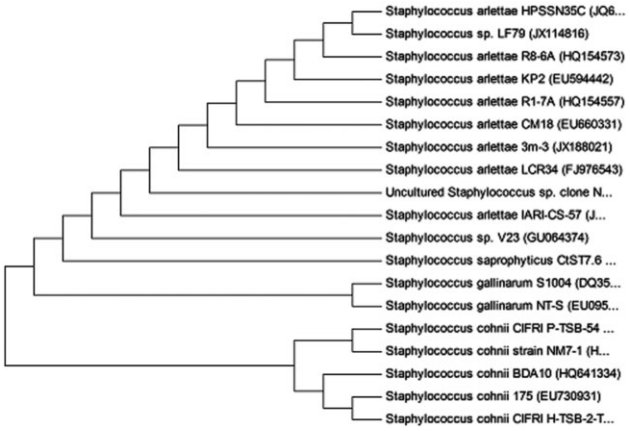

Figure 2. Phylogenetic affiliation of *Staphylococcus arlettae* HPSSN35C based on nucleotide sequence of 16S rRNA gene.

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Table 5. Antibiotic sensitivity of *S. arlettae* HPSSN35C.

Symbol	Antibiotic tested	Concentration	Zone diameter (mm)
OX	Oxacillin	1 mcg	32
AMP	Ampicillin	10 mcg	>40
CLR	Clarithromycin	15 mcg	20
GEN	Gentamicin	10 mcg	12
AMC	Amoxyclav	30 mcg	20
VA	Vancomycin	30 mcg	18
CEP	Cephalothin	30 mcg	24
AK	Amikacin	30 mcg	>40
NV	Novobiocin	5 mcg	>40
E	Erythromycin	15 mcg	38
TEI	Teicoplanin	10 mcg	>40
COT	Co-trimoxazole	25 mcg	>40
AZM	Azithromycin	15 mcg	>40
P	Penicillin	10 units	>40
OF	Ofloxacin	5 mcg	>40
MET	Methichillin	5 mcg	28
LZ	Linezolid	30 mcg	>40
CD	Clindamycin	2 mcg	>40
TE	Tetracycline	30 mcg	>40
C	Chloramphenicol	30 mcg	>40

hypersaline soils near Alicante [32] in which some Gram positive cocci from hypersaline soils with salinity of 5–10.7% w/v NaCl but growth was seen above 20% NaCl. It is highly likely that such groups might have migrated to these environments from surroundings during the course of time. However, the optimum salt concentration for growth of *S. arlettae* HPSSN35C ranged from 5 to 20% w/v NaCl. This culture produced pigment in response to salt content in the medium. It has been reported that pigment production in halophiles is stimulated as salt concentration approaches saturation [33]. It has also been proposed that these pigments in halophiles protect their DNA against oxidative damage by scavenging free radicals [34]. The isolate utilized most of the carbohydrates, did not utilize aminoacids, was incapable of growing anaerobically, did not utilize aromatic compounds and did not reduce nitrate. The isolate was highly sensitive to most of the antibiotics tested.

This is the first report indicating that: (i) *S. arlettae* can tolerate extremely high salt concentration upto 5.98 M.

Table 6. Comparison of closely related *Staphylococcus* strains to *S. arlettae* HPSSN35C.

	S. arlettae[a]	*S. xylosus*[b]	*S. arlettae* ITI-164[c]	*S. arlettae* HPSSN35C	*S. lentus*[d]	*S. gallinarium*[d]
Anaerobic growth	−	±	+	−	ND	ND
Oxidase	−	−	−	−	+	−
Catalase	+	+	+	+	ND	ND
Coagulase	−	−	−	ND	−	−
Nitrate reduction	−	±	+	ND	+	+
Phosphatase	(+)	±	+	+	ND	ND
Urease	−	+	−	−	−	+
Antibiotic resistance:						
Novobiocin	+	+	+	−	+	+
Penicillin G	+	−	−	−	ND	ND
Tetracycline	+	±	−	−	ND	ND
Chloramphenicol	+	±	−	−	ND	ND
Hydrolysis of:						
Casein	−	±	+	−	ND	ND
Tween 80	−	±	−	ND	ND	ND
Sugar fermentation:						
Lactose	+	±	−	+	±	±
Maltose	+	+	−	+	±	+
Xylose	+	+	(+)	−	W	+
Trehalose	+	+	+	+	+	+
Mannitol	+	+	−	+	+	+
Arabinose	+	±	−	+	±	+
Sucrose	+	+	+	+	+	+
Raffinose	+	−	−	−	+	+
Growth characteristics:						
Optimal temperature	30–42 °C	38 °C	32 °C	32 °C	ND	ND
NaCl growth range	0–4.5 M	0–4.3 M	ND	0–6 M	ND	ND

+, positive test; −, negative test; ±, may be positive or negative; (+), delayed positive reaction; w, weak reaction; ND, test not determined.
[a]Data from Schleifer *et al.* [17] and Kloos *et al.* [29].
[b]Data from Kloos *et al.* [18, 23] and McMeekin *et al.* [30].
[c]Data from Vilhelmsson *et al.* [31].
[d]Data from Bergey's Manual of Systematic Bacteriology [23].

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Reports till date record maximum salt tolerance of *S. arlettae* in the range of 0.06–4.5 M [31]. (ii) It can produce peach/pink pigment at high salt concentration. However, the type strain *S. arlettae* is known to produce yellow pigment [23]. (iii) *S. arlettae* has been reported from fermented foods [35] but not from saline soils.

The extreme halotolerance of the organism posed several difficulties throughout the experiment. Due to the restricted growth of organism at low salt concentration faint results were obtained for several tests. The salt tolerance and versatile nature of isolate can be exploited for environmental biotechnological applications. The bioremediation in saline environments inevitably requires the application of halotolerant and halophilic microorganisms. High concentration of salt is commonly used to force reactive dyes out of solution and bind onto substrates. Such high salinity may later have an inhibitory effect on the dye reduction process in effluent, thereby causing problems for conventional biological treatments. Thus testing effectiveness of halotolerant bacteria in decolorization of textile effluents might be promising. *S. arlettae* has been reported to degrade dye [19] but substantial work has not been done on it. Thus, this extremely halotolerant *S. arlettae* HPSSN35C could be further tested for its effectiveness in dye degradation and may prove to be promising candidate for dye reduction in wide range of saline conditions.

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References

[1] Gomes, J., Steiner, W., 2004. The biocatalytic potential of extremophiles and extremozymes. Food Technol. Biotechnol., **4**, 223–235.

[2] Margesin, R., Schinner, F., 2001. Potential of halotolerant and halophilic microorganisms for biotechnology. Extremophiles, **5**, 73–83.

[3] Hezayen, F.F., Younis, M.A.M., Haggigi, N.S.A., Shabeb, M.S.A., 2010. *Oceanobacillus aswanensis* strain FS10 sp. nov., an extremely halotolerant bacterium isolated from salted fish sauce in Aswan City. Egypt Global J. Mol. Sci., **5**, 1–6.

[4] Tiquia, S.M., Davis, D., Hadid, H., Kasparian, S. et al., 2007. Halophilic and halotolerant bacteria from river waters and shallow groundwater along the rouge river of southern Michigan. Environ. Technol., **28**, 297–307.

[5] Larsen, H., 1986. Halophilic and halotolerant microorganisms – an overview and historical perspective. FEMS Microbiol. Rev., **39**, 3–7.

[6] Oren, A., 2008. Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Syst., **4**, 2.

[7] Yakimov, M.M., Timmis, K.N., Wray, V., Fredrickson, H.L., 1995. Characterization of a new lipopeptide surfactant produced by thermotolerant and halotolerant subsurface *Bacillus licheniformis* BAS50. Appl. Environ. Microbiol., **61**, 1706–1713.

[8] Moreno, J., Vargas, M.A., Olivares, H., Rivas, J. et al., 1998. Exopolysaccharide production by the cyanobacterium *Anabaena* sp. ATCC 33047 in batch and continuous culture. J. Biotechnol., **60**, 175–182.

[9] Philippis, R.D., Margheri, M.C., Materassi, R., Vincenzini, M., 1998. Potential of unicellular cyanobacteria from saline environments as exopolysaccharide producers. Appl. Environ. Microbiol., **64**, 1130–1132.

[10] Oren, A., 2010. Industrial and environmental applications of halophilic microorganisms. Environ. Technol., **31**, 825–834.

[11] Calvo, C., Martínez-Checa, F., Toledo, F.L., Porcel, J. et al., 2002. Characteristics of bioemulsifiers synthesized in crude oil media by *Halomonas eurihalina* and their effectiveness in the isolation of bacteria able to grow in the presence of hydrocarbons. Appl. Microbiol. Biotechnol., **60**, 347–351.

[12] Arias, S., Moral, A.D., Ferrer, M.R., Tallon, R. et al., 2003. Mauran an exopolysaccharide produced by the halophilic bacterium *Halomonas maura*, with a novel composition and interesting properties for biotechnology. Extremophiles, **7**, 319–326.

[13] Llamas, I., Moral, A.D., Martínez-Checa, F., Arco, Y. et al., 2006. *Halomonas maura* is a physiologically versatile bacterium of both ecological and biotechnological interest. Antonie van Leeuwenhoek, **89**, 395–403.

[14] Béjar, V., Llamas, I., Calco, C., Quesada, E., 1998. Characterization of exopolysaccharides produced by 19 halophilic strains of the species *Halomonas eurihalina*. J. Biotechnol., **61**, 135–141.

[15] Setati, M.E., 2010. Diversity and industrial potential of hydrolase producing halophilic/halotolerant eubacteria. Afr. J. Biotechnol., **9**, 1555–1560.

[16] Kuznetsov, V.D., Zaitseva, T.A., Vakulenko, L.V., Filippova, S.N., 1992. *Streptomyces albiaxalis* sp. nov., a new petroleum hydrocarbon degrading species of thermo and halotolerant *Streptomyces*. Microbiology, **61**, 62–67.

[17] Schleifer, K.H., Kilpper-BiilL, R., Deverise, L.A., 1984. *S. arlettae* sp. Nov. *S. equorum* sp. nov. and *S. kloosii* sp. nov: three new coagulase-negative, novobiocin resistant species from animals. Syst. Appl. Microbiol., **5**, 501–509.

[18] Kloos, W.E., Schleifer, K.H., Götz, F., 1992. The genus Staphylococcus, in: Balows, A., Trüper, H.G., Dworkin, M., Harder, W. Schleifer, K.H. (Eds.) The Prokaryotes, 2nd edn, vol. **II**, Springer Verlag, New York, pp. 1369–1420.

[19] Sarkar, P., Fakhruddin, A.N.M., Pramanik, K., Al-Mahin, A., 2011. Decolorization of methyl red by *Staphylococcus arlettae* PF4 isolated from garden soil. Int. J. Environ., **1**, 35–40.

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[20] Kouadjo, G., Zeze, A., 2011. Chromium tolerance and reduction potential of staphylococci species isolated from a fly ash dumping site in South Africa. Afr. J. Biotechnol., **10**, 15587–15594.

[21] Sagar, S., Dwivedi, A., Yadav, S., Tripathi, M. et al., 2012. Hexavalent chromium reduction and plant growth promotion by *S. arlettae* strain Cr11. Chemosphere, **86**, 847–852.

[22] Srivastava, S., Verma, P.C., Chaudhry, V., Singh, N. et al., 2012. Influence of inoculation of arsenic-resistant *Staphylococcus arlettae* on growth and arsenic uptake in *Brassica juncea* (L.) Czern. Var. R-46. J. Hazard Mater. http://dx.doi.org/10.1016/j.jhazmat.2012.08.019

[23] Kloos, W.E., Schleifer, K.H., 1986. Genus IV Staphylococcus, in: Bergey´s Manual of Systematic Bacteriology, Vol **2**, Williams and Wilkins, Baltimore, pp. 1015–1035.

[24] Altschul, S.F., Gish, W., Miller, W., Myers, E.W. et al., 1990. Basic local alignment search tool. J. Mol. Biol., **215**, 403–410.

[25] Tamura, K., Dudley, J., Nei, M., Kumar, S., 2007. MEGA4; molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol., **24**, 1596–1599.

[26] Sheikh, A.F., Mehdinejad, M., 2012. Identification and determination of coagulase-negative staphylococci species and antimicrobial susceptibility pattern of isolates from clinical specimens. Afr. J. Microbiol. Res., **6**, 1669–1674.

[27] Nanjani, S.G., Soni, H.P., 2012. Effect of salt concentration and isolation of exopolysaccharide producing halotolerant bacteria. J. Sci. Technol. Res., **3**, 140–141.

[28] Murray, P.R., Baron, E.J., Jorgensen, J.H., Pfaller, M.A., Yolken, R.H. (Eds.), 2003. Manual of Clinical Microbiology, 8th edn., American Society for Microbiology, Washington, DC.

[29] Kloos, W.E., Schleifer, K., Gotz, F., 1992. The Genus *Staphylococcus*, in: Balows, A., Truper, H.G., Dworkin, M., Harder, W., Schleifer, K.H. (Eds.), The Prokaryotes, Springer-Verlag, New York, pp. 1369–1420.

[30] McMeekin, T.A., Chandler, R.E., Doe, P.E., Garland, C.D., et al., 1987. Model for combined effect of temperature and salt concentration/water activity on the growth of *Staphylococcus xylosus*. J. Appl. Bacteriol., **62**, 543–550.

[31] Vilhelmsson, O., Hafsteinsson, H., Kristjansson, J.K., 1996. Isolation and characterization of moderately halophilic bacteria from fully cured salted cod (bachalao). J. Appl. Microbiol., **81**, 95–103.

[32] Ventosa, J.J., Nieto, J., Oren, A., 1998. Biology of moderately halophilic aerobic bacteria. Microbiol. Mol. Biol. Rev., **62**, 504–544.

[33] Oren, A., 2009. Microbial diversity and microbial abundance in salt-saturated brines: why are the waters of hypersaline lakes red? Nat. Resour. Environ. Issues, **15**, 247–255.

[34] Kish, A., DiRuggiero, J., 2012. DNA replication and repair in halophiles, in: Vreeland, R.H. (Ed.), Advances in Understanding the Biology of Halophilic Microorganisms, Springer, The Netherlands, pp. 163–198.

[35] Nychas, G.J.E., Arkoudelon, J.S., 1990. Staphylococci: their role in fermented sausages. J. Appl. Bacteriol., **69**, 167–188.

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