Molecular Profiling of Rhizosphere Bacterial Communities Associated with *Prosopis juliflora* and *Parthenium hysterophorus*

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*Prosopis juliflora* and *Parthenium hysterophorus* are the two arid, exotic weeds of India that are characterized by distinct, profuse growth even in nutritionally poor soils and environmentally stressed conditions. Owing to the exceptional growth nature of these two plants, they are believed to harbor some novel bacterial communities with wide adaptability in their rhizosphere. Hence, in the present study, the bacterial communities associated with the rhizosphere of *Prosopis* and *Parthenium* were characterized by clonal 16S rRNA gene sequence analysis. The culturable microbial counts in the rhizosphere of these two plants were higher than bulk soils, possibly influenced by the root exudates of these two plants. The phylogenetic analysis of V1_V2 domains of the 16S rRNA gene indicated a wider range of bacterial communities present in the rhizosphere of these two plants than in bulk soils and the predominant genera included Acidobacteria, Gammaproteobacteria, and Bacteriodetes in the rhizosphere of *Prosopis*, and Acidobacteria, Betaproteobacteria, and Nitrospiriae in the *Parthenium* rhizosphere. The diversity of bacterial communities was more pronounced in the *Parthenium* rhizosphere than in the *Prosopis* rhizosphere. This culture-independent bacterial analysis offered extensive possibilities of unraveling novel microbes in the rhizospheres of *Prosopis* and *Parthenium* with genes for diverse functions, which could be exploited for nutrient transformation and stress tolerance in cultivated crops.

**Keywords:** 16S rRNA gene, metagenomic DNA, *Prosopis*, *Parthenium*, rhizosphere bacterial community

The microbial communities in rhizosphere soil are functionally more active and structurally more complex than non-rhizosphere (bulk) soil [27]. The rhizosphere constitutes a chemically complex niche that may be exploited by a wide variety of bacteria that in turn exert many beneficial effects including nutrient transformations, plant growth promotions, disease suppression, and tolerance to abiotic stresses like drought and salinity [30]. The variability in root structure and functioning among the plant species cause the spatial and temporal heterogeneities in a microbial community and resource exchange [11]. The rhizosphere microbial and faunal communities have very diverse membership, including symbiotic and saprotrophic bacteria and fungi, grazing protozoa, nematodes, mites, enchytraeid worms, etc. [7].

*Prosopis juliflora*, an evergreen woody plant introduced from South America that can grow in most of the Indian agroclimatic conditions and withstand severe stress condition, is useful for afforestation of wastelands especially affected with salinity [5]. Ragweed (*Parthenium hysterophorus*) is an aggressive herbaceous weed of the Asteraceae with an almost worldwide occurrence [46]. In India, it is spread over all parts of the country including the Himalayas and causes severe health hazards to human and animals and reduces agricultural productivity. It has very high adaptability and can grow in wastelands and soilless hard surfaces [38]. Bioprospecting the rhizosphere of those plants growing under extreme ecological conditions, like the salt marsh of Atlantic coasts [1, 2] or the desert ecosystem [9], is a unique approach to find beneficial microorganisms with high ecological adaptability. As these plants survive extreme variations in temperature and moisture and grow well in nutritionally poor soils with low organic contents, it is believed that microorganisms living in association with the plants should possess adaptive mechanisms to manage with frequent stress conditions [9]. Hence, it is hypothesized that the rhizosphere of these two plants should also harbor some novel bacterial communities in their rhizosphere with adaptability to severe drought, high salinity, and nutritionally poor conditions. In our earlier study, we have demonstrated the diversity potential of rhizosphere soils of...
Prosopis and Parthenium in terms of diazotrophic bacterial communities [10].

The diversity of soil microorganisms has been exploited for many years based on the cultivation and isolation of microbial species in general or specific nutritional media. It has been estimated that 0.1–1% of the total soil population can be cultured by applying these techniques, and the vast majority of genetic diversity present in this population is still unexplored owing to difficulties in enriching and isolating these microorganisms [43]. These unknown microorganisms represent an untapped reservoir of novel species and strains [37]. Hence, culture-based methodology is inadequate to serve the needs of microbial ecologists seeking to describe the diversity of bacterial communities in environmental samples [26]. Alternatively, culture-independent molecular techniques provide relatively new avenues for studying bacterial communities of soil in general as well as specific functional groups [14, 22, 24]. For studying microbial communities of soil, the marker of choice is highly conserved ribosomal genes (16S rRNA gene) [15], and the community profile of an ecosystem can be studied either by sequence polymorphisms and phylogenetic diversity using clonal libraries [47] or by fingerprinting methods such as denatured gradient gel electrophoresis [33], terminal restriction fragment length polymorphism [32], and length heterogeneity of hypervariable regions of 16S rRNA gene [44].

As continuation of our earlier investigation on rhizospheres of Prosopis and Parthenium [10], the present investigation was mainly focused to identify the rhizosphere bacterial community profile as revealed by the 16S rRNA gene sequences of these two plants by a culture-independent metagenomic approach. No reports are available so far in this regard, and this is the first molecular study of bacterial community structure and diversity in Prosopis juliflora and Parthenium hysterophorus by 16S rRNA gene sequence analysis.

### Materials and Methods

#### Soil Sampling

Three replicated samples each from rhizosphere and bulk (non-rhizosphere) soils of both Prosopis juliflora and Parthenium hysterophorus plants were collected from wastelands near the Wetland Experimental Station of Tamil Nadu Agricultural University, Coimbatore, India (11°16' N; 76°58' E) during August 2010. In the case of Prosopis, the active roots at a depth of about 50 cm were collected at four locations of the same tree, and soil adhering to the roots were carefully collected in sterile polythene bags and pooled for each sample. The Parthenium plants were uprooted and rhizosphere soil samples were collected. The non-rhizosphere (bulk) soils were collected away from the root systems and not influenced by the roots of the plants in the same location at a depth of 0–30 cm. The microbiological analysis of the soil samples was carried out on the same day of sampling to minimize the storage effects. After removing stones and stubbles, the powdered soils were packed in water-tight plastic bags and stored at −20°C for physico-chemical and metagenomic analyses. The soil pH, electrical conductivity (EC), and total macro- and micronutrients including nitrogen, phosphorus, potassium, copper, manganese, iron, and zinc were analyzed by following standard procedures [4]. The number of culturable bacteria, total fungi, and diazotrophs were quantified using the dilution plating, agar media, and incubation times and temperatures described by Weaver et al. [48].

#### DNA Extraction and Quantification

DNA was extracted from each soil sample using the Fast-DNA Spin Kit for Soil (Qiogene, Irvine, CA, USA) from 500 mg of soil, following the manufacturer’s specifications. High-molecular-weight DNA was quantified using a spectrophotometer (Nonodrop ND1000; Thermo Scientific, USA) and diluted to 20 ng/µl working stock.

#### PCR Amplification of Hypervariable Domains of 16S rRNA Gene

Hypervariable domains of the 16S rRNA gene (V1_V2) were amplified for each sample using universal bacterial primers, 27F (5′-AGA GTT TGA TCM TGG CTC AG-3′) [44] and 35R (5′-GCT GCC TCC CGT AGG AGF-3′) [12]. The PCR reaction was performed using 1× PCR buffer, 2.5 mM MgCl₂, 250 µM dNTPs, 0.5 µm DNA polymerase (Fermentas, USA), 1 µM each of forward and reverse primers (Chromous Biotech, Bangalore, India), 1.0% bovine serum albumin fraction V (Fisher Scientific), 25 ng of metagenomic DNA, and diethylpyrocarbonate-treated water to a final volume of 40 µl. Amplification was performed using a thermocycler (Eppendorf, Germany) with the following parameters: initial denaturation at 95°C for 10 min; 35 cycles of denaturation at 94°C, annealing at 55°C, and extension at 72°C (each for 1 min); and a final elongation at 72°C for 10 min.

16S rRNA Gene Clonal Library Construction and ARDRA

PCR products were purified using a GenElute PCR clean up kit (Sigma-Aldrich Co., Germany) and the purified products were cloned into a T/A cloning vector, pTZ57R (Fermentas, USA), according to manufacturer’s protocol. The cloned vectors were transformed to E. coli DH5α by using the chemical transformation method [41]. The metagenomic clonal libraries for each plant from rhizosphere- and non-rhizosphere (bulk) soils were stored in 300 µl volume 96-well microtiter plates with 20% glycerol at −80°C for further analysis. All the clones from each library were subjected to colony PCR by using M13F and M13R primers (Fermentas, USA), and the amplified ribosomal DNA restriction analysis (ARDRA) of recombinants containing the expected insert size (500 bp) was carried out employing HaeIII enzyme (Fermentas, USA), followed by 2% (w/v) agarose gel electrophoresis. Clones showing unique ARDRA patterns were selected and sequenced using an ABI prism terminator cycle sequencing ready reaction kit, and electrophoresis of the products was carried out on an Applied Biosystems (Model 3100) automated sequencer (Bangalore-Genei, Bangalore, India).

#### Phylogenetic Analysis

The identity of 16S rRNA gene sequences was performed by similarity search using the BLAST tool (http://blast.ncbi.nlm.nih.gov/BLAST.cgi). The closest species, strain, and per cent similarity to the isolates were obtained from the BLAST result. The phylogenetic tree was constructed with existing 16S rRNA gene sequences from different bacteria, obtained from the NCBI GenBank database. The phylogenetic
The rhizosphere of bulk soil. Among the two, *Prosopis* harbored more number of culturable heterotrophic bacteria, fungi, and diazotrophic bacteria than *Parthenium*.

### Bacterial Community Composition of *Prosopis* Rhizosphere as Revealed by 16S rRNA Clonal Library from Metagenomic DNA

A total of 96 and 98 ribosomal RNA gene clones from clonal libraries of the *Prosopis* rhizosphere and bulk soil, respectively, were analyzed for distribution of different bacterial communities. The ARDRA profiling was done for both the samples to identify tentative clustering of different bacterial groups. Unique banding patterns were scored, and similarities between the clones of each clonal library were used for grouping, and from each group two or three representative clones were sequenced (data not shown).

The overview of phylogenetic grouping of both clonal library sequences was summarized as Fig. 1. The rhizosphere of *Prosopis* was dominated by Acidobacteria (43%), followed by Alphaproteobacteria (33%) and Bacteriodetes (9%), in contrast to bulk soil in which Alphaproteobacteria (25%) and Actinobacteria (38%) were predominant along with Acidobacteria (36%). The Actinobacteria level was drastically reduced in rhizosphere than in bulk soils. The neighbor-joining tree of 16S rRNA sequences obtained from both rhizospheres of *Prosopis* also confirmed that the rhizosphere bacterial communities were distinct and diversified (Fig. 2).

### Bacterial Community Composition of *Parthenium* Rhizosphere as Revealed by 16S rRNA Clonal Library from Metagenomic DNA

The 16S rRNA gene clones from the rhizosphere and bulk soils of *Parthenium* as clonal libraries were used for bacterial community analysis. A total of 136 and 128

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**Table 1.** Physico-chemical and microbiological properties of the soil samples collected for this study.

<table>
<thead>
<tr>
<th>Soil properties</th>
<th><em>Prosopis</em> Bulk</th>
<th><em>Prosopis</em> Rhizosphere</th>
<th><em>Parthenium</em> Bulk</th>
<th><em>Parthenium</em> Rhizosphere</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.46 (± 0.02)</td>
<td>8.19 (± 0.02)</td>
<td>8.44 (± 0.10)</td>
<td>8.08 (± 0.13)</td>
</tr>
<tr>
<td>EC (dS/m)</td>
<td>0.15 (± 0.003)</td>
<td>0.24 (± 0.002)</td>
<td>0.12 (± 0.001)</td>
<td>0.57 (± 0.003)</td>
</tr>
<tr>
<td>Organic C (mg/kg)</td>
<td>0.38 (± 0.09)</td>
<td>0.41 (± 0.10)</td>
<td>0.41 (± 0.08)</td>
<td>0.47 (± 0.07)</td>
</tr>
<tr>
<td>Available N (kg/ha)</td>
<td>176 (± 12)</td>
<td>311 (± 15)</td>
<td>184 (± 15)</td>
<td>255 (± 11)</td>
</tr>
<tr>
<td>Available P (kg/ha)</td>
<td>14.30 (± 2.15)</td>
<td>26.7 (± 2.79)</td>
<td>17.3 (± 3.14)</td>
<td>29.2 (± 2.11)</td>
</tr>
<tr>
<td>Available K (kg/ha)</td>
<td>201 (± 20)</td>
<td>257 (± 22)</td>
<td>205 (± 16)</td>
<td>292 (± 22)</td>
</tr>
<tr>
<td>Copper (mg/kg)</td>
<td>1.42 (± 0.02)</td>
<td>1.48 (± 0.02)</td>
<td>1.45 (± 0.01)</td>
<td>2.38 (± 0.02)</td>
</tr>
<tr>
<td>Manganese (mg/kg)</td>
<td>14.2 (± 1.12)</td>
<td>74.3 (± 1.17)</td>
<td>15.4 (± 3.15)</td>
<td>17.7 (1.94)</td>
</tr>
<tr>
<td>Iron (mg/kg)</td>
<td>4.10 (± 0.78)</td>
<td>5.56 (± 0.55)</td>
<td>4.14 (± 1.15)</td>
<td>7.68 (± 1.89)</td>
</tr>
<tr>
<td>Zinc (mg/kg)</td>
<td>0.60 (± 0.09)</td>
<td>3.98 (± 0.19)</td>
<td>0.58 (± 0.07)</td>
<td>1.54 (± 0.10)</td>
</tr>
<tr>
<td>Total bacteria (× 10⁷ CFU/g dw soil)</td>
<td>73 (± 8.5)</td>
<td>484 (± 34.0)</td>
<td>62 (± 5.52)</td>
<td>333 (± 22.55)</td>
</tr>
<tr>
<td>Total fungi (× 10⁷ CFU/g dw soil)</td>
<td>31 (± 6.5)</td>
<td>54 (± 2.20)</td>
<td>34 (± 8.15)</td>
<td>49 (± 3.80)</td>
</tr>
<tr>
<td>Total diazotrophs (× 10⁷ CFU/g dw soil)</td>
<td>18 (± 2.5)</td>
<td>68 (± 9.1)</td>
<td>61 (± 7.25)</td>
<td>13 (± 2.80)</td>
</tr>
</tbody>
</table>

Values are mean (±SE) (n=6); Values in each row followed by different letters are significantly different at p < 0.05, according to Duncan’s Multiple Range Test.
clones from rhizosphere and bulk soils were analyzed by ARDRA and sequencing. The detailed phylogenetic tree derived from 16S rRNA gene sequences of this study were closely related to different phylogenetic groups using the neighbor-joining method (Fig. 3). The results revealed that the rhizosphere of *Parthenium* harbor more diversified bacterial communities than bulk soil as well as the *Prosopis* rhizosphere. In the *Parthenium* rhizosphere, nearly 30% each of the ribotypes were of Alphaproteobacteria and Acidobacteria, followed by Nitrospirae (21%) and Betaproteobacteria (12%). Lower percentages of Gammaproteobacteria (5%) and Bacteriodetes (5%) were reported in the rhizosphere of *Parthenium* (Fig. 1). As expected, the bacterial community in bulk soil was similar to that of bulk soil of *Prosopis*, comprising Alpha and Betaproteobacteria, with a very low level of Acidobacteria.

**In Silico Analysis of 16S rRNA Clone Sequences**

To distinguish and to identify the novel bacterial species present in both plants’ rhizospheres, *in silico* analysis of cloned sequences was performed using BLAST search of the NCBI GenBank followed by the PubMed database. For this, the species and its phylogenetic affiliation along with the ecological significance were collected and presented as Table 2. The sequences of the *Parthenium* rhizosphere were close to some of the unique bacterial species, like *Lysobacter*, an antagonistic bacteria isolated from coastal sand dune plant species; *Chaetococcus*, a bug used for textile-dye bioremediation, isolated from wastewater collected from a textile-dye works in Korea; and members of oxalic acid utilizing (Oxalobacteraceae) and phenol-degrading *Novospringobium* from Taihu Lake of China. Likewise, some sequences of the *Prosopis* rhizosphere were close to the multidrug-resistant bacterial species *Inquilinus* from clinical samples and polyphosphate-accumulating Gemmatimonadetes from wastewater (Table 2). The unique and rare species occurrence was more in *Parthenium* than in *Prosopis*.

**DISCUSSION**

In the present study, we have used culture-independent ribosomal gene sequencing to analyze the bacterial community in the rhizosphere soil of *Prosopis juliflora* and *Parthenium hysterophorus*, which grew well under severe drought- and nutritionally poor environments. The concept behind this study is that there may be some novel soil bacterial communities that interact with the roots of these plants with wider ecological adaptability, and identifying those groups would pave the way for bioprospecting of beneficial bacteria for agricultural applications. The soils collected from rhizosphere and non-rhizosphere (bulk) soils of *Prosopis juliflora* and *Parthenium hysterophorus* were characterized for physical and chemical attributes in order to assess the rhizosphere influence. The results revealed that there is a significant impact of the rhizosphere of these two plants on soil physico-chemical properties. The increased level of macro- and micronutrients such as manganese, iron, and zinc in the rhizosphere of these plants over that in the bulk soil was attributed to the enhanced microbial activities in the root region, as documented by Singh et al. [42]. This was further authenticated by increased levels of total culturable bacteria, fungi, and diazotrophs in the present study. In our previous study, we have also documented that rhizospheres of these two plants were colonized by more diversified diazotrophs than in bulk soil [10]. The increased nutrient availability through root exudates and soil microbe-root symbiotic relationships are thought to be the responsible factors, and this is supported by soil analysis showing increased nutrient contents in the rhizosphere soil compared with non-rhizosphere soils [6]. The microhabitats and spatial separation provided by the roots, which limit competition and equitable microbial population [51], are the possible reasons for enhanced microbial counts in the

![Fig. 1. 16S rRNA gene sequence types affiliated with individual phylogenetic groups present in rhizosphere and bulk soils of *Prosopis* and *Parthenium*.](image-url)

The grouping was done based on the BLAST analysis of 16S rRNA sequences. The values on the top of the bars give the number of clones analyzed in the respective clonal library.
Fig 2. Neighbor-joining phylogenetic tree based on the V1_V2 hypervariable domain of the 16S rRNA gene of clones from rhizosphere soil of *Prosopis* showing highest similarity with bacteria from different phyla. The percentages of 1,000 bootstrap replicates are shown at the left nodes when at least 50%. The scale bar indicates five changes per 100 bp nucleotides.
Fig 3. Neighbor-joining phylogenetic tree based on the V1-V2 hypervariable domain of the 16S rRNA gene of clones from rhizosphere soil of Parthenium showing highest similarity with bacteria from different phyla. The percentages of 1,000 bootstrap replicates are shown at the left nodes when at least 50%. The scale bar indicates two changes per 100 bp nucleotides.
The increased microbial and nutritional properties in the *Prosopis* rhizosphere compared with the *Parthenium* rhizosphere may be due to metabolites diversity in the root exudates of each plant rhizosphere, which significantly differentiated the microbial activity [27].

Recent molecular techniques clearly revealed that different plants support different bacterial, fungal, and archaeal communities [20, 31, 35]. Several investigations are in progress to analyze the rhizosphere of plant species that are grown in relatively stressed conditions in terms of nutrients and environments [1, 9, 23, 34]. *Spartina alterniflora* collected from a salt marsh of the Atlantic coast was also reported to have more diversified bacterial communities in the rhizosphere [2]. The grass plant *Lasiusurus sindicus*, collected from Thar desert of India, harbors more than 121 ribotypes, comprising 8 different phyla of bacteria [9]. Similar observations were made in *Taxus* species rhizosphere, collected from a naturally grown temperate forest region of China that also revealed that the 16S rRNA gene phylotype distribution was significantly changed owing to the rhizosphere effects of that plant compared with the non-rhizosphere bulk soil [23]. In the present study, as reported by earlier workers, the rhizospheres of *Parthenium hysterophorus* and *Prosopis juliflora* were colonized by more diversified bacterial communities than their respective bulk soils.

Acidobacteria are the most represented phylum commonly found in both rhizosphere and bulk soils of *Parthenium* and *Prosopis*. Acidobacteria are observed and identified frequently using molecular surveys based on 16S rRNA gene sequences in community DNAs [13, 39]. Lee et al. [29] found that their proportion is more than 50% of PCR-derived clone libraries in the rhizosphere of chestnut. In the present study, we have also observed the increased proportion of Acidobacteria in rhizospheres of *Prosopis* and *Parthenium* relative to bulk soils. Considering their phylogenetic diversity and ecological distribution pattern, the Acidobacteria are a more metabolically and genetically diverse group than Proteobacteria [25]. The proportion of Acidobacteria to Proteobacteria, observed in the present study, is also in conformity with earlier reports [1, 9]. The pH of rhizosphere and bulk soils of these plants is saline, and predominance of Acidobacteria in the rhizosphere of these plants may help in the salinity tolerance of the plants owing to the inherent ability of acid production by these bacteria. However, this ubiquitous bacterial taxon is known as an uncultured bacterial group and its ecological function remains unknown.

The percentage distribution of bacterial phylotypes estimated for rhizosphere of plants grown naturally under stress conditions varies from plant to plant, as these surveys were made at geographically different locations. Interestingly, the *Taxus* rhizosphere comprises 34% of Acidobacteria, 31% of Gammaproteobacteria, 16% of Alphaproteobacteria, followed by 9% of Actinobacteria [23]. In contrast to this, *Lasiusurus sindicus*, grass from the Thar desert, harbors 43% of Actinobacteria, followed by 31% of Firmicutes and 9% and 6% of Beta- and Alphaproteobacterial sequences [9]. The high-arid and high-altitude grass plants rhizosphere community by DGGE indicated bacterial predominance in the order of Acidobacteria > Gammaproteobacteria > Alphaproteobacteria > Bacteriodetes in the case of *Prosopis*.

### Table 2. Affiliation of some uncommon bacterial sequences reported in rhizospheres of *Parthenium* and *Prosopis*.

<table>
<thead>
<tr>
<th>Sequence (Accession No.)</th>
<th>Affiliation</th>
<th>Rhizosphere</th>
<th>Source of closest sequencea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysobacter sp. (GQ301047)</td>
<td>Gammaproteobacteria</td>
<td><em>Parthenium</em></td>
<td>Antagonistic bacteria from coastal sand dune plant species [28]</td>
</tr>
<tr>
<td>bacteroidetes (GQ301056, 74, 77)</td>
<td>New Phylum: Bacteriodetes</td>
<td><em>Parthenium</em> and <em>Prosopis</em></td>
<td>Aquatic habitat [36]</td>
</tr>
<tr>
<td>Chaetococcus (GQ301053)</td>
<td>Alphaproteobacteria</td>
<td><em>Parthenium</em></td>
<td>Wastewater collected from a textile-dye works in Korea [49]</td>
</tr>
<tr>
<td>Oxalobacteraceae (GQ301055)</td>
<td>Betaproteobacteria</td>
<td><em>Parthenium</em></td>
<td>Oxalic acid utilizers [18]</td>
</tr>
<tr>
<td>Novosphingobium (GQ301057)</td>
<td>Alphaproteobacteria</td>
<td><em>Parthenium</em></td>
<td>Phenolic-compounds-degrading bacteria from Taihu Lake, China [19]</td>
</tr>
<tr>
<td>Inquilinus sp. (GQ301069)</td>
<td>Alphaproteobacteria</td>
<td><em>Prosopis</em></td>
<td>Clinical isolate with multidrug resistance [8]</td>
</tr>
<tr>
<td>Gemmatimonadetes (GQ301070)</td>
<td>New Phylum: Gemmatimonadetes</td>
<td><em>Prosopis</em></td>
<td>Polyphosphate-accumulating bacterial groups from wastewater [50]</td>
</tr>
</tbody>
</table>

*aReported based on best match (98%) during BLAST Search for 16S rRNA genes cloned and sequenced.*
and Alphaproteobacteria > Acidobacteria > Betaproteobacteria > Nitrospirae. Interestingly, no Firmicutes (Gram +ve rods) were reported in any of our clonal libraries. Hence, it is clearly evident that the rhizosphere bacterial community is unique for each plant, even when they grow in the same soil and environmental conditions [42]. In contrast to these reports, few studies have also indicated a large degree of bacterial homogeneity and decreased bacterial diversity compared with non-rhizosphere soils [16, 21, 26].

As reviewed by Singh et al. [42], the rhizosphere of any plant is a biologically active zone of soil, rich in organic carbon and other plant nutrients referred to as rhizodeposits, regulating the soil microbial community in the immediate vicinity of roots, thereby encouraging beneficial symbioses and protective associations, ensuring supply of vital nutrients and changing the chemical and physical properties of the soil [3]. Most of the sequences obtained in this study showed similarities with sequences from cultured or uncultured bacterial species from environmental samples associated with soil and plant roots. It is evident from our results that the plant root associated clones showed a greater diversity (Fig. 4) and represented sequences from α-, β-,γ-Proteobacteria, Bacteroidetes, Acidobacteria, and Actinobacteria (Fig. 1). Interestingly, the Actinobacteria, the antagonistic phylum, had a drastic decline in the rhizosphere compared with the bulk soil, and the phyla Nitrospirae and Gammatimonads were exclusively found in the rhizosphere of Parthenium and Prosopis, respectively. In silico analysis of some of the uncommon bacterial species reported in this study also revealed that the rhizosphere of the plants grew in extreme nutritional-poor environments that harbor functionally diversified microflora. For example, the rhizosphere of Parthenium has Lysobacter sp., which is known to be an antagonistic Gammaproteobacteria, from coastal sand dune plant species [28]; Chaetococcus, an Alphaproteobacteria known for bioremediation of textile dyes [49]; Oxalobacter (Alphaproteobacteria), an oxalic acid utilizing [18]; and Novosphingobium (Alphaproteobacteria), a phenol-degrading bacterium [19]. Likewise, Prosopis also harbors the uncommon bacteria such as Inquilinus species and Gemmatimonads responsible for multidrug resistance [8] and polyphosphate accumulation [50], respectively. Thus, it appears that the roots of Prosopis juliflora and Parthenium hysterophorus might offer a suitable microenvironment in their rhizosphere for the survival of more diversified bacteria than the bulk soil. The coverage of two libraries represented an approximate coverage of bacterial diversity, which is in agreement with similar studies [9, 17].

In the present investigation, we found that the rhizospheres of Prosopis and Parthenium harbor more diversified bacterial communities from different phyla than bulk soil. Our study clearly showed that the exotic plants growing under severe stress conditions and nutritionally poor environment were enriched with novel bacterial communities, and bioprospecting these microorganisms for agriculture and environment could be a practically viable approach. It would be very interesting to investigate the molecular understanding between these plants and microbes in rhizospheres for further exploitation.

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REFERENCES


