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ORIGINAL PAPER

Characterization of the predominant bacterial population of different mangrove rhizosphere soils using 16S rRNA gene-based single-strand conformation polymorphism (SSCP)

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Abstract Variations in chemical parameters and bacterial populations in mangrove rhizosphere samples were noted for different sites. The C, N, P and K contents as well as pH, EC and salinity showed variation between sites. Significant differences in soil properties were also found in sampling sites. Two types of soil were noted among sites. Guesthouse had significantly higher organic matter and nutrient content (N) than other three sites suggesting that human discharges, litter deposition and surface runoff were major nutrient inputs. This contaminated site was located at the landward edges. Positive correlations between organic matter, N, P and K contents were found suggesting that these nutrients were from similar input sources. Effects of sampling sites on microbial diversity were also analyzed via SSCP. *Porteresia coarctata* and *Rhizophora mucronata* did not show any variation in the banding patterns among replicates sampled in short distance within site. But *Sonneratia apetala* showed variation among replicates sampled in distance within site. A significant variation was noted in the SSCP profile among replicates between sites. The majority of dominant SSCP band sequences were related to bacterial genera of root and root-free soil environments, namely *Bacillus*, *Planococcus, Planomicrobium*, low G+C Gram-positive bacterium, glacial ice bacterium and unidentified bacteria. In the analysis of 16S rRNA sequences, members belonging to the phylum *Firmicutes* dominated the sequence collection. The phylogenetic analysis of 16S *r*RNA gene sequences showed close relationships to a wide range of clones or bacterial species of phylum *Firmicutes* and unidentified bacteria.

Keywords Mangroves · PCR-SSCP · Bacillus · 16S r DNA gene

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Introduction

The mangroves in India are productive ecosystems that are very sensitive to environmental changes (Krishnamurthy 1993). Pichavaram mangrove is one of the rare mangrove forests in southeast coast of India. It is located in the Vellar–Coleroon estuarine complex and has many islands separated by intricate waterways. It covers an area of about 1,350 ha and is traversed by a large number of channels and creeks, which connect the Coleroon estuary in the south and Vellar estuary in the north. It represents 14 exclusive mangrove species (Kannupandi and Kannan 1998). *Avicennia marina* alone constitutes nearly 30% of the total population followed by *Bruguiera cylindrica* (17%) and *Avicennia officianalis* (16%). The population density of other species is poor and many of the species are on the verge of total extinction.

According to a recent statistics, it is found that nearly 62.8% of the Pichavaram mangrove forests were degraded between 1897 and 1994 (Nayak 1993). It has been found that the breadth of the beach protecting the mangrove areas

from wave action at Pichavaram mangrove has reduced by 550 m between 1970 and 1992. Many plants previously recorded from Pichavaram mangrove have completely vanished. For example, the pollen analysis of the sediments from Pichavaram showed that *Sonneratia* sp. was abundant here in the past (Caratini et al. 1973), but it is on the verge of extinction at present. Certain species like *Xylocarpus granatum, Rhizophora stylosa* and *Bruguiera gymnorrhiza*, which were once collected from this mangrove are not available at present. It is also found that most of the individuals of *Rhizophora* sp. are aged and the rate of reproduction is also low in Pichavaram mangrove. It seems to be on its way to extinction at this mangrove, being replaced by the much more dynamic *Avicennia marina* (Kannupandi and Kannan 1998).

Also, mangroves are dominant features of the coastal areas of tropical countries and form a productive ecosystem that supports abundant life through a food chain that starts with the trees and the habitat's unique micro-biota (Smith et al. 1991). Knowledge of the diversity of microbial communities inhabiting this unique swampy, saline, partially anaerobic environment is useful since it provides clues about the type of bacteria able to adapt and exploit such habitats (Semenov et al. 1999). Very little is known about the association of rhizobacteria with mangroves. (Petra et al. 2004). PCR-based 16S rRNA profiling provides a rapid characterization of the most abundant phylotypes present within an environmental sample without the prerequisite of first culturing the microbe in the laboratory. PCR-single-strand conformation polymorphism (SSCP) has been a useful tool for monitoring microbes within a variety of environments (Schmalenberger et al. 2001).

In this study, we have chosen five different mangrove rhizospheres and the rationale for choosing these plants the main reason is that they were approaching extinction. The study on their rhizospheric bacteria (PGPRs) would help in the reforestation of mangroves. The chosen mangroves *viz. Porteresia coarctata* (Roxb) Takeoka (family Poaceae)-mangrove associated plants, *Avicennia marina* var. *marina* (Forssk.) Vierh. (Family Avicenniaceae)-true mangrove, *Sonneratia apetala* Buch Ham., (family Sonneratiaceae)-endangered species in Pichavaram mangroves. *Excoecaria agallocha* L. (family Euphorbiaceae)-mangrove associated plants and *Rhizophora mucronata* Poir. (Family Rhizophoraceae)-true mangrove was present in Pichavaram forest, southern India.

Rhizospheric soil chemical parameters were determined as well as bacterial community structures in stable environmental conditions (before monsoon). SSCP method was used in this study which is potentially more simple and straightforward than DGGE or TGGE. The SSCP technique has been used to study the structural analysis of natural bacterial communities (Lee et al. 1996).

Materials and methods

Study area

The study was conducted at Pichavaram mangroves on the south east coast of India, situated about 250 km South of the city of Chennai at Latitude: 11°27′ N Longitude: 79°47′ E. Pichavaram mangrove is the rare and degrading mangrove forest in southeast coast of India. These experiments were conducted pre-monsoon.

Study sites, sampling and MPN count

Rhizosphere soil samples (0–10 cm) were collected in different sites at Pichavaram mangrove forest during pre-monsoon using a soil core (10 cm long and 4.5 cm in diameter) after scraping off the first top centimeter of the sediments. The distance between collection sites ranged around 3–4 Km from each site and the sampling within sites was done at 200 m distance from each sampling spot towards sea.

Sampling was done from four different sites and all the sites were man-disturbed. Tide elevation was high and the soil type was sandy clay except for the location Guesthouse where it was sandy.

Sampling was done from *Porteresia coarctata* in site-1 (Tandavarayacholarpet), from *Sonneratia apetala* and *Avicennia marina* in Site: 2 (Kodiyampalayam), from *Rhizophora mucronata, Avicennia marina* and *Excoecaria agallocha* in Site: 3 (Pillumedu) and from *Rhizophora mucronata*, *Avicennia marina, Excoecaria agallocha* Site: 4 (Guesthouse).

The sampling sites had extensive vegetations of mangrove species such as Rhizophora apiculata, R. mucronata, and the occasional mangroves are Sonneratia apetala, Avicennia marina, A. officinalis, Bruguiera cylindrica, Ceriops decandra, Aegiceras corniculatum. Plant rhizospheres were completely covered by water and the soil type varied (sandy-clay type) in collection sites. The sampling plant species had different root types i.e., thick creeping rhizome stilt root system (P. coarctata), sporadically and profuse stilt roots (A. marina and R. mucronata, respectively) and superficial spreading horizontal roots (S. apetala and E. agallocha). The samples were placed in zip-bags and transferred to the laboratory. Sub-samples were immediately used for enrichment and isolation of bacterial cultures. Yet another part of the samples were freeze-dried and filtered through a 2 mm sieve, and analyzed for electrical conductivity (E.C), pH and content of nitrogen, phosphorus and potassium at SPIC Agric. Input Diagnostic center, Chennai, India. Three replicates were maintained for all analysis. Total counts of heterotrophic bacteria,

diazotobacters and phosphate solubilizers were estimated by serial dilution and plating (Holt et al. 1994).

Total heterotrophic count was taken by dilution plating on nutrient agar, supplemented with 1% NaCl, and the plates were incubated for a week and colony forming units were counted. The Most Probable Number (MPN) was estimated. For isolation of diazotrophs, LGI semi-solid medium (Cavalcant and Dobereiner 1988) was used. Isolation of phosphate solubilizers were done using Pikovskaya's medium (Pikovskaya 1948). It was inoculated with serial diluted 0.1 ml of soil solution. The colonies which showed hallow zone around them were taken and purified again on the same medium.

DNA extraction from rhizosphere samples

DNAs were extracted from rhizosphere samples (100 mg) using SoilMaster^{TM} DNA Extraction kit as manufacture's instructions. Final DNA extracts were subjected to electrophoresis in Tris-acetate-EDTA (TAE) buffer containing 0.5 mg of ethidium bromide per ml in 0.7% agarose gels containing DNA standards of 500 ng of lambda phage DNA. The DNA concentrations and A260/A280 ratios were determined spectrophotometrically.

PCR amplification

PCR amplification targeting bacterial 16S rRNA genes was performed with the Com1 (forward) (5'CAGCAGCC GCGGTAATAC3', positions 519 to 536) and Com2-Ph (reverse) (5'CCGTCAATTCCTTTGAGTTT3', positions 907–926). Each PCR was performed in a total volume of 50 µl in 0.2 ml micro tubes. Reaction mixtures were contained 1 × PCR buffer with 1.5 mM $MgCl_2$, deoxynucleoside triphosphate solution (2 mM each dATP, dCTP, dGTP and dTTP), primers Com1 and Com2-Ph (50 µM each) (Schwieger and Tebbe 1998), and 2 U of *Taq* DNA polymerase. The total amount of genomic DNA added to PCR mixtures was 10 ng and thermocycling, which was conducted in a Mini cycler 150 instrument (MJ research), started with an initial denaturation for 3 min at 94°C. A total of 30 cycles, each including 60 s at 94°C, 60 s at 50°C, and 90 s at 72°C, was followed by a final primer extension step of 5 min at 72°C. PCR products were purified with a QIAquick PCR cleanup kit (Qiagen, Germany). Amplified products were separated by electrophoresis on agarose gels (1.5% agarose gel, including 0.5 µg ethidium bromide) to quantify the amplicon and to verify that the correct size products was produced. The purity and amount of PCR products were analyzed with 10 µl of the reaction mixture after agarose gel electrophoresis.

SSCP analysis

In order to obtain single-stranded DNA from PCR products, the phosphorylated strand was removed by lambda exonuclease digestion. For the digestion of the phosphorylated strand, 40 U of lambda exonuclease (New England Biolabs) was mixed with 10 µl of the resuspended PCR product in a total volume of 25 µl containing a final concentration of 1× lambda exonuclease buffer (New England Biolabs). The reaction mixtures were incubated at 37°C for 2 h, and then the volume made up to 100 µl with sterile double-distilled water. Proteins were removed by phenol-chloroform extraction (Orita et al. 1989). DNA was precipitated with ethanol and centrifuged (15 min at 27,000 × g), and finally single-stranded DNA was resuspended in 12.5 µl of TE, pH 7.6. Before electrophoretic analysis, 8 µl of denaturing loading buffer (95% formamide 10 mM NaOH, 0.25% bromophenol blue, 0.25% xylene cyanol) was added. Samples were incubated at 95°C for 2 min and immediately cooled in ice. After 5 min, samples were loaded onto the gels. The samples were electrophoresed in a 6% acrylamide-bisacrylamide (39:1) gel with 1 × TBE buffer. Gels were run at 300 V for 4 h at 20°C. The gels were silver-stained according to the procedure by Bassam et al. (1991).

Recovery of bands from SSCP gels and sequence analysis

Prominent SSCP bands were selected and used for excision and nucleotide sequence determination (Fig. 2). The elution of bands from the gel was done by a "crush and soak" method (Sambrook et al. 1989). The eluted DNA was used as the template for a PCR performed under the condition described above for environmental samples. Reamplified samples were sequenced by Big Dye Terminator (BDT) Chemistry version 3.0 of Applied Biosystems using Com1 and Com2 primers. The GenBank database searched using BLAST (NCBI) to determine sequence similarities. The Phylogenetic tree was constructed using Tree Top-phylogenetic Tree Prediction tool available at www.genebee.msu.su/services/phtree_reduced.htm/.

Nucleotide sequence accession numbers

Band sequences determined in this study have been deposited in the NCBI database under accession numbers DQ 322699 and DQ 322700; DQ 336196 to DQ 336202 and DQ 356474 to DQ 356479.

Results and discussion

Chemical parameters

The pre-monsoon season was chosen for a first survey as it represents the most stable conditions, with relatively uniform salinity within the network of mangrove creeks, and terrestrial (i.e., upland) inputs are expected to be minimal during this season. The variations in the dissolved and particulate nutrient pools in mangrove soils are regulated by several factors, such as tidal elevation, soil type, redox status, rate of plant and microbial growth, and bioturbation intensity (Steinke et al. 1990). Tables 1 and 2 illustrate the variations in environmental conditions and organic contents as well as bacterial populations in rhizosphere samples between sites. Bouillon et al (2004) have studied the variation in sediment organic matter across different mangrove sites in Indian subcontinent. The average of pH and EC values varied between 7.3–7.7 and 16.9–21.4 mS/cm, respectively, among sites. The carbon content (% dry weight basis) varied from 0.44% to 2.44%, being lowest in the sample taken at Pillumedu (0.15%) and highest at Guesthouse (2.44%). The averages of nitrogen (N) and phosphorus (P) contents ranged from 90 to 125 Kg/ha and from 7.5 to 12.5 Kg/ha, respectively. At Guesthouse, the rate of N-content was much more. Guesthouse soil showed high richness in organic and nitrogen contents since it is highly man-disturbed site compared with the other three sites and also high rate of decomposition in sandy soil (Kristensen et al. 2000). The potassium content (K) ranged from 863 to 1,575 Kg/ha, being lowest in the soil taken at Guesthouse (863 Kg/ha) highest at Kodiyampayalam (1,575 Kg/ha). The slight or lack of variation in organic and nutrient contents between Tandavaraycholarpet and Pillumedu indicates that these nutrients were from similar input sources and the microbial contribution in litter decomposition in that area (Davis et al. 2003). Concentrations of soil organic matter, total and extractable nitrogen, phosphorus and potassium were high in landward sites while pH and salinity (3–27 ppt) increased with distance from landward to seaward sites.

Bacterial population

Total heterotrophic bacterial (THB) density varied 3.1×10^4 to 2.8×10^5 between sites. The variation in number of THB in rhizosphere samples within and between sites was noted as very less. There was less variation in Tandavaraycholarpet (1.2×10^5 to 2.3×10^5), Pillumedu (1.0×10^5 to 2.8×10^5) and Kodiumpalayam (3.1×10^4 to 4.2×10^4). Slightly high variation was noted in Guesthouse (4.7×10^4 to 2.2×10^5). The population of diazotrophs varied from 1.7×10^1 to 1.8×10^3 between sites. The diazotroph population was noted to be similar between sites and within a site. Phosphate solubilizers varied from 1.3×10^3 to 4.0×10^5 in their population between sites, being highest in Rhizosphere samples of Avicennia marina taken in Pillumedu site (4.0×10^5) and lowest in samples collected from Porteresia coarctata samples (1.3×10^3) in Tandavaraycholarpet. There was much less variation in the number of phosphate solubilizers within Tandavaraycholarpet (1.0×10^3 to 6.9×10^3) and Guesthouse (6.6×10^3 to 8.1×10^3) compared to Kodiyampalayam (4.1×10^3 to 1.0×10^4) and Pillumedu (6×10^3 to 4×10^5)

According to Holmer et al. (2001) the variation in bacterial population density depends mainly on degradation rate, which relies upon species-specific and initial chemical composition of leaves, particularly the C:N ratio. *Avicennia* species (Lacerda et al. 1995) decompose at a faster rate than *Rhizophora* and *Sonneratia* species (Mongias and Ganeshamurthy 1989). Moreover, the smaller difference in the THB population between different rhizospheric samples within sites suggests that the rate of leaf breakdown had no significant difference in mixed species litter (Wardle et al. 1997). The highest THB population in rhizospheres of *Rhizophora* (tannin-rich species) than other mangrove species indicates that there is a negative correlation with tannin levels and microbial counts (Kathiresan et al. 1998). Moreover, according to Woitchik et al. (1997) the rate of decomposition and nitrogen enrichment will be higher in *Rhizosphora* during dry season. The abundance of phosphate solubilizers in all samples from all sites indicate the efficient nutrient cycling in this forest ecosystem.

Table 1 Chemical analysis of the soil samples

S. No	Sample sites	Total organic content (%)	pH*	E.C mS/cm	Available Nutrients (Kg/ha)		
					N	P*	K
1	Guesthouse	2.44[a]	7.3	17.5[b]	125	7.5	863[c]
2	Kodiyampalayam	1.32[b]	7.5	16.9[b]	90	12.5	1575[a]
3	Tandavaraycholarpet	0.44[c]	7.5	21.4[a]	95	7.5	1438[b]
4	Pillumedu	0.15[c]	7.7	19.2[b]	95	7.5	1463[b]

* Values are not significantly different

The values are means of three replicates. Values with same letters in a column do not differ significantly at $P = 0.05$

Table 2 Sequence analysis of bands excised from SSCP gels bacterial 16S rRNA genes extracted from five different rhizosphere samples from Pichavaram mangrove forest

Band (s)[a]	Most closely related bacterial sequence[b]	Identity	Accession no.	References
A	*Planococcus* sp.	99%	AY582938	Li (Unpublished)
	Uncultured Bacilli bacterium	98%	AY607217	Lueders *et al.* (2004)
B	Unidentified Hailaer soda lake bacterium	97%	AF275705	Mao et al. (Unpublished)
	Bacillus aquimaris	96%	AY505499	Schneegurt et al. (Unpublished)
C	*Planomicrobium chinense*	99%	AJ697862	Dai et al. (2005)
	Uncultured low G+C Gram-positive bacterium	98%	AY642546	Lopez-Garcia et al. (2005)
D	Uncultured bacterium	97%	DQ12944	Piceno et al. Unpublished
	Bacillus thuringiensis	96%	DQ328627	Gao et al. (Unpublished)
E	*Planococcus maritimus*	94%	AF500007	Yoon et al. (2003)
	Pseudomonas sp.	94%	AJ842253	Li (Unpublished)
F	Bacillus licheniformis	96%	DQ144421	Srivastava, et al. Unpublished
	Uncultured soil bacterium	96%	AF423304	Valinski et al. (2002)
G	*Bacillus catenulatus*	95%	AY523411	Wang et al. Unpublished
	Bacillus subtilis	95%	DQ420172	Neilan et al. Unpublished
H	Uncultured low G+C Gram-positive bacterium	99%	AY642551	Lopez-Garcia et al. (2005)
	Uncultured bacterium	98%	DQ088812	Onstott et al. (Unpublished)
I	*Bacillus pumilus*	97%	AY462205	Joe Unpublished
	Bacillus sp.	97%	AJ831842	Suresh and Shivaji (Unpublished)
J	*Planomicrobium okeanokoites*	100%	AY730709	Millar et al.(Unpublished)
	Uncultured Bacillaceae bacterium	99%	AY387381	Harry et al.(Unpublished)
K	*Bacillus* sp.	97%	DQ305300	Li and Yang Unpublished
	Uncultured Bacillaceae bacterium	95%	AM159318	Conrad et al.(Unpublished)
L	Bacillus cohnii	99%	AF140014	Venkateswaran and Nealson (Unpublished)
	Uncultured soil bacterium	98%	AF423258	Valinski et al. (2002)
M	Planococcus citreus	98%	AF500008	Yoon et al. (2003)
	Uncultured low G+C Gram-positive bacterium	97%	AY642551	Lopez-Garcia et al. (2005)
N	Uncultured Bacilli bacterium	97%	AY607212	Lueders et al. (2004)
	Uncultured Bacilli bacterium	96%	AY360610	Lueders et al. (2004)
O	*Bacillus* sp.	98%	AF414443	Oppong et al. (2003)
	Uncultured bacterium	97%	AM085483	Zhao et al. (Unpublished)

[a] the lowercase letters indicate bands derived from 16S rRNA genes

[b] The database entry with the highest level of identity is shown. When the most similar sequence was the sequence of an unidentified bacterium or environmental clone, the value for the most closely related identified bacterium is also given

SSCP profile

The heterogeneity of PCR products amplified from community DNA with eubacterial primers spanning the V4 and V5 regions of 16S rRNA genes was analysed by the SSCP method. The SSCP patterns of the rhizosphere samples consisted of 18–29 distinguishable bands of different intensities (De Troch and Vanderleyden 1996). The SSCP pattern showed greater spatial variations between sites among the same rhizosphere samples (Fig. 1). *P. coarctata* rhizosphere samples collected in short distance within Tandavaraycholarpet showed no difference in the SSCP banding pattern. Likewise, *R. mucornata* rhizosphere samples collected from Pillumedu showed similar pattern. *S. apetala* samples collected in distance within Kodiyampalayam showed significant differences in the SSCP profile. Rhizosphere samples of *A. marina*, *R. mucornata* and *E. agallocha* showed entirely different profiles between sites. There could be many reasons for the lack of variation within site and significant variation between sites among same rhizosphere samples. The five mangrove species in the study possess various types of root systems (Cragg 1983) and the soil type is not the same between sites. These two characters play an important role in the adaptation of bacterial communities with root systems (Kuske et al. 2002). In all SSCP profiles the presence of a number of dominant bands suggest that these major populations are better adapted to their conditions (Zhou et al. 2002), in which it is dominant.

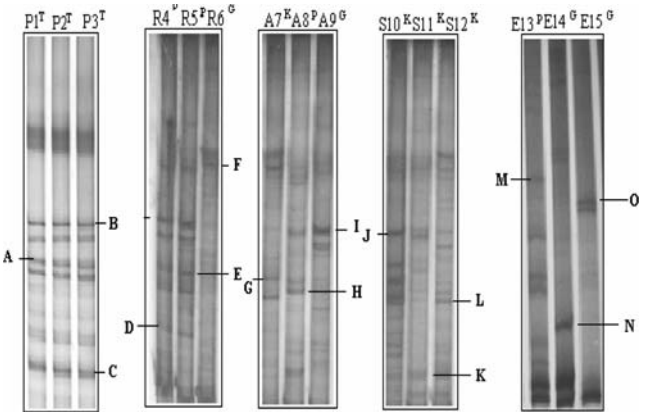

Fig. 1 SSCP profiles produced from PCR-amplified *16S rRNA* gene of rhizosphere samples collected from four different sites in Pichavaram mangrove forest Host plants taken: *Porterseia coarctata*-P, *Rhizophora mucornata*-R, *Avicennia marina*-A, *Sonnerratia apetla*-S *Excoecaria agallocha*-E Samples were taken from Tandavaraycholarpet-^{T,} Kodiyampalayam-^{K,} Pillmedu-^{P}, Guest house-^{G} These locations were present in mangrove region and they vary in 1 Km distance from each other. Arrows indicates the major bands excised for sequencing

To gain insight into the identities of major bacterial populations, prominent SSCP bands from different rhizosphere profiles were excised and nucleotide sequence analysis was carried out (Fig. 2). Sequencing of SSCP bands revealed that the majority of the dominant populations detected had 16S rRNA gene sequences that were most closely related to those of previously described marine and soil bacteria (i.e., *Planococcus* and *Bacillus*) and unidentified bacteria detected as environmental clones or bacteria. The highest level of identity between a SSCP band and a previously defined sequence was the level of identity observed for band J (Table 2). This band showed 100% identity to a sequence recovered from coastal sediment (AY730709) (Engelhardt et al. 2001) and 99% identity to a previously isolated uncultured Bacillaceae bacterium (AY387381). The band sequences that were least similar to previously recovered sequences were the sequences obtained from bands A, B, C, D, F, G, H, I, K, L, M, N, and O, which showed 95–99% identity to the most closely related database sequences. The sequence obtained from band E had the lowest level of identity with sequences of cultured bacterial species.

Phylogenetic analysis of these 16S rRNA gene sequences revealed that they displayed close relationships to a wide range of clones or bacterial species of phylum firmicutes and unidentified bacteria (Fig. 2). In the sequence analyses, genera *Planococcus* and *Bacillus* prevailed in all five rhizosphere samples and unidentified bacteria were also noted among them. Moreover, the bacterial populations of these two genera existed in all the different rhizosphere soils in different sites. The majority of band sequences appeared to cluster in bacterial taxonomic groups, which are generally found in seawater or sediments. In most analyses of 16S rRNA gene sequences from coastal region, members belonging to the phylum Firmicutes dominated the sequence collection (Dai et al. 2005). It was remarkable that a number of sequences, e.g., those belonging to the gamma proteobacteria, resemble sequences previously found in rice rhizosphere samples (AM159318). However, others have also detected sequences affiliated with isolates or clones from water and extreme environments, such as hydrothermal vents (Lueders et al. 2004) and paper mill slimes (Oppong et al. 2003). These sequence results of major bands in the SSCP

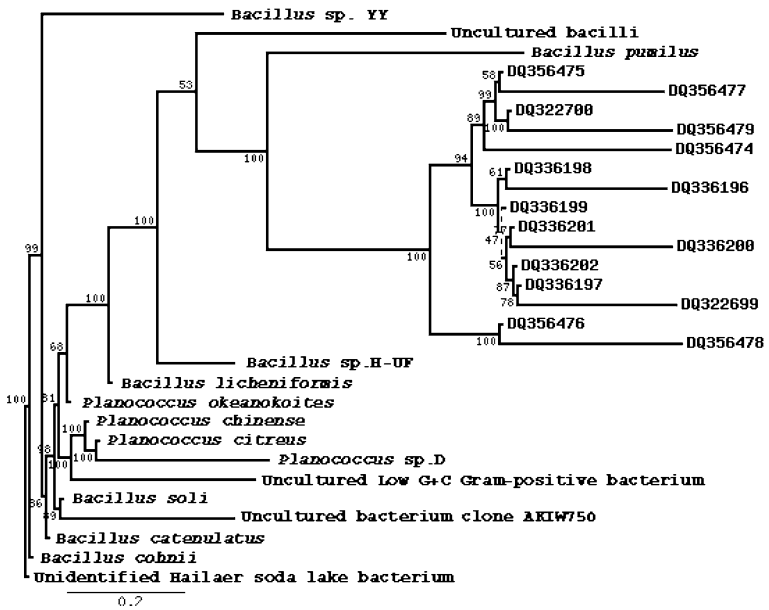

Fig. 2 Phylogenetic tree representing the relationships of the predominant band *16S rRNA* gene sequences from five different mangrove rhizosphere samples to various closely related clone and isolate*. *Sequences obtained from Blast searches (band sequences detected in this study are given in their accession number with plant and site name)

showed only a narrow taxonomic group of bacteria in the different rhizosphere samples and sites during pre-monsoon. The results of the bacterial diversity analysis indicate that the rhizosphere soil of the different mangrove species in various sites contained dominant bacterial species, which is characteristic of disturbed or harsh environments (Atlas et al. 1991).

Conclusions

The microbial diversity analysis gives better understanding on the microbial structure of specific habitats such as the mangrove ecosystem. The dominant bacteria obtained in the study could be explored to use as plant growth-promoting bacteria (PGPB). As mangroves are being deforested on an alarming scale, it is imperative to consider strategies for their preservation and reforestation. Especially in semi-arid regions of the world where mangroves do not efficiently reforest themselves, artificial reforestation might be a possible solution. As a starting point, it is assumed that mangrove seedlings might benefit from artificial inoculation with PGPB as do many other plant species (Bashan 1998). As possible beneficial features of the native microflora of semi-arid mangroves are unknown, we have proposed, as a first step, establishing the structure of bacterial population associated with mangrove rhizospheres via SSCP technique. This kind of study has scarcely begun and therefore we believe that this work will contribute to the current status of knowledge in the area.

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