Proceedings of the Pakistan Academy of Sciences:
B. Life and Environmental Sciences 55 (2): 65–70 (2018)

ISSN: 2518-4261 (print), ISSN 2518-427X (online)

Pakistan Academy of Sciences

Research Article

Exploring the Rhizospheric Bacterial Communities of *Mangifera indica*

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Abstract: Rhizosphere soil plays an important role in providing environment conducive for growth of plants, therefore the knowledge about its living constituents is of paramount importance. The present study has partially determined the dynamics of bacterial communities in the rhizosphere soil of *Mangifera indica*, an indigenous fruit tree. Soil samples from the rhizosphere of *Mangifera indica* were collected from various locations and analysed for bacterial load and communities. The experiments were performed in two distinct phases under two different growth conditions such are aerobic and anaerobic. The data of aerobic phase of this study revealed presence of spore forming and non-spore forming aerobic bacterial species belonging to *Bacillus, Enterobacter, Pseudomonas, Proteus,* and *Serratia genera*, while the findings of anaerobic phase yielded members of genus *Lactobacilli* only. *Lactobacilli* occupied an average 17% of total anaerobic communities of bacteria in the rhizosphere of *Mangifera indica*, while aerobic bacilli occupied only 8.33% of total aerobic bacterial communities. Data indicated that the rhizosphere soil of *Mangifera indica* is rich in bacterial communities belonging to both aerobic and anaerobic groups. However, the load of bacterial isolates varied dramatically from sample to sample suggesting that in addition to type of plant other factors such as soil environment and nutrients may influence the bacterial communities in the rhizosphere soil of a plant.

Keywords: Rhizosphere, soil, Bacterial communities, *Mangifera indica.*

1. INTRODUCTION

Rhizosphere is a compartment where soil is under the direct influence of plants via their roots. The organic compounds such as amino acids, proteins, fatty acids and flavonoids are usually secreted by plant roots in its rhizosphere soil which in turn shape the colonization of microbial communities (fungal, archeal and bacterial) in this compartment. These microbial communities of rhizosphere are collectively called as rhizobiome [1]. The rhizobiome has critical role in the nutrient cycling by assisting the plant in the uptake of several vital nutrients, such as phosphorous, potassium and nitrogen from the soil. The type of plant and soil affects the community structure of the rhizobiome. In addition, various environmental factors including regional climate and pollutants have been reported to influence the structure and diversity of bacterial communities of the rhizobiome [2]. *Mangifera indica*, commonly called as mango belongs to genus *Mangifera,* of the flowering plant family Anacardiaceae. It has been produced in tropical Asia and Pakistan is its fifth largest producer in the world. It has also been exported from Pakistan to different regions globally.

The bacterial communities of the rhizobiome have been shown to play a vital role in development of a plant by production of molecular signals. These signals act as communication tools for interaction between plant roots and root bacteria. This interaction exerts considerable beneficial influence upon fitness and development of the plant [3]. One of the best examples of plant fitness by bacterial signals, is the production of iron-binding compounds (siderophores) by genus *Pseudomonas* which determines quality of the growth of plant and responsible for more yield of plant [4]. Moreover, Pseudomonas spp. may cause suppression of some plant diseases [5]. It is also well known that plants have significant influence on the diversity,

Received, April 2018; Accepted, June 2018
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spatial distribution and abundance of soil microbes through the rhizospheres. It has been suggested that measurement of the microbial community structure of soil indicates the status of a system and help in understanding the ecological process [6].

A previous study has shown that plant species and soil type significantly affect the structure of Pseudomonas and Bacillus communities of rhizobiome [7]. In this context, the root microbiome of *Arabidopsis thaliana* plant was found more diverse than the bulk soil indicating that a plant genotype determines the pattern of colonization of specific microbial community inhabiting in roots [8]. Therefore, a better understanding of the microbial ecology of the rhizosphere of a plant may allow discovering potentially useful secondary metabolites for exploiting them as antagonists of pathogens. The present study was devised to assess the dynamics of bacterial communities in the rhizobiome of indigenous *Mangifera indica* (mango tree) growing under the common conditions of agriculture practices.

2. MATERIAL AND METHODS

2.1 Sources of Samples and their Collection

Soil samples were collected from the rhizosphere of indigenous mango tree cultivated at seven distinct sites of Jamshoro, Sindh. Samples were collected in a sterile beaker or flask with sterile spoon and immediately covered with cotton plugs or aluminum foil to avoid environmental contamination. Rhizosphere soil adhered to the roots of plant was collected by hand shaking off the soil against the sides of beaker as described previously [9]. The samples were transferred immediately to the laboratory for microbiological analysis.

2.2 Isolation of Aerobic Bacteria from Rhizosphere Soil Samples

One gram of soil sample was mixed into 5ml of sterile nutrient broth (OXOID, England). The tubes were incubated overnight aerobically. Following the incubation (enrichment stage), ten folds serial dilutions in sterile nutrient broth were made [10]. The last 3 dilutions (10^{-2}, 10^{-3}, and 10^{-4}) were pipette out and poured onto nutrient agar (OXOID, England) and then incubated overnight at 37°C aerobically. After incubation, discrete, and morphologically distinct colonies were streaked onto fresh nutrient agar to obtain pure culture colonies.

2.3 Isolation of Anaerobic Bacteria from Rhizosphere Soil Samples

For isolation of anaerobic bacteria from rhizosphere soil samples de Man Rogosa and Sharpe (MRS) medium was used [11]. One gram of soil sample was mixed into 5ml of sterile MRS broth (OXOID, England) and incubated for 3 days anaerobically. Following the incubation (enrichment stage), ten folds serial dilutions was made using 0.85% NaCl solution. The last 3 dilutions (10^{-7}, 10^{-8}, and 10^{-9}) were pipette out and poured onto MRS agar (OXOID, England) plates supplemented with 1% $CaCo_3$, and then incubated for 3-5 days at 37°C anaerobically. After incubation, discrete colonies with a halo zone around them were streaked onto fresh MRS agar plates to obtain pure culture colonies.

2.4 Identification of Bacterial Isolates

The isolated bacteria were identified by conventional methods including Gram staining, colonial/cultural, microscopic and biochemical characteristics i.e. fermentation of lactose, H_2S production, ability to produce indole, citrate utilization, urease production, motility of organism and ability to produce cytochrome oxidase enzyme. Gram's staining was performed according to standardized method [12]. Spore staining was performed according to Schaeffer-Fulton method [13].

3. RESULTS

3.1 Bacterial Load in the Rhizosphere Soil of *Mangifera indica*

The bacterial Load analysis of culture-able aerobic and anaerobic bacteria of the rhizosphere soil samples was determined by counting colony forming units (CFU) (Table 01). Varied CFU count was observed from the samples, suggesting that the type of plant is not only factor influencing microbiome of plants; however other factors of soil also have influential role.

3.2 Bacterial Community Dynamics of the Rhizosphere of *Mangifera indica*

The data of Gram staining reaction performed on all bacterial isolates (n=60) of aerobically processed samples showed that proteobacteria were numerically dominant with average 90% of total bacteria. Some samples also yielded the growth of Firmicutes. In order to understand the diversity and community dynamics of bacteria, large creamy colonies grown anaerobically on MRS agar plates (n=100) from each of the samples were selected and subjected to microscopic observation using Gram's staining. It was found that 17% isolates in average were non spore forming Gram-positive bacilli. Whereas, colonies of aerobic bacteria showing distinct morphology on nutrient agar (n=60) were randomly selected for colonial characteristics (Table 02). The data demonstrated that they include Gram-positive bacilli, Gram-positive cocci, and Gram-negative bacilli (Fig 01). All aerobic and anaerobic isolates of this study were further identified on the basis of biochemical tests (Table 03). The results of biochemical characteristics of Gram-negative isolates (Table 04) showed that the bacterial communities of rhizosphere soil comprised mainly *Serratia* spp, *Enterobacter* spp, and *Pseudomonas* spp, *Acinetobacter* spp, and *Proteus* spp while Gram positive isolates included mainly *Bacillus* species (Table 05). The *Enterobacter* species were found dominant among the bacterial communities of rhizosphere soil of *Mangifera indica* plants. Furthermore, *Bacillus* isolates were capable of spore formation (Fig 02).

Table 1 Colony forming units (CFU) of bacteria grown on MRS agar and Nutrient agar from the rhizosphere soil samples **.**

Soil samples	CFU/g of soil on MRS agar (Anaerobic)[a]	Log10 of CFU/g of soil on MRS agar	CFU/g of soil on NA (Aerobic)[b]	Log10 of CFU/g of soil on NA
S1	9.5×10^7/ml	7.977	2.0×10^6/ml	6.301
S2	7.9×10^7/ml	7.897	6.8×10^6/ml	6.832
S3	2.9×10^6/ml	6.462	6.3×10^6/ml	6.799
S4	1.32×10^6/ml	6.120	3.4×10^6/ml	6.531
S5	1.24×10^6/ml	6.093	2.4×10^6/ml	6.380
S6	1.6×10^7/ml	7.204	9.0×10^6/ml	6.954
S7	2.0×10^7/ml	7.301	6.4×10^7/ml	7.806

[a] Plates incubated in anaerobic conditions
[b] Plates were incubated in aerobic conditions

Table 2 Colonial characteristics of aerobic bacterial isolates grown on Nutrient agar

Isolates Label	Colony morphology	Color	Elevation	Surface	Size
NA01- 06	Irregular	Yellowish	Flat	Rough	Large
NA07-19	Circular	Grayish white	Raised	Mucoid	Moderate
NA20- 25	Irregular	Yellow green translucent	Flat	Smooth	Large
NA26- 46	Circular	White creamy	Convex	Mucoid	Small-moderate
NA47- 51	Circular	White	Umbonate	Mucoid	Small
NA52- 60	Irregular	Opaque	Flat	Smooth	Large

Table 3 Biochemical characteristics of aerobic and anaerobic isolates of this study

Isolates	Gram's staining reaction	Catalase test	Oxidase test	Spore staining	Vancomycin sensitivity
MRS1-16	+	-	-	-	+
MRS17	+	+	-	-	+
NA1-5	+	+	-	+	-
NA06	+	+	+	-	-
NA07-19	-	+	-	-	-
NA20-25	-	+	+	-	-
NA26-51	-	+	-	-	-
NA52-60	-	+	+	-	-

Table 4 Biochemical characteristics of aerobic Gram-negative isolates

Isolates	TSI			Simmon's citrate	Urease	H_2S	Indole	Motility
	Butt	Slants	Gas					
Type 1 isolates	Acidic	Acidic	-	+	-	+	+	+
Type 2 isolates	Alkaline	Alkaline	+	+	-	-	-	+
Type 3 isolates	Acidic	Acidic	-	+	-	-	-	+
Type 4 isolates	Acidic	Alkaline	-	+	+	-		+
Type 5 isolates	Alkaline	Alkaline	-	+	-	-	-	+

Table 5 Types of aerobic bacteria isolated from the rhizosphere soil of *Mangifera indica*

Type of Bacteria	Occurrence (% of aerobic bacteria)
Bacillus species	8.33
Micrococcus species	1.66
Citrobacter species	21.66
Pseudomonas species	10
Enterobacter species	35
Serratia species	8.33
Proteus species	15

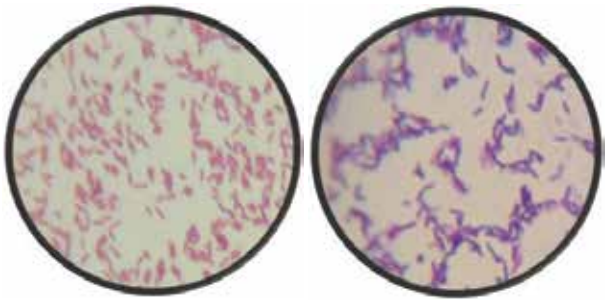

Fig. 1. Representative result of microscopic characteristics of aerobic Gram-negative (left) and Gram-positive (right) bacteria isolated from rhizosphere soil of *Mangifera indica* plant.

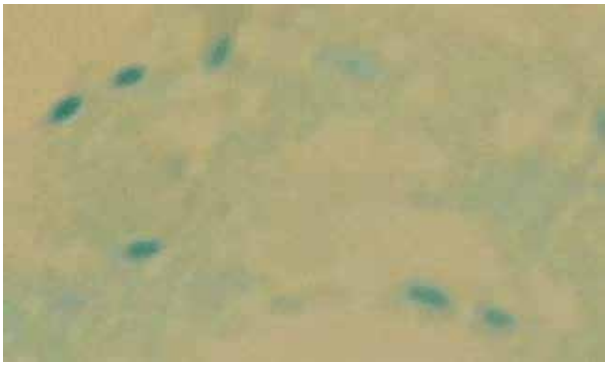

Fig. 2. Spore staining test result of aerobic *Bacillus* spp. isolated in this study.

Lactobacilli are LAB, because they produce lactic acid as an end product of fermentation of sugar on MRSA medium. Upon addition of $CaCO_3$ in media, lactate react with it and forms Ca-lactate as aresult a clear area/zone appears around the colonies of LAB. The appearance of a clear zone around the bacterial colony was considered as indicative of lactic acid production by the LAB isolate (Fig 03). Vancomycin sensitivity test has been suggested to differentiate between *Lactobacilli* species [14] since some *Lactobacilli* species (*L. rhamnosus*) are naturally resistant to vancomycin due to presence of D-Lac amino acid in place of D-ala in their cell wall structures. The present study has shown that majority of Lactobacilli isolates were sensitive to vancomycin (Fig 04) suggesting that the isolated strains were presumably *L. acidophilus* [14], and not *L. rhamnosus* which are naturally resistant to vancomycin [15].

4. DISCUSSION

Rhizosphere soil accommodates numerous bacterial communities due to its direct interaction with plant roots. Bacterial community structure of the rhizosphere soil of plants plays vital role in growth of the plant. However, the composition and quality of soil is debated. Since plant roots secrete many organic nutrients required for processing and functioning of these bacterial communities, they proliferate and propagate at their extreme. Alternatively, bulk soil lacks sufficient nutrients for

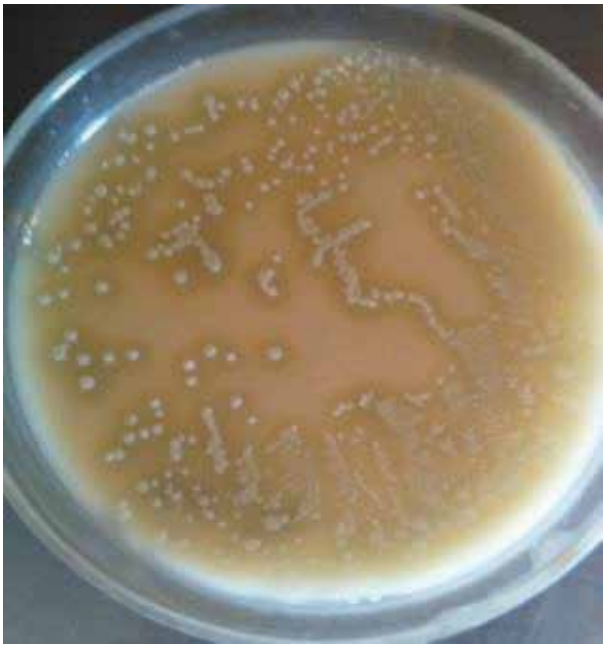

Fig. 3. MRS agar supplemented with $CaCO_3$ showing the lactic acid production by anaerobic *Bacilli* isolates. A clear zone around the bacterial colonies was considered as indicative of lactic acid production.

Fig. 4. Vancomycin sensitivity test to differentiate between *Lactobacilli* species. Some *Lactobacilli* species are naturally resistant to Vancomycin.

the growth of bacteria. A number of studies have investigated bacterial community dynamics of rhizosphere soil including in-depth study [16-18], analysis of such communities in rhizosphere soil of indigenous plant *Mangifera indica* is of paramount importance for recovering bacterial strains capable of performing unique functions including production of novel antibacterial compounds.

In a recent study, it has been shown that plant species and pH of soil may affect the microbial community structure in the rhizosphere soil [19]. Therefore, the present study was conducted to determine the influence of local weather and environment of soil on the rhizopheric microbiome of *Mangifera indica,* an important and second largest produced fruit in Pakistan. Knowing the bacterial population of the rhizosphere of *Mangifera indica* plant would be of greatest interest for understanding microbial ecology of rhizosphere of indigenous plants as well as it may help in planning future strategies to increase yield of this plant. Findings of this study can be exploited in discovery of biocontrol agents to cope with pathogens of this plant. In this study we observed that 17% of anaerobic population comprised Lactobacilli while others were not identified in this study. However, the communities of aerobic bacteria were of different types of Gram-positive and Gram-negative bacteria. It was found that the microbial load varied from sample to sample suggested that other factors of soil environment affect the microbial community dynamics of rhizosphere soil apart from plant type since all the samples in this study were collected from same type of plant.

5. CONCLUSION

In conclusion, knowing about bacterial communities of rhizosphere soil of particular locality is significant for ecosystem and the data obtained in this study provided insights into active bacterial population of *Mangifera indica* plant which may lead discovery of novel bacterial strains capable of providing an opportunity to treat challenging plant pathogens.

6. ACKNOWLEDGEMENTS

Authors are thankful to all gardeners of University of Sindh, Jamshoro for their help and cooperation during sampling process.

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