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Isolation and characterization of endophytic bacteria colonizing halophyte and other salt tolerant plant species from coastal Gujarat

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Endophytic bacteria were isolated from leaves of four dominant halophyte and salt tolerant plant species of coastal Gujarat. The bacterial counts on nutrient agar were found to be maximum in *Spharanthus indicus* (40%) and were minimum in *Salicornia brachiata* (10%). Twenty (20) bacterial isolates were selected and were characterized through morphological characters and biochemical tests. Three were pigmented and 17 were non-pigmented and 50% isolates exhibited amylase activity and only 15% isolates showed urease activity. Six (30%) and two (10%) isolates showed positive results for ammonia production and phosphate solubilization activity. Salt tolerance of the endophytes was also tested. Of the 20 endophytic bacteria, seventeen (85%) isolates showed growth at 7.5% NaCl and fifteen (75%) tolerated upto 10% NaCl concentration. Overall, the growth rate of endophytes decreased with increasing concentration of NaCl in media. The endophytic bacteria were identified through 16S rRNA sequencing and mostly the isolated endophytic bacteria belong to genera *Bacillus* spp.

Key words: Endophyte, halophyte, halophilic bacteria, coastal region, salt tolerance.

INTRODUCTION

About 1% of the species of the land plants can grow and reproduce in coastal or inland saline soils (Manousaki and Kalogerakis, 2011). These remarkable plants, halophytes, are able to survive and reproduce in environments where the salt (NaCl) concentration is around 200 mM or more and tolerate salt concentrations that kill 99% of other species (Flowers and Colmer, 2008).

Among these salt-adapted halophytes are annuals and perennials, monocotyledonous and dicotyledonous species, shrubs, and some trees. Halophytes are highly adaptable plants, which can accrue relatively large amounts of salts.

The halophytes may be productive under harsh conditions of high salt contents of soil, which they manage by balancing their internal osmotic potential through salt accumulation in foliage. So far, over 2,000 halophytic plant species from more than 550 genera in over 100 families have been identified.

Various studies indicate that more than fifty salt-tolerant

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Figure 1. Map showing the study area.

economically useful roots, trunks, bark, stems, leaves, flowers, fruit, and seeds. The vegetative yields of halophytes and other salt tolerant species could have great potentials particularly as a source of livestock fodders (El Shaer, 2010). The sustainable cultivation of halophytes and other salt-tolerant crops on appropriate lands can serve commercial purposes without the degradation associated with large-scale annual mono-cultures and modern industrial agriculture in general.

Microbes that colonize living, internal tissues of plants such as leaf, root, stem and seeds without causing any immediate, over-negative effects are termed as endophytes (Bacon and White, 2000). It is noteworthy that, of the nearly 3, 00,000 plants species that exist on the earth, each individual is a host to endophytes (Petrini, 1991). Each plant has been reported to harbor one or more endophytes (Verma et al., 2007; Kharwar et al., 2008). Endophytes are viewed as outstanding source of secondary metabolites bioactive antimicrobial natural products.

The endophytic microbes were studied in terrestrial plants (Petrini, 1991; Saikkonen et al., 1998; Tan and Zou, 2001) which are found to possess antibacterial (Sessitsch et al., 2004; Wiyakrutta et al., 2004; Long et al., 2003), antifungal (Sessitsch et al., 2004), anticancer (Wiyakrutta et al., 2004; Strobel et al., 1993), anti-malarial (Wiyakrutta et al., 2004), antiviral (Guo et al., 2000), antioxidant (Harper et al., 2003; Strobel et al., 2002) and anti-diabetic (Zhang et al., 1999) activities. In fact, endophyte carrying plants grow more vigorously and are toxic to herbivores. Furthermore, such plants are more drought tolerant than non-infected plants. Also salt tolerance is observed in plants infected with endophytes (Waller et al., 2005). Endophytes acts as biological triggers to activate the stress response more rapidly and strongly than non symbiotic plants. An endophyte with near negligible biomass relative to plants, possesses the the capacity to alter plant community structure, and this process would have been in operation throughout its expanding range (Johri, 2006).

Endophytes are largely unexplored component of biodiversity, especially in the tropics. Endophytes are constantly exposed to intergeneric-genetic exchange with the host plant. Isolation of a potent anticancer agent, taxol from *Pestalotiopsis microspora*, an endophyte of the Yew tree and the phytohormone producing fungus from rice plant, *Gibberella fujikuroi* suggests the potential of endophytes as a source of useful metabolities (Strobel and Long, 1998; Stierle et al., 1993).

Although the presence of endophytic fungi in leaves of some of the halophytes from coastal region is known but endophytic bacteria and their bioprospecting potential from dominant halophytes and/or salt tolerant plant species like *Salicornia brachiata*, *Spharanthus indicus*, *Cressa cretica* and *Suaeda nudiflora* is largely unknown. Hence, the present attempt was to isolate, characterize and explore the biological activity of endophytic bacteria from the leaves of 4 different halophyte and salt tolerant plant species dominant in coastal region of Gujarat. The study was undertaken to test the salt tolerance of the enophytic bacteria and screen them for their plant growth promoting characters.

MATERIALS AND METHODS

Study area and sample collection

The Bara tract that lies between Gulf of Khambhat in Gujarat state covers 3 Tehsils namely: Vagra, Jambusar and Amod of Bharuch district. It lies between 21° 40' to 22° 13'N latitude and 72° 32' to 72° 55' E longitude at level of 5-9 m above mean sea level. The Bara tract experiences a tropical climate. The annual rainfall ranges from 275 to 1484 mm with an average of 737 mm (cv = 37.2%). The onset of monsoon is erratic which normally affects crop seeding operations, germination and seedling establishment. There is at least one critical dry spell of three to four weeks during the months of July-September. The land is having a gentle slope towards the coastal side. The region is also affected by poor quality of ground water which can be used for irrigation in conjunction with surface water.

The coastline of Gujarat is 1,663 km long with total coastal area of 30,022.25 km^2 stretching upto 20 km from the shoreline. The Bara Tract of Bharuch district falls near the Gulf of Cambay (Figure 1). Healthy leaf samples of four different species of halophytic plants namely: *S. brachiata*, *S. indicus*, *C. cretica* and *S. nudiflora* were collected from coastal salt affected soils of Gujarat, India. The details of location of each sampling site are presented in Table 1. Five sub-samples of each plant species growing in different locations were collected. Also, the rhizospheric soils (0-30 cm) of these plant species were collected for estimating soil properties and nutrient status.

All the samples were collected in sealed sterile plastic bags and transported aseptically to the laboratory. A portion of collected leaf samples of each plant species was separated and after washing, air dried followed by oven drying at 65°C for 24 h. The dried samples were grinded through Wiley mill and passed through sieve. The grinded samples were digested in di-acid mixture and the extract was analyzed for Na and K content through flame photometer and Ca and Mg content in the acid extract was determined through

Table 1. Details and locations of the halophyte and salt tolerant plant samples collected from halophyte and salt tolerant plant species.

Plant species	Common name	Family	Type	Location	Site
Cressa cretica	Luni	*Convolvulacea*	Herb	Inland	Occhan, Pahaj
Salicornia brachiata	Marchar	*Chenopodiaceae*	Herb	Coastal	Hatab, Bhavnagar
Suadea nudiflora	Moras	*Chenopodiaceae*	Herb	Coastal	Aladar
Sphaeranthus indicus	Gorakh mundi	*Asteraceae*	Herb	Coastal	Gandhar

titration method (Singh et al., 1999).

Isolation and characterization of endophytic bacteria

For the isolation of endophytic bacteria, the fresh leaf samples were subjected to pretreatment as per the method described by Sun et al. (2006). Fresh leaf samples were washed in running tap water, followed by 2 min wash in 70% ethanol. Then the leaf samples were washed in 2% sodium hypochlorite for 1 min. Finally, leaf were washed in sterile distilled water for 2 min and dried. After pretreatment, leaves were crushed in sterile distilled water using mortar and pestle.

About 1 ml of crushed samples was serially diluted and 0.1 ml of aliquot from 10^{-2} to 10^{-4} dilutions were taken and spread onto nutrient agar medium using sterilized glass L-rod. Plating was done in duplicates and all the plates were incubated at 28°C for 5 days. After incubation morphologically different bacterial colonies were selected and streaked on nutrient agar plants and incubated at 28°C for 48 h. From the total isolates, based on the difference in cultural morphology such as colour, texture, consistency and size limited numbers of representative isolates were selected from all the samples for further investigations. All the selected isolates were sub-cultured in nutrient agar slants and preserved in a refrigerator at 4°C. Phenotypic characteristics, such as Grams' reaction, motility, catalase and oxidase activity of all the isolates were performed following standard procedures.

Screening of endophytic bacteria for enzymatic activity

All the endophytic bacterial isolates were screened for 2 enzymes, amylase and urease as per the method described by Sahu et al. (2005). For the screening of amylase activity all the isolates were spot inoculated on starch agar plates and incubated at 28°C for 5 days. After incubation, plates were flooded with Lugol's iodine. Clear colourless zone around the growth indicates amylase production. Urease activity was determined by inoculating 0.1 ml of each culture into 5 ml urea broth and incubating at 28°C for five days. Purple red colour throughout the medium indicates alkalinezation and urea hydrolysis.

Screening of endophytic bacteria for production of plant growth promoting substances

Ammonia production by endophytic bacteria was studied by inoculating culture into 5 ml Peptone Nitrate Broth and incubating at 28°C for 48 h. The change of red litmus to purple or blue indicates ammonia production. Phosphate solubilizing activity of endophytic bacteria was studied by the method described by Pandey et al. (2008) using Pikovskaya's agar medium. After incubation, the presence of clear halo around the growth indicates phosphate solubilization.

The Methyl red test was used to detect mixed acid fermentation by endophytic bacteria. Endophytic cultures were inoculated into 5 ml of GPB broth individually and incubated at 28°C for 48 h. After incubation, 5 drops of methyl red indicator was added to the medium. Only mixed acid fermenters produce sufficient quantities of acids during the initial phase of incubation which was detected by methyl red indicator.

The Voges-Proskauer test was used as a qualitative method for the detection of acetoin. Endophytic cultures were inoculated into 5 ml of MR-VP broth individually and incubated at 28°C for five days. After incubation, to 1 ml of bacterial culture, 3 ml of freshly prepared 5% α-naphthol in absolute ethanol and 1 ml of 40% KOH were added and the mixture was stirred vigorously. The formation of red colour was indicative of the presence of acetoin. For the screening of indole acetic acid (IAA), about 0.1 ml of 24 h old culture was inoculated into each 5 ml of 1% Tryptone broth and incubated at 28°C for five days. After incubation, three to four drops of xylene was added and mixed vigorously. Two layers were allowed to separate followed by slow addition of 1 ml Ehrlich's reagent so as to form the layer on the surface of xylene. The formation of pink coloured ring at the lower surface of xylene layer indicated the production of IAA.

Screening of endophytic bacteria for salt (NaCl) tolerance

Endophytic bacteria were inoculated onto Nutrient agar medium supplemented with different concentrations of NaCl (2.5, 5.0, 7.5 and 10%). All the plates were incubated at 28°C for 5 days and bacterial growth was observed at every 24 h.

Identification of endophytic bacteria

The twenty endophytic bacterial isolates were submitted for molecular identification where Fast MicroSeq 500 16S rDNA Bacterial identification kit was used for extraction. Sequencing of the 16S rRNA gene was carried out using primers in 3130 Genetic analyzer and submitted to NCBI Genebank database.

RESULTS

Properties of rhizosphere soil

The rhizosphere soils of the 4 different dominant halophytes and salt tolerant plant species were found to be medium black to coastal alluvium and moderate to highly saline. The soils are clayey in texture with swell-shrink properties thus high water holding capacity. The rhizosphere soils are dominated by high soluble salt content where Na and Ca ions were present in high concentrations. The soils are found to be low in N and P while sufficient to high in sulphate content. The details of the soil properties are presented in Table 2.

Table 2. Properties of rhizosphere soil of halophyte and salt tolerant plant species.

Soil properties	Range	Mean
pH (1:2 w/v)	7.85-9.25	8.40
EC (dS/m)	2.05-35.50	17.05
Org. C. (g/kg)	1.3-6.7	4.1
Available P (mg/kg)	1.41-2.62	1.98
Available S (mg/kg)	24.4-302.10	124.5
NH_4-N (mg/kg)	3.10-10.08	7.91
NO_3-N (mg/kg)	2.64-7.06	4.17
Exch. Na (mg/kg)	968.7-5171.8	2568.5
Exch. K (mg/kg)	387.5-1775.0	935.2
Exch. Ca (mg/kg)	3400-7100	5600
Exch. Mg (mg/kg)	240-2160	1080
Water holding capacity (%)	43.7-59.4	51.6

EC = Electrical conductivity; Org. C. = organic carbon content; Exch. = exchangeable ion.

Table 3. Ionic content in leaves of halophyte and salt tolerant plant species.

Plant species	K (mg/g)	Na (mg/g)	Ca (mg/g)	Mg (mg/g)	S content (mg/g)
Cressa cretica	5.91	50.50	20.0	16.8	3.97
Salicornia brachiata	11.92	17.76	24.0	16.8	7.33
Suadea nudiflora	10.32	21.42	12.0	4.8	0.53
Spharanthus indicus	7.66	15.30	14.0	4.8	2.42

Ionic content of leaf samples

Plant leaf samples were analysed for various ionic elemental contents to get an idea about the uptake of these elements. By the analysis of the leaf samples of various halophyte and salt tolerant plant species, it was found that *S. brachiata* contain maximum amount of potassium content in their leaves. *S. nudiflora* leaves contain higher K than plant species studied (Table 3). The highest Na (50.50 mg/g) content was found in the leaves of *C. cretica* and the lowest amount (15.30 mg/g) was found in the leaf samples of *S. indicus*. It was found that the leaf samples of all the plant species contain calcium in the range of 12.0 to 24.0 mg/g, in which the highest Ca (24.0 mg/g) was found in the leaves of *S. brachiata* (24.0 mg/g) (Table 3). The highest content of magnesium (16.8 mg/g) was found in the leaves of *C. cretica* and *S. brachiata* while the lowest value of Mg (4.80 mg/g) was observed in the leaves of *S. indicus*. The sulphur content of leaf samples of different plant species varied from 0.53 to 7.33 mg/g on dry weight basis (Table 3). The leaves of *S. brachiata* contain the highest sulphur (7.33 mg/g), while the leaf samples of *Suadea nudiflora* contain the lowest sulphur (0.53 mg/g).

Isolation of endophytic bacteria

Nutrient agar plates inoculated with leaf extracts of four dominant halophytes or salt tolerant plants showed morphologically different bacterial colonies. Twenty isolates were selected for further investigations based on their fast growth. The bacterial counts were found maximum in *S. indicus* (40%) and were minimum in *S. brachiata* (10%) (Figures 2 and 3).

Characterization of endophytic bacteria

Morphological characteristics of endophytic bacteria isolated from leaves of different halophyte and salt tolerant plant species are shown in Table 4.

Of the 20 isolates selected, 3 were pigmented and 17 were non-pigmented isolates. Regarding cell shape and Gram's staining, 7 were Gram-negative cocci, 2 Gram-positive cocci, 4 Gram-negative bacilli and 7 Gram-positive bacilli. Motility test results depicted that 18 isolates were motile while only 2 isolates were non-motile. In total, 11 isolates showed positive results for oxidase test whereas all endophytic bacterial cultures showed negative catalase test.

Enzymatic activity

The enzymatic activity of endophytic isolates revealed that 50% isolates exhibited amylase activity and only

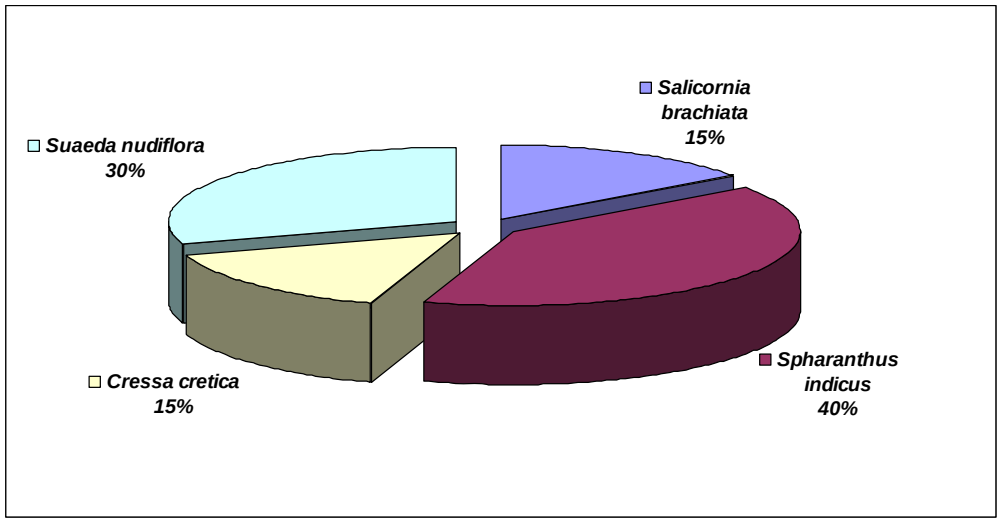

Figure 2. Percent distribution of endophytic bacteria in leaves of halophytes and salt tolerant plant species from Coastal Gujarat.

Figure 3. Dominant halophytes of coastal Gujarat.

15% isolates showed urease activity. Detailed results are shown in Table 5.

Production of plant growth promoters

Of 20 endophytic bacterial isolates screened for plant growth promoting substances, 6 (30%) and 2 (10%) isolates showed positive results for ammonia production and phosphate solubilization activity. Only 4 (20%) were mixed acid fermenters, 5 (25%) showed the production of acetoin and none of the isolates exhibited IAA production (Table 6).

Molecular identification of endophytic bacteria

The selected bacterial isolates were submitted for 16S rRNA gene sequencing and it was observed that *Acinetobacter baumannii, Bacillus cereus*, *Bacillus firmus, Bacillus aerius*, *Pseudomonas fluorescens* and *Bacillus subtilis* were positive for ammonia production while phosphate solubilization was positive for *A. baumannii* and *P. fluorescens* (Table 7).

Tolerance to sodium chloride

All the 20 endophytic bacteria showed good growth at 2.5% NaCl concentration while 18 (90%) isolates grow upto 5% NaCl, seventeen (85%) isolates showed growth at 7.5% NaCl and fifteen (75%) tolerated upto 10% NaCl concentration. *Bacillus foraminis* and *Bacillus gibsonii* could tolerate upto 7.5% NaCl while *A. baumannii* and *Paenibacillus xylanisolvens* tolerated only upto 2.5% NaCl concentration and *P. fluorescens* upto 5% NaCl. All

Table 4. Morphological characteristics of endophytic bacteria isolated from leaves of different salt tolerant plant species.

Isolate no.	Colony morphology				Grams' staining	Shape	Motility
	Form	Elevation	Margin	Colour			
EB1	Circular	Pulvunate	Entire	White	-	Cocci	M
EB2	Circular	Convex	Entire	Yellow	-	Cocci	HM
EB3	Irregular	Raised	Wavy	White	+	Bacilli	M
EB4	Irregular	Effused	Wavy	Off- white	-	Cocci	HM
EB5	Circular	Convex	Entire	White	-	Cocci	HM
EB6	Circular	Convex	Entire	Off- white	+	Bacilli	M
EB7	Irregular	Flat	Wavy	White	-	Cocci	M
EB8	Circular	Pulvunate	Entire	White	-	Cocci	HM
EB9	Irregular	Flat	Wavy	Off- white	+	Bacilli	M
EB10	Circular	Convex	Entire	Off- white	+	Cocci	HM
EB11	Circular	Flat	Entire	Dew drop	+	Bacilli	HM
EB12	Irregular	Effused	Wavy	Off- white	+	Bacilli	NM
EB13	Circular	Capitate	Entire	Dew drop	+	Bacilli	M
EB14	Circular	Effused	Wavy	Off-white	-	Bacilli	HM
EB15	Circular	Umbonate	Entire	White	+	Cocci	HM
EB16	Irregular	Flat	Wavy	White	+	Bacilli	M
EB17	Circular	Convex	Entire	Off-white	-	Bacilli	NM
EB18	Circular	Convex	Entire	Orange	-	Bacilli	M
EB19	Round	Convex	Entire	Light yellow	-	Cocci	HM
EB20	Round	Flat	Erose	Off-white	-	Bacilli	M

- Negative; + positive; M motile; HM highly motile; NM non-motile.

Table 5. Enzyme activity of endophytic bacteria isolated from leaves of halophyte and salt tolerant plant species.

Isolate no.	Oxidase test	Catalase test	Amylase activity	Urease activity
EB1	-	-	+	-
EB2	-	-	-	-
EB3	+	-	+	-
EB4	+	-	+	-
EB5	-	-	-	+
EB6	+	-	-	-
EB7	+	-	-	+
EB8	-	-	-	-
EB9	+	-	-	-
EB10	-	-	+	-
EB11	+	-	+	-
EB12	+	-	+	-
EB13	+	-	+	-
EB14	-	-	+	+
EB15	+	-	-	-
EB16	+	-	+	-
EB17	-	-	+	-
EB18	-	-	-	-
EB19	-	-	-	-
EB20	+	-	-	-

Table 6. Plant growth promotion properties of endophytic bacteria isolated from leaves of halophytes and salt tolerant plant species.

Isolate no.	MR test	VP test	Ammonia production	Indole production	Phosphate solubilization
EB1	-	-	+	-	+
EB2	+	-	-	-	-
EB3	+	+	+	-	-
EB4	-	-	+	-	-
EB5	+	+	-	-	-
EB6	-	-	-	-	-
EB7	-	-	-	-	-
EB8	+	+	-	-	-
EB9	-	+	-	-	-
EB10	-	-	-	-	-
EB11	-	-	-	-	-
EB12	-	-	-	-	-
EB13	-	-	-	-	-
EB14	-	+	+	-	-
EB15	-	-	+	-	+
EB16	-	-	+	-	-
EB17	-	-	-	-	-
EB18	-	-	-	-	-
EB19	-	-	-	-	-
EB20	-	-	-	-	-

+ Positive; - negative

Table 7. Molecular characterization of endophytic bacteria.

Isolate ID	Endophytic bacteria
EB1	*Acinetobacter baumannii*
EB2	*Kocuria flavus*
EB3	*Bacillus cereus*
EB4	*Bacillus firmus*
EB5	*Staphylococcus pasteuri*
EB6	*Paenibacillus xylanisolvens*
EB7	*Bacillus horneckiae*
EB8	*Paenibacillus xylanisolvens*
EB9	*Bacillus licheniformis*
EB10	*Bacillus foraminis*
EB11	*Virgibacillus picturae*
EB12	*Oceanobacillus picturae*
EB13	*Bacillus subtilis*
EB14	*Bacillus aerius*
EB15	*Pseudomonas fluorescens*
EB16	*Bacillus subtilis*
EB17	*Bacillus aryabhattai /megaterium*
EB18	*Arthrobacter luteolus*
EB19	*Bacillus gibsonii*
EB20	*Paenibacillus sp.*

the other isolates were able to tolerate 10% NaCl concentration in media. Overall, the growth rate of endophytes decreased with increasing concentration of NaCl (Table 8) in the media.

DISCUSSION

In general Na^+ depresses K^+ uptake, but Hardikar et al. (2011) observed significant increase of K^+ in all tissue of seedlings with the increasing soil salinity in *Salvadora oleoides*. There was high selectivity of *S. oleoides* for K^+ over Na^+.

In practice, Na^+ is largely compartmentalised in vacuoles in halophytes (Flowers, 1977; Flowers et al., 1986). A range of metabolically inert organic compounds is also present and utilized to adjust the osmotic potential of the cytoplasm.

Maggigo et al. (2000) also observed increased growth in *S. persica* under saline conditions. Although the presence of NaCl is rarely an obligate requirement for growth of many halophyte (Flowers, 1977). The absence of salt in the nutrient solution strongly inhibited the growth of *S. persica* and other halophytes this was observed by (Maggigo et al., 2000).

In *Salvadora persica,* sodium content of leaves increased by 10% when imposed salinity was raised up to 30 dSm^{-1}, while potassium content in leaves reduced up to 18% at this salinity level. Maggio et al. (2000) also

Table 8. Salt tolerance of endophytic bacteria isolated from leaves of halophytes and salt tolerant plant species.

Isolate no.	Salt tolerance (NaCl %)			
	2.5%	5.0%	7.5%	10%
EB1	+	-	-	-
EB2	+	+	+	+
EB3	+	+	+	+
EB4	+	+	+	+
EB5	+	+	+	+
EB6	+	-	-	-
EB7	+	+	+	+
EB8	+	+	+	+
EB9	+	+	+	+
EB10	+	+	+	-
EB11	+	+	+	+
EB12	+	+	+	+
EB13	+	+	+	+
EB14	+	+	+	+
EB15	+	+	-	-
EB16	+	+	+	+
EB17	+	+	+	+
EB18	+	+	+	+
EB19	+	+	+	-
EB20	+	+	+	+

+= Growth; - = no growth

reported that in *S. persica* sodium content of plant grown under salinity showed 40 fold increase as compared to non-saline conditions. The increased Na^+ content plays an important role in osmotic adjustments. Contrary, in *S. oleoides,* K^+ and Na^+ content significantly increased in leaves in response to increasing soil salinity. There was a positive relationship between salt concentration applied and K^+ content in leaf. Similarly, a positive relationship was obtained between salt concentration and Na^+ content of leaves.

The high Na^+ content in leaf is due to compartmentation in leaf vacuoles in *Suaeda* (Maathuis et al., 1992). Whereas, K^+ content was reported to be very high in the leaves of *Salvadora oleoides*.

Endophytic bacteria are poorly investigated group of micro organism that represent an abundant and dependable source of bioactive and chemically novel compounds with potential for exploitation in a wide variety of medical, agriculture, and industrial areas (Strobel and Daisy, 2003). The mechanisms through which endophytes exist and respond to their surrounding must be better understood in order to be more predictive about which higher plants to seek study and spend time for isolating microfloral components. This may facilitate the product discovery process (Ravikumar et al., 2010). In the present study, from four different halophytes or salt tolerant plant leaves, 20 bacterial isolates were selected based on different morphological characters and salt tolerance. Other parts of the plant such as roots (Asraful et al., 2010; Zhang et al., 2010), stems, seeds (Zhang et al., 2010; Magani et al., 2010), petioles, tubers tissues, and flowers (Reiter and Sessitsch, 2000) can also be used in isolation of endophytes. Morphologically different 36 bacterial isolates has been isolated from leaves of mangrove and salt-marsh plants (Gayathri et al., 2010). Leaves of Gaynura procumbent plants have been used for isolation of cultivatable bacterial endophytes (Bhore et al., 2010).

It is well established that plant bacterial endophytes are to be found in most healthy plant tissues (Frommel et al., 1993; McInroy and Kloepper, 1995; Sturz, 1995). This particular host endophyte interaction has been variously defined as altruism, commensalisms, symbiosis or passivity to pathogenicity. Whatever the specific relationship involved, internal plant colonization by bacteria constitutes a vast and as yet little mapped ecological niche.

The diversity of a collection of twenty putative endophytic bacteria isolated from different tissues of the host was assessed using phenotypic characterization methods. Colony morphology gave an indication of the variation among the endophytes. The isolates studied were chosen for their dominance as well as uniqueness or differences with other in colony morphology. Interestingly, the proportion of Gram positive and Gram negative isolates in our study was almost similar. Earlier researchers have reported a predominance of Gram negative bacteria in the tissues of various plants (Stoltzfus et al., 1997; Elbeltagy, 2000). However, Zinniel et al. (2002) reported an equal presence of Gram negative and Gram positive bacteria.

Our observation revealed that, 85% of endophytic bacterial isolates were non-pigmented whereas only 15% were pigmented. Results of endophytes from mangrove leaves revealed 75% pigmented and 25% non-pigmented isolates (Gayathri et al., 2010).

Motility test results showed that majority of the isolates that is, 90% are highly motile. Studies on *Glycine max* and *Glycine soja* revealed that when grown on 2% agar, 78% of the endophytic isolates were found to be motile (Hung and Annapurna, 2004). Due to motility of these endophytes, there is an advantage for spreading of endophytes into the host plant.

Enzymes are the most important products. In the present study, while screening the endophytic bacterial isolates for two different enzymes, 10 isolates exhibited amylase and three isolates the urease activity. The assemblage of endophytes in young, mature and senescent leaves of *R. apiculata* and its possible role in mangrove litter degradation have been reported (Kumaresan and Suryanarayanan, 2001).

Endophytic bacteria residing within plant tissues have been reported to be promoting the plant growth directly or

indirectly through production of phytohormones, bio-control of host plant diseases and improvement of plant nutritional status (Pandey et al., 2008; Rosenblueth and Romero, 2006). They possess the capacity to solubilize phosphates as shown with the endophytic bacteria of soybean in phosphate assimilation (Hung and Annapurna, 2004). Phosphate solubilization by *Bacillus* sp. isolated from salt stressed environment had been observed by earlier researchers (Son et al., 2006). It is also evident in the present study that endophytic bacterial isolates showed four growth promoting activities, particularly ammonia and acetoin production by 6 and 5 isolates, respectively. Volatile substances, such as 2,3 butanediol and acetoin produced by bacteria are responsible for plant growth promotion, which is newly discovered mechanism (Ryu et al., 2003). Mixed acid fermenters produce complex mixture of acids like acetic, lactic, succinic and formic acids. Majority of the bacterial isolates were identified as *Bacillus* spp. Earlier studies also indicated the dominance of genera *Bacillus sp* and *Pseudomonas* spp. having PGP activity in salt stress (Tank and Saraf, 2010).

Occurrence of halophilic bacteria is well known in coastal marine biotopes including mangrove and salt marsh ecosystems. There are only two reports on halophilic endophytes in the coastal plants, one of them is the report of Kamalraj et al. (2008) who showed the effect of NaCl on endophytic fungal assemblage in the leaves of a mangrove *C. roxburghiana*. In the study, none of the endophyte showed growth above 300 mM NaCl concentration. Secondly, the endophytic bacteria isolated from five mangrove and two salt marsh leaves are reported to tolerate salt concentration upto 10% NaCl (Gayathri et al., 2010). However, in the present study, salt tolerance of endophytic bacteria was also observed upto 10% NaCl concentration and the growth rate of endophytes decreased with the increase in the salt concentration. Upadhyay et al. (2009) had reported that bacteria isolated from saline environment are more likely to withstand salt stress. Furthermore, if such bacterial strains also possess plant growth promoting properties they would be beneficial for use in mitigation of salt stress to enable agricultural crop production in saline soils (Egamberdiyeva and Islam, 2008).

Conclusion

This study demonstrated the occurrence and diversity of culturable endophytic bacteria from leaves of 4 different dominant halophytes and salt tolerant plant species dominant in coastal region of Gujarat. The successful colonization of these plants with such microbes suggests that they can be utilized in future applications, such as delivery of degradative enzyme for controlling certain plant diseases, plant growth promoting substances or other useful products. Also the halophilic endophytic bacteria having potential for plant growth promotion and phosphate solubilization can be possibly utilized for bio-remediation of salt affected soils for agricultural crop production. Therefore, further studies are needed for possible commercial utility of these potential bio-chemicals.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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