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# Study of Bacterial Diversity of Mangroves Rhizosphere

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## Abstract

**Microbial diversity has been an important facet of scientific research, since microbes promise a plethora of biomolecules which are otherwise not found in nature. Microbes are subjected to high level of competition for survival in the environment, and hence develop mechanisms of defense. The biomolecules produced by these microbes as part of their defense or survival mechanism, are of importance for human and animal drugs and many other industrial and environmental applications. The marine counterparts of these terrestrial microbes have yet higher potential, since the marine environment has higher biotic and abiotic stresses, leading to new molecule discovery. In the current study, a bacterial diversity study of the culturable bacteria of the mangrove rhizosphere of *Avicennia marina* has been undertaken, to understand the flora diversity. Mangroves are unique ecosystems which are under a combination of marine and terrestrial influence. Mangroves are seaward, inland and also found in creek areas. This diversity in their habitat, leads them to produce variable root exudates, which support the growth of different types of organisms. This study has revealed that certain species are dominant in these ecosystems irrespective of the biotic and abiotic stresses, whereas certain species appear only at neutral pH. The study will help select organisms for further biomolecule discovery programs, based on their environment of isolation and other growth parameters.**

## Keywords

**Rhizosphere, Mangroves, Bacteria, Microbial Diversity, 16S rRNA Sequencing**

## 1. Introduction

Mangroves are unique coastal plants which have originated due to the tectonic land shifts because of which ter-

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restrial plants got bared to the open sea with ecological and economic importance. They not only provide socio-economic benefits to local tribes, but also provide protection to coastal areas against natural disasters and facilitate the formation of land by trapping sediments [1] [2]. Around 34 major and 20 minor mangrove species belonging to about 20 genera in over 11 families have been recorded globally [3]. Mangroves constitute a significant part of tropical coastal biodiversity which occupy less than 1% of the world's surface [4] and are mainly found between the Tropic of Cancer and the Tropic of Capricorn on all continents covering an estimated 75% of the tropical coastline worldwide. Mangroves of South and Southeast Asia form the most extensive and diverse mangrove system comprising 41.4% of global mangroves in the world [5]. Among them Indian mangroves make up 3.1% of the total global cover and are distributed along all the maritime states except the union territory of Lakshwadeep covering an area of about 4461 $km^2$ along the 7500 km long Indian coastline [6]. Gujarat state, with a coastline of about 1650 km, harbors approximately 960 $km^2$ of mangroves [6]. Gujarat mangrove forest covers 1103 $km^2$; 175 $km^2$ are moderately dense and 928 $km^2$ are open mangrove forests (FSI, 2013). Mangroves in Gujarat are mostly confined to three regions a) Indus deltaic region *i.e.* Kori creek and Sir Creek area, b) The Gulf of Kachchh and c) The Gulf of Khambhat.

Bacterial diversity from these ecosystems has been studied worldwide for their unique biochemical processes. The present study includes isolation, morphological characterization and identification of rhizospheric bacteria using biochemical and molecular biology techniques [7] [8]. Molecular biology techniques like 16S rRNA techniques are an important tool in final identification of bacteria sequencing this gene, and provide genus and species identification for isolates that do not fit any recognized biochemical profiles. It gives acceptable identification which otherwise according to conventional system of taxonomy is not possible [9].

## 2. Materials and Methods

### 2.1. Study Area and Sample Collection

The study was conducted at three different sites of southern part of Gulf of Kachchh, Gujarat. The geographical location of collection sites are: Station 1—Sikka (Latitude 22˚26.406'N Longitude 069˚50.029'E) (coastal wetland, port activity), Station 2—Valsura (Latitude 22˚33.578'N Longitude 070˚02.502'E) (coastal wetland, ship breaking activity) and Station 3—Khijadiya (Latitude 22˚31.375'N Longitude 070˚08.099'E) (inland wetland, bird sanctuary) (see **Figure 1**). It contains about 30 - 35 ppt (parts per trillion) soil salinity and has a temperature around 26˚C - 30˚C. The pH of the three location varied from 6.0 - 8.0. *Avicennia marina* (true mangrove plant) rhizosphere soil samples were collected carefully by sterile spatula in sterile polypropylene tubes, properly labeled. These were transported in an ice box to the laboratory and processed within 2 - 4 h of collection.

### 2.2. Isolation of Bacteria

About 1g of rhizosphere soil of *Avicennia marina* was transferred to 50 mL test tube containing 10 mL sterile distilled water and vortexed vigorously for 10 min. The resulting solution containing the rhizosphere bacteria was serially diluted up to $10^{-4}$ using sterile distilled water. 100 µl aliquot was taken from each dilution and plated in triplicate onto Zobell's marine agar 2216 (ZB agar) (Himedia, India), MPM (Synthetic sea water media) agar medium [10] and incubated at 28˚C for 24 - 96 h. After incubation, colony counts were recorded and colonies with distinctive morphologies were selected for further studies. The isolated bacteria were purified by streak plate technique [11]. The 35 isolates obtained through this process of isolation were subjected to various biochemical tests according to "Bergey's Manual of Determinative Bacteriology" (Volume-4) and molecular techniques of identification.

### 2.3. Identification of Bacterial Isolates

#### 2.3.1. Morphological Characterization

The morphological characterization of the bacterial colonies were carried out on the basis of their shape, size, colour, margin, elevation on the media and Gram staining were performed to decide the further determinative protocol.

#### 2.3.2. Biochemical Analysis

The pure culture were subjected to identification by Bergey's Manual of Determinative Bacteriology (Volume-4)

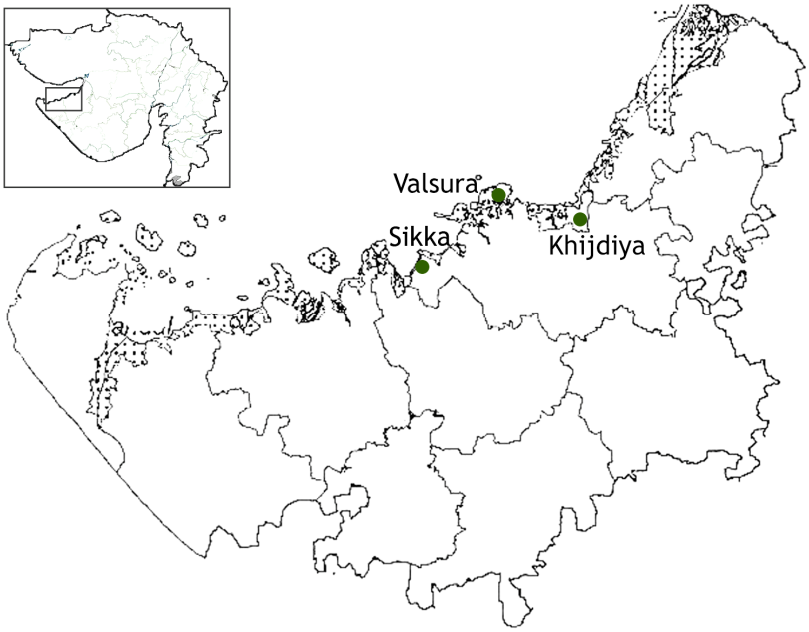


**Figure 1.** Sample collection sites in Gulf of Kachchh, Gujarat.

using catalase test with 3% hydrogen peroxide, Anaerobic Test, Oxidative Fermentative (O/F test), methyl red test, Voges Proskaeur test, glucose test, sorbitol test and mannitol test.

### 2.3.3. Molecular Analysis

**Genomic DNA Isolation**

Culture was centrifuged at 10,000 rpm for 5 min. Pellet was collected and resuspended by adding 9 ml of STE buffer (0.1 mM NaCl, 10 mM Tris, 10 mM EDTA) 1 ml of SDS (10% Stock Solution). The suspension was incubated at 70˚C for 1 hr. and centrifuged at 6000 rpm for 10 min at room temperature. The supernatant was collected in fresh tube and add equal volume of Phenol:Chloroform:Isoamyl alcohol (PCI mix) (25:24:1) was added and mixed slowly. The suspension was centrifuged at 6000 rpm for 10 min. The aqueous phase in fresh tube. Equal vol. of Chloroform: Isoamyl alcohol (24:1) and mix slowly and centrifuged at 6000 rpm for 10 min. The aqueous phase was collected and added double the vol. of absolute alcohol was added. The tube was subjected to overnight incubation in −20˚C. The solution was centrifuged at 6000 rpm 4˚C for 10 min and the pellet was resuspended in $1/10^{th}$ ml of 3M sodium acetate and 10 ml of absolute alcohol and centrifuged at 6000 rpm 4˚C for 10 min. The supernatant was discarded and the pellet was air dried. The pellet was dissolved in 1 ml sterile TE buffer. The DNA quality was checked using Agarose gel electrophoresis and quantified using Nanodrop.

**PCR Amplification and Phylogenetic Analysis**

The 16S rRNA gene from the genomic DNA was amplified using primers F 5'-AGAGTTTGATCCTGG CTAG-3', R 5'-CGGTTACCTTGTTACGACTT-3' and F5'-TGGAGAGTTTGATCCTGGCTCAG-3', R 5'-GGTTACCTTGTTACGACTT-3' (at Xcelris Genomics Pvt. Ltd., Ahmedabad and Junagadh Agricultural University, Junagadh). The PCR reaction mixture (25 µl) comprised of bacterial DNA (50 ng), 10 pmol each of the two oligonucleotide primers, 2.5 mM of each deoxynucleoside triphosphate, 1.5 U of Taq polymerase and 2.5 µl of 10× buffer. The amplification was performed using following PCR cycle: the initial denaturation at 94˚C for 5 min, followed by 35 cycles of 94˚C for 30 s, 60˚C for 30 s and 72˚C for 90 s and a final extension of 72˚C for 10 min. PCR product was electrophoresed on 1.5% agarose gel. PCR products varied from 1200 bp to 1500 bp. Sequencing was performed by ABI 3730xl Genetic Analyzer 96 well capillary system.FASTA files were subjected to BLAST for further analysis [12]. A phylogenetic tree was constructed using MEGA 5.1 software. The top ten alignment matches were presented according to percent similarity and the nearest distances.

Identification utilizing GenBank database was carried out by internet-based 16S rRNA gene sequence comparison software utilizing the basic local alignment search tool with default settings. The closest species level match (% identity) was considered the identification.

# 3. Results

## 3.1. Isolation and Enumeration of Bacteria

The average number of culturable heterotrophic bacteria in soil samples from the three different stations was $2.41 \times 10^8$ CFU/g. Among the collection station, soil sample from the Khijadiya mangroves recorded the highest bacterial counts $2.81 \times 10^8$ CFU/g and Sikka recorded the lowest bacterial count of $1.97 \times 10^8$ CFU/g.

## 3.2. Identification & Phylogeny

The morphological and biochemical studies of the 35 isolates were performed according to (see **Table 1**). Molecular characterization was done using 16S rRNA gene sequencing. Nineteen (19) out of thirty five (35) isolates had >99% similarity with known species. The isolates belongs to the phyla Firmicutes (62.86%), followed by Proteobacteria (22.86%) and Actinobacteria (14.29%). The phylogenetic tree of the three different locations is as in (see **Figure 2**).

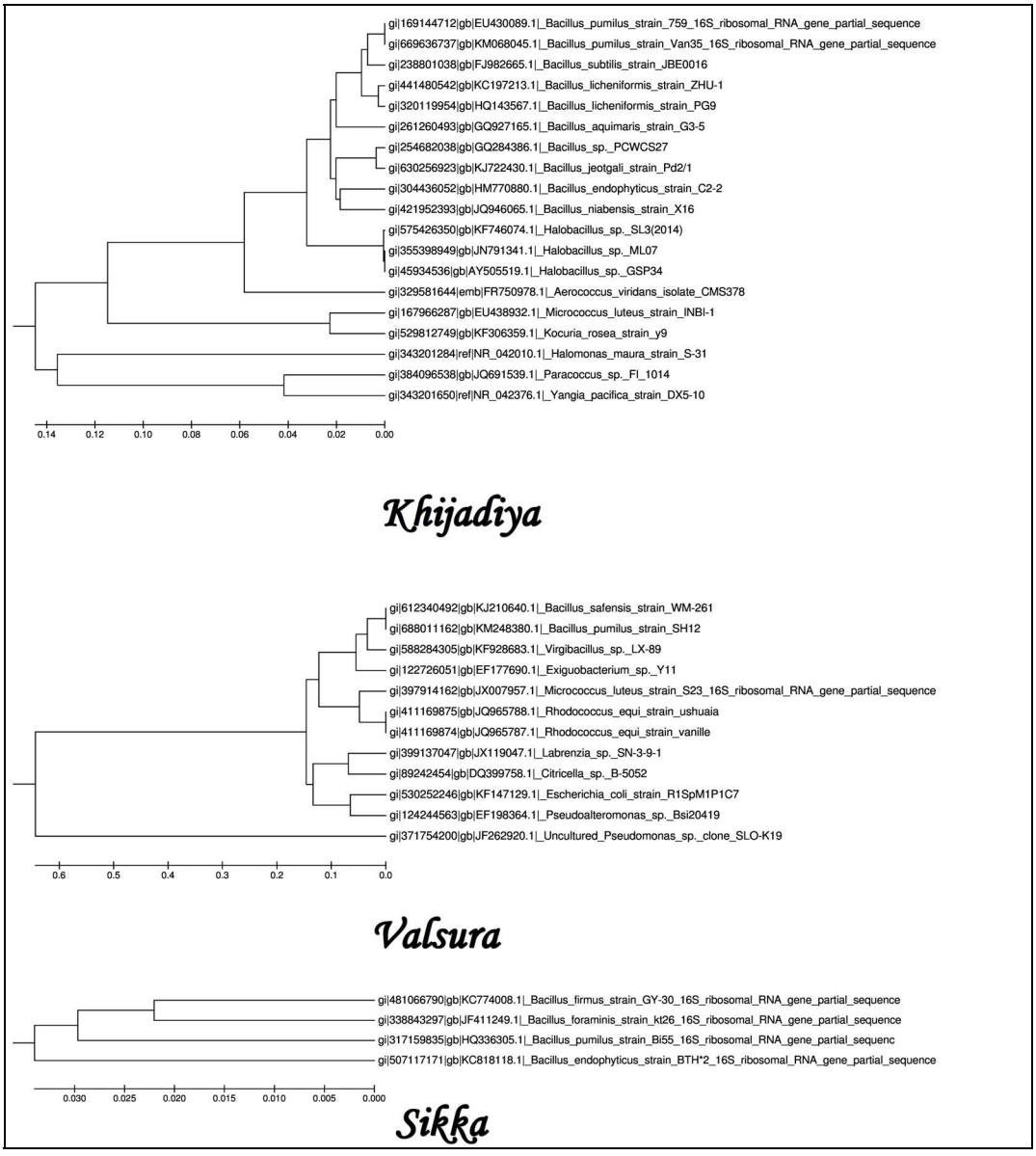


**Figure 2.** The phylogenetic tree indicates the phylogenetic relationship of the 35 isolates.

**Table 1.** Colony characterization and biochemical test.

| | | Colony Characteristics | | | | | | | Biochemical Test | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sr. No. | Sample | Form | Elevation | Margin | Pigment | Texture | Transperency | Gram's Staining | Spore Staining | Motility | Catalase | Manitol | Glucose | Anaerobic (Hugh-Leifson's O-F) | Acid Fast staining | Hemolysis | Starch hydrolysis | Sorbitol Fermentation | Voges Proskauer's test | Methyl Red test | Biochemical identified |
| 1 | Kh1MS17z | Circular | Raised | Entire | Buff | Mucoid | Translucent | Gm+ve, rods | Negative | Motile | + | NR | + | NR | - | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 2 | Kh1MS16z | Circular | Raised | Entire | Yellow | Waxy | Opaque | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | NR | NR | NR | NR | NR | NR | ***Arthrobacter* sp.** |
| 3 | Kh2MS3z | Circular | Raised | Entire | Buff | Mucoid | Translucent | Gm+ve, rods | Negative | Motile | - | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 4 | Kh1MS10z | Circular | Convex | Entire | Dew drop | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | + | NR | - | NR | NR | - | NR | NR | NR | NR | ***Bacillus* sp.** |
| 5 | Kh1MS5z | Circular | Flat | Undulate | Buff | Mucoid | Opaque | Gm+ve, rods | Positive | Motile | - | NR | - | NR | NR | - | NR | NR | NR | NR | ***Bacillus* sp.** |
| 6 | Kh1MS18z | Irregular | Convex | Entire | Creamy | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | NR | NR | NR | + | NR | NR | NR | NR | NR | NR | ***Clostridium* sp.** |
| 7 | Kh1MS3z | Irregular | Flat | Lobate | Red | Waxy | Opaque | Gm+ve, rods | Negative | Motile | + | NR | NR | - | NR | - | NR | NR | NR | NR | ***Bacillus* sp.** |
| 8 | Kh2MS7z | Circular | Raised | Entire | Colorless | Mucoid | Transparent | Gm+ve, cocci | Negative | Non-motile | + | - | NR | NR | NR | NR | NR | NR | NR | NR | ***Staphylococcus* sp.** |
| 9 | Kh1MS9z | Irregular | Flat | Undulate | Creamy | Mucoid | Opaque | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | - | NR | - | NR | NR | NR | ***Corynebacterium* sp.** |
| 10 | Kh3MS5z | Circular | Raised | Entire | Brown | Mucoid | Translucent | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | - | NR | - | NR | NR | NR | ***Corynebacterium* sp.** |
| 11 | Kh3MS6z | Circular | Raised | Entire | Colourless | Mucoid | Transparent | Gm+ve, rods | Negative | Motile | - | - | - | NR | - | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 12 | Kh1MS6m | Irregular | Raised | Entire | Creamy | Waxy | Opaque | Gm+ve, rods | Negative | Motile | - | - | + | NR | NR | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 13 | Kh1MS3m | Circular | Raised | Entire | Colourless | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | - | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 14 | Kh3MS3m | Irregular | Raised | Undulate | Colourless | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | + | NR | NR | - | NR | NR | + | NR | - | - | ***Bacillus* sp.** |
| 15 | Kh3MS4m | Rhizoid | Flat | Lobate | Colourless | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | + | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 16 | Kh1MS7m | Circular | Raised | Entire | Colourless | Mucoid | Transparent | Gm+ve, cocci | Negative | Motile | + | NR | NR | - | NR | NR | - | NR | NR | NR | ***Enterococcus* sp.** |
| 17 | Kh2MS1m | Circular | Flat | Entire | Colourless | Waxy | Translucent | Gm+ve, rods | Positive | Motile | - | NR | NR | NR | NR | - | NR | - | NR | NR | ***Bacillus* sp.** |
| 18 | Kh2MS5m | Circular | Raised | Entire | Colourless | Mucoid | Transparent | Gm+ve, cocci | Negative | Non-motile | - | NR | NR | NR | NR | - | NR | - | NR | NR | ***Streptococccus* sp.** |
| 19 | Kh1MS2m | Circular | Raised | Entire | Creamish | Mucoid | Transparent | Gm+ve, rods | Positive | Motile | + | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 20 | Vl1MS6z | Circular | Raised | Entire | Buff | Mucoid | Transparent | Gm+ve, rods | Positive | Motile | + | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 21 | Vl3MS2z | Irregular | Raised | Undulate | Creamy white | Waxy | Opaque | Gm+ve, rods | Positive | Motile | + | NR | NR | + | NR | NR | NR | NR | NR | NR | ***Clostridium* sp.** |
| 22 | Vl1MS8z | Circular | Raised | Entire | Dew drop | Mucoid | Translucent | Gm+ve, rods | Positive | Motile | + | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 23 | Vl1MS4z | Circular | Raised | Entire | Creamy | Mucoid | Opaque | Gm+ve, cocci | Negative | Non-motile | + | + | NR | NR | NR | NR | NR | NR | NR | NR | ***Staphylococcus aureus*** |
| 24 | Vl2MS6z | Circular | Raised | Entire | Creamy buff | Mucoid | Translucent | Gm+ve, cocci | Negative | Non-motile | + | + | NR | NR | NR | NR | NR | NR | NR | NR | ***Staphylococcus aureus*** |
| 25 | Vl1MS1z | Circular | Convex | Entire | Brown | Mucoid | Translucent | Gm+ve, rods | Negative | Motile | - | NR | NR | - | NR | NR | - | NR | NR | NR | ***Bacillus* sp.** |
| 26 | Vl2MS1z | Circular | Raised | Entire | Yellow | Mucoid | Transparent | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | - | NR | - | NR | NR | NR | ***Corynebacterium* sp.** |
| 27 | Vl1MS3z | Circular | Raised | Entire | Red | Mucoid | Transparent | Gm+ve, rods | Negative | Motile | - | - | - | NR | - | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 28 | Vl3MS3z | Circular | Raised | Entire | Brown | Mucoid | Transparent | Gm+ve, rods | Negative | Motile | - | - | - | NR | - | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 29 | Vl3MS5z | Circular | Raised | Entire | Yellowish | Mucoid | Transparent | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | - | NR | - | NR | NR | NR | ***Corynebacterium* sp.** |
| 30 | Vl3MS6z | Circular | Convex | Entire | Creamy | Mucoid | Translucent | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | - | NR | - | NR | NR | NR | ***Corynebacterium* sp.** |
| 31 | Vl2MS1m | Circular | Raised | Entire | Buff | Mucoid | Translucent | Gm+ve, rods | Negative | Non-motile | + | - | NR | NR | NR | NR | NR | NR | NR | NR | ***Staphylococcus* sp.** |
| 32 | Si1MS2z | Circular | Convex | Entire | Buff | Mucoid | Transparent | Gm+ve, rods | Negative | Motile | NR | NR | NR | NR | NR | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 33 | Si1MS10z | Irregular | Convex | Undulate | Pinkish White | waxy | Opaque | Gm+ve, rods | Negative | Non-motile | + | NR | NR | NR | NR | NR | NR | NR | NR | NR | ***Arthrobacter* sp.** |
| 34 | Si1MS12z | Circular | Convex | Entire | Buff | Mucoid | Transparent | Gm+ve, rods | Negative | Motile | - | - | - | NR | - | NR | NR | NR | NR | NR | ***Lactobacillus* sp.** |
| 35 | Si1MS7m | Irregular | Convex | Undulate | Dirty white | Waxy | Opaque | Gm+ve, cocci | Cocci | Non-motile | - | NR | NR | NR | NR | - | NR | - | NR | NR | ***Streptococcus* sp.** |

NR = not required.

### 3.3. Comparison of Classical and Conventional Identification Techniques

Comparison of biochemical identification and molecular identification shows the similarities and dissimilarities in final identification of the organism shown in (see **Table 2**).

**Table 2.** Comparison of classical and conventional identification techniques.

| Sr. No. | Sample | Identified by 16S rRNA gene sequencing | % Similarity in alignment | Accession number | Identified by biochemical tests |
|---|---|---|---|---|---|
| 1 | Kh1MS17z | *Paracoccus* sp. *F*1 1014 | 100 | JQ691539.1 | *Lactobacillus* sp. |
| 2 | Kh1MS16z | *Bacillus aquimaris strain G*3-5 | 99 | GQ927165.1 | *Arthrobacter* sp. |
| 3 | Kh2MS3z | *Halobacillus* sp. *ML*07 | 100 | JN791341.1 | *Bacillus* sp. |
| 4 | Kh1MS10z | *Bacillus endophyticus strain C*2-2 | 99 | HM770880.1 | *Bacillus* sp. |
| 5 | Kh1MS5z | *Halomonas maura strain S*-31 | 100 | NR_042010.1 | *Bacillus* sp. |
| 6 | Kh1MS18z | *Bacillus licheniformis strain PG*9 | 99 | HQ143567.1 | *Clostridium sp* |
| 7 | Kh1MS3z | *Bacillus niabensis strain X*16 | 99 | JQ946065.1 | *Bacillus* sp. |
| 8 | Kh2MS7z | *Bacillus licheniformis strain ZHU*-1 | 99 | KC197213.1 | *Staphylococcus* sp. |
| 9 | Kh1MS9z | *Bacillus* sp. *PCWCS*27 | 95 | GQ284386.1 | *Corynebacterium* sp. |
| 10 | Kh3MS5z | *Bacillus pumilus strain*759 | 96 | EU430089.1 | *Corynebacterium* sp. |
| 11 | Kh3MS6z | *Bacillus pumilus strain Van*35 | 97 | KM068045.1 | *Lactobacillus* sp. |
| 12 | Kh1MS6m | *Yangia pacifica strain*: *DX*5-10 | 99 | NR_042376.1 | *Lactobacillus* sp. |
| 13 | Kh1MS3m | *Micrococcus luteus strain INBI*-1 | 99 | EU438932.1 | *Bacillus* sp. |
| 14 | Kh3MS3m | *Bacillus subtilis strain JBE* 0016 | 99 | FJ982665.1 | *Bacillus* sp. |
| 15 | Kh3MS4m | *Kocuria rosea strain Y*9 | 99 | KF306359.1 | *Bacillus* sp. |
| 16 | Kh1MS7m | *Halobacillus* sp. *SL3(*2014*)* | 99 | KF746074.1 | *Enterococcus* sp. |
| 17 | Kh2MS1m | *Bacillus jeotgali strain pd*2/1 | 98 | KJ22430.1 | *Bacillus* sp. |
| 18 | Kh2MS5m | *Halobacillus* sp. *GSP*34 | 99 | AY505519.1 | *Streptococccus* sp. |
| 19 | Kh1MS2m | *Aerococcus viridians isolate CMS*378 | 90 | FR750978.1 | *Bacillus* sp. |
| 20 | Vl1MS6z | *Labrenzia* sp. *SN*-3-9-1 | 99 | JX119047.1 | *Bacillus* sp. |
| 21 | Vl3MS2z | *Citreicella* sp. *B*-5052 | 99 | DQ399758.1 | *Clostridium* sp. |
| 22 | Vl1MS8z | *Rhodococcus equi strain ushuaia* | 99 | JQ965788.1 | *Bacillus* sp. |
| 23 | Vl1MS4z | *Escherichia coli strain R*1*SpM*1 | 100 | KF147128.1 | *Staphylococcus aureus* |
| 24 | Vl2MS6z | *Rhodococcus equi strain vanille* | 99 | JQ965787.1 | *Staphylococcus aureus* |
| 25 | Vl1MS1z | *Virgibacillus* sp. *LX*-89 | 96 | KF928683.1 | *Bacillus* sp. |
| 26 | Vl2MS1z | *Bacillus safensis strain WM*-261 | 97 | KJ210640.1 | *Corynebacterium* sp. |
| 27 | Vl1MS3z | *Bacillus pumilus strain SH*-12 | 98 | KM248380.1 | *Lactobacillus* sp. |
| 28 | Vl3MS3z | *Pseudoalteromonas* sp. *Bsi*20419 | 98 | EF198364.1 | *Lactobacillus* sp. |
| 29 | Vl3MS5z | *Uncultured Pseudomonas* sp. *Clone SLO-K*19 | 97 | JF262920.1 | *Corynebacterium* sp. |
| 30 | Vl3MS6z | *Micrococcus luteus strain S*23 | 96 | JX007957.1 | *Corynebacterium* sp. |
| 31 | Vl2MS1m | *Exiguobacterium* sp.*Y*11 | 99 | EF177690.1 | *Staphylococcus* sp. |
| 32 | Si1MS2z | *Bacillus firmus strain GY*-30 | 99 | KC774008.1 | *Lactobacillus* sp. |
| 33 | Si1MS10z | *Bacillus endophyticus strain BTH#*2 | 100 | KC818118.1 | *Arthrobacter* sp. |
| 34 | Si1MS12z | *Bacillus pumilus strain Bi*55 | 96 | HQ336305.1 | *Lactobacillus* sp. |
| 35 | Si1MS7m | *Bacillus foraminis strain kt*26 | 99 | JF411249.1 | *Streptococcus* sp. |

## 4. Discussion

The 35 culturable bacteria from three different locations of *Avicennia marina* were collected and studied for the representative diversity. The bacterial diversity as seen in **Figure 2** shows that as the pH increases from 6.0 to 8.0 the diversity decreases. The family Bacillaceae (60%) is the predominant family along all the three pH values and the existence of this family is found to be exclusive at higher pH. The family Rhodobacteraceae constitutes 11.43%; Micrococcaceae constitutes 8.57%; and the families Nocardiaceae and Pseudoalteromonadaceae constitute 5.71% (see **Table 3**). At pH 6.0 the diversity includes families like, Bacillaceae, Rhodobacteraceae,

**Table 3.** Taxonomic groups present in mangrove rhizosphere.

| Sr. No. | Sample | Identified by 16 s Sq. | Phylum | Family |
|---|---|---|---|---|
| 1 | Kh1MS17z | *Paracoccus* sp. *F*1 1014 | Proteobacteria | Rhodobacteraceae |
| 2 | Kh1MS16z | *Bacillus aquimaris strain G*3-5 | Firmicutes | Bacillaceae |
| 3 | Kh2MS3z | *Halobacillus* sp. *ML*07 | Firmicutes | Bacillaceae |
| 4 | Kh1MS10z | *Bacillus endophyticus strain C*2-2 | Firmicutes | Bacillaceae |
| 5 | Kh1MS5z | *Halomonas maura strain S*-31 | Proteobacteria | Halomonadaceae |
| 6 | Kh1MS18z | *Bacillus licheniformis strain PG*9 | Firmicutes | Bacillaceae |
| 7 | Kh1MS3z | *Bacillus niabensis strain X*16 | Firmicutes | Bacillaceae |
| 8 | Kh2MS7z | *Bacillus licheniformis strain ZHU*-1 | Firmicutes | Bacillaceae |
| 9 | Kh1MS9z | *Bacillus* sp. *PCWCS*27 | Firmicutes | Bacillaceae |
| 10 | Kh3MS5z | *Bacillus pumilus strain*759 | Firmicutes | Bacillaceae |
| 11 | Kh3MS6z | *Bacillus pumilus strain Van*35 | Firmicutes | Bacillaceae |
| 12 | Kh1MS6m | *Yangia pacifica strain*: *DX*5-10 | Proteobacteria | Rhodobacteraceae |
| 13 | Kh1MS3m | *Micrococcus luteus strain INBI*-1 | Actinobacteria | Micrococcaceae |
| 14 | Kh3MS3m | *Bacillus subtilis strain JBE* 0016 | Firmicutes | Bacillaceae |
| 15 | Kh3MS4m | *Kocuria rosea strain Y*9 | Actinobacteria | Micrococcaceae |
| 16 | Kh1MS7m | *Halobacillus* sp. *SL*3 (2014) | Firmicutes | Bacillaceae |
| 17 | Kh2MS1m | *Bacillus jeotgali strain pd*2/1 | Firmicutes | Bacillaceae |
| 18 | Kh2MS5m | *Halobacillus* sp. *GSP*34 | Firmicutes | Bacillaceae |
| 19 | Kh1MS2m | *Aerococcus viridians isolate CMS*378 | Firmicutes | Aerococcaceae |
| 20 | Vl1MS6z | *Labrenzia* sp. *SN*-3-9-1 | Proteobacteria | Rhodobacteraceae |
| 21 | Vl3MS2z | *Citreicella* sp. *B*-5052 | Proteobacteria | Rhodobacteraceae |
| 22 | Vl1MS8z | *Rhodococcus equi strain ushuaia* | Actinobacteria | Nocardiaceae |
| 23 | Vl1MS4z | *Escherichia coli strain R*1*SpM*1 | Proteobacteria | Enterobacteriaceae |
| 24 | Vl2MS6z | *Rhodococcus equi strain vanille* | Actinobacteria | Nocardiaceae |
| 25 | Vl1MS1z | *Virgibacillus* sp. *LX*-89 | Firmicutes | Bacillaceae |
| 26 | Vl2MS1z | *Bacillus safensis strain WM*-261 | Firmicutes | Bacillaceae |
| 27 | Vl1MS3z | *Bacillus pumilus strain SH*-12 | Firmicutes | Bacillaceae |
| 28 | Vl3MS3z | *Pseudoalteromonas* sp. *Bsi*20419 | Proteobacteria | Pseudoalteromonadaceae |
| 29 | Vl3MS5z | *Uncultured Pseudomonas* sp. *Clone SLO-K*19 | Proteobacteria | Pseudomonadaceae |
| 30 | Vl3MS6z | *Micrococcus luteus strain S*23 | Actinobacteria | Micrococcaceae |
| 31 | Vl2MS1m | *Exiguobacterium* sp. *Y*11 | Firmicutes | Bacillales Family XII. Incertae Sedis |
| 32 | Si1MS2z | *Bacillus firmus strain GY*-30 | Firmicutes | Bacillaceae |
| 33 | Si1MS10z | *Bacillus endophyticus strain BTH#*2 | Firmicutes | Bacillaceae |
| 34 | Si1MS12z | *Bacillus pumilus strain Bi*55 | Firmicutes | Bacillaceae |
| 35 | Si1MS7m | *Bacillus foraminis strain kt*26 | Firmicutes | Bacillaceae |

Halomonadaceae, Micrococcaceae, Aerococcaceae; at pH 7.0 the diversity includes Bacillaceae, Rhodobacteraceae, Micrococcaceae, Nocardiaceae, Enterobacteriaceae, Pseduoalteromonadaceae, families and at pH 8.0 the diversity is restricted to Bacillaceae family.

Bergy's Manual for determinative microbiology is an established key for identification of microbes. The key has successfully produced results which have shown 98% harmony with 16S rRNA sequencing when limited to a specific genus in case of marine microbes [13]. Also, in case of clinical samples like Mycobacteria and Vibrio, the biochemical identification shows a harmony in results making the score to 70% [14]. In another study working with 47 samples using biochemical tests and 16S rRNA gene PCR, in 33 cases (70.2%), biochemical tests were confirmed by PCR [15]. In case of environmental samples, the samples are subjected to 16S rRNA gene sequencing, and biochemical tests of the genus are then used to confirm the genus and species identification [13]. In the current study unknown environmental samples were subjected to biochemical testing for the first stage of elimination, isolation and preservation. The identification of the isolated samples using 16S rRNA gene sequencing revealed a 51.43% match up to the phylum level, 48.57% up to class, 20% up to order and 17.14% up to family level. The match at genus level is at a low of 3%. This shows that for environmental samples, where biochemical tests are an important tool for first stage identification, genus and species level identification can be confirmed by 16S rRNA gene sequencing. For 10 samples (28.57%), namely, Kh1MS17z, Kh2MS3z, Kh1MS9z, Kh1MS7m, Kh1MS5m, V11MS6z, V13MS2z, V11MS1z, V13MS3z and V12MS1m, 16S rDNA gene sequencing could give an identification up to genus. Further determinative biochemical tests and MALDI-TOFF are needed to be applied to these samples. Biochemical tests and molecular techniques are complementary tools, and both are important for conclusive identification of unknown environmental samples. This diversity study has been able to cultivate an uncultured *Pseudomonas* sp. *Clone SLO-K*19, reported through 16S rRNA sequencing, on minimal media.

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