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**ORIGINAL ARTICLE** **OPEN ACCESS**

# Enumeration and isolation of marine microbes from rhizosphere and nonrhizosphere of *Avicennia marina*

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**ABSTRACT**
Mangroves are native to a unique harsh ecosystem, and mangrove-associated bacteria may be one of the reasons for this plant's survival in this environment. Exploration of mangrove microbial companions may allow full to filter such prospective specialized microbial strains that are as distinct as their habitat, thus it is vital to examine the microbial diversity from the unstudied mangrove ecosystem. The purpose of this study was to discover an indigenous diazo trophic bacterial population for improving nursery procedures in mangrove restoration. The plant species *Avicennia marina* was chosen for study in geographically distinct sections of the Punnakayal mangrove forest. The current study found that the undiscovered Punnakayal mangrove habitats are a potential source of antagonistic actinomycetes and so merit bioprospecting. The actinomycetes population was greater in the *Avicennia marina* rhizosphere than in the non-rhizosphere zone. The greatest bacterial and fungal population was recorded up to a depth of 5 cm, and the population dropped in deeper samples. Among 31 isolates only 22 isolates demonstrated action against test organisms exposed to the first screening. Six isolates were active solely against gram-negative organisms, five against gram-positive organisms, seventeen against both gram positive and negative species, and three isolates exhibited no inhibitory effect against both gram positive and negative organisms. 22 of the isolates tested positive for *Bacillus cereus* and *Staphylococcus aureus*, 14 for *Escherichia coli*, 12 for *Pseudomonas aeruginosa*, and 11 for *Enterobacter aerogenes* and *Klebsiella pneumoniae*. The actinobacterial strain SDMRI-3 will be a strong candidate for antibiotic molecule separation since it demonstrated good activity in preliminary and secondary screening assays.
Keywords : Mangroves, Marine microbes, Avicennia marina, Antibiotics Punnakayal forest

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## INTRODUCTION

Mangrove related bacteria populate mangrove soil (1)(2) and mangrove detritus (3), Mangrove rhizosphere (4) pneumatophores (5)roots(6) and leaves (7). Many unique halotolerant enzymes, including amylase, cellulase, lipase, and protease, are produced by mangrove-associated microorganisms (8). Actinomycetes are important members of the microbial community because they recycle organic matter and produce innovative medications, nutritional materials, cosmetics, enzymes, antitumor drugs, enzyme inhibitors, immunological modulators, and vitamins (9). In general, root colonization and rhizosphere competence isimportant requirements for selecting a successful plant growth promoting rhizosphere (PGPR) candidate (10) (11). There is a lot of interest in using PGPR as bio-inoculants to boost halophytic plant development (12). Because the maritime environment differs significantly from the terrestrial environment, it must be investigated and utilized for new biological products. So far, microorganisms that produce diverse beneficial chemicals have mostly been identified from neritic settings (13).Microorganisms in the mangrove ecosystem play an important role in the growth and development of mangrove plants by regenerating nutrients via biological processes such as decomposition or by fixingnitrogen and phosphorus (14). Microbes from harsh habitats have received a lot of interest in recent years. Actinomycetes are known to produce nearly 80% of the world's antibiotics, primarily from the genera Streptomyces and Micromonospora (15). Production of novel pharmaceuticals, nutritional materials, cosmetics, enzymes, antitumor agents, enzyme inhibitors, immune modulators, and vitamins.

Streptomyces are very prolific, producing a wide range of antibiotics (up to 80% of total antibiotic output) as well as active secondary metabolites (16).
The Punnakayal mangrove environment is little unexplored, although it may be a rich source of actinomycetes that produce new and effective antibacterial chemicals. (17). As a result, the current examination was conducted to enumerate and isolate actinomycetes from sediment samples from the Punnakayal mangrove region.

## MATERIAL AND METHODS

### Sampling area

The Punnakayal estuary (N' 80 38. 266 E' 780 07. 317) is the only one in the Tuticorin area of the Gulf of Mannar. This estuary has two species of mangrove trees: *Rhizophora mucronata* and *Avicennia marina*. *Avicennia marina* is the major mangrove species in the environment inside this ecosystem. The samples were taken from the Punnakayal mangrove region on the Tuticorin coast of the Gulf of Mannar in Tamil Nadu, India.

### Sample collection

Sediment samples were taken from the rhizosphere and non-rhizosphere regions of juvenile *Avicennia marina* plants at depths of 5, 10, and 15 cm. The samples (around 500g) were gathered in clean, dry, and sterile polythene bags with the use of a sterile spatula and other tools. The collecting location was chosen with many factors in mind, including organic matter, moisture content, particle size, and soil colour, as well as being free of contamination. Samples were carried in ice boxes to the laboratory and maintained in a refrigerator at 4°C until analysis.

### Determination of sediment temperature

A thermometer was used to determine the temperature of the silt. The thermometer was placed into the soil to a depth of 5 cm and left for 15 minutes before recording the temperature measurement (18).

### Determination of sediment pH and Electrical conductivity (EC)

The soil pH and electrical conductivity (EC) were measured using a digital pH meter and the Watson and Brown techniques. 3 g of sediment was placed in a beaker with 6 ml of distilled water, swirled for five seconds, and left to stand for ten minutes. The pH and EC meter electrode was then gently put into the slurry and spun, and the measurement was recorded. (19)

### Microbial analysis

In a 250 ml Erlenmeyer's flask, 10g of rhizosphere and non-rhizosphere silt was transferred to 100 ml sterile water blank. The flask contexts were properly combined in a shaker for 5 minutes before being serially diluted up to $10^6$with sterile 50%seawater blanks. Enumerating bacteria, fungus, and actinomycetes required one ml of$10^6$ , $10^5$, $10^4$ dilutions with sterile 50 % sea water blanks. 20 mL of the appropriate media was aseptically put onto petri dishes, which were then spun clockwise and anticlockwise and allowed to harden. Three duplicates were kept for each dilution. The inoculation plates were incubated at 37°C for a day and 6 - 11 days for bacteria and actinomycetes, and at 28°C for 3 - 5 days for fungi. Following the incubation time, the microbial colonies were counted using a colony counter. The countable colonies in the petri dishes were counted and represented as the number of colony forming units per gram or ml (CFU/ gram) of the material examined.(20)

### Sample treatment

The obtained sediment samples were separated into two halves, with one half utilized for bacteria and fungal isolation. The second half of the sediment sample was utilized for actinomycetes separation and was air dried aseptically. After a week, sediment samples were incubated for 5 minutes at 55°C to aid in the isolation of actinomycetes (21).

### Isolation of bacteria, fungi and actinomycetes

For bacteria, fungi, and actinomycetes, 100 microlitres of $10^6$ , $10^5$, $10^4$ dilutions were dispersed over the surface of nutritional agar plates, Sabourauds dextrose agar plates, and Starch casein nitrate agar plates, respectively. The inoculation plates were incubated at 37°C for 24 hours, 28°C for 3-5 days, and 37°C for 6-11 days for bacteria, fungi and actinomycetes respectively (22).

### Isolation of actinomycetes

To isolate actinomycetes, one gram of three different oven dried sediment samples, such as Sample A, Sample B, and Sample C, were serially diluted and 100: l of the diluted samples were added from each of the dilutions and spread plated on the starch casein nitrate (SCN) agar medium plates containing starch, 10.00 (g/l); potassium nitrate, 2.00 (g/l); di-potassium hydrogen phosphate. To inhibit the development of bacteria and fungi, nalidixic acid (20 g/ml) and cycloheximide (50 g/ml) were used. The plates were incubated at 30°C for 10 days. (23).

**Maintenance of Culture**

Isolated colonies from the plates were subcultured on appropriate agar slants and incubated at 37°C for 24 hours before being kept at 4°C for further investigation.

**Morphological characterization of marine actinomycetes**

The colonies of chosen marine actinomycetes were streaked on starch casein nitrate plates and cultured for a week. Color, uniformity, and development pattern of the colony were studied.

**Screening of Actinobacteriafor antibiotic production**

Using drug-resistant bacterial pathogens collected from MTCC Chandigarh, India, isolated actinobacteria were tested for their potential to produce antibiotic metabolites. The following test pathogens used in this study respectively *Bacillus cereus* (645), *Staphylococcus aureus* (96), *Escherichia coli* (443), *Pseudomonas aeruginosa* (424),*Enterobacter aerogenes*(111) *Klebsiella pneumonia* (432)

**Primary Screening for antagonistic activity**

The agar plug technique was used to assess the antagonistic activity of actinobacterial isolates (Mohanraj*et al.,* 2011). This study *employed Escherichia coli, Bacillus cereus, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, and Klebsiella pneumoniae* as test bacterial pathogens. The bacterial isolates were all acquired from the MTCC in Chandigarh. Agar plugs were extracted with a 5 mm diameter core from actino-bacteria cultures cultivated on ISP2 agar medium for 10 days. To get just the disseminated microbial metabolites in the agar plugs, the surface growth on agar was scraped using a sterile knife. The agar plugs were put on a nutrient agar plate that had been swabbed with the test bacterial pathogens earlier. The plates were then incubated for 24 hours at 37°C. Following incubation, antimicrobial activity was demonstrated by the creation of an inhibitory zone around the agar plug, which may have been caused by disseminated antimicrobial metabolites generated by the expanding actinobacterial culture. The absence of an inhibitory zone suggested a negative outcome for diffusible metabolite formation in the solid growth medium (24).

**Secondary Screening**

The isolates that demonstrated broad spectrum activity after initial screening were chosen for subsequent screening. The isolates were produced in bulk on Modified Glycerol Yeast Extract Agar. The spores were extracted. Fermentation took place in Erlenmeyer flasks. Harvested active isolate spores were cultured in a 500 ml flask containing 100 ml of culture media (0.8g NaCl, 1g $NH_4Cl$, 0.1g KCl, 0.1g $KH_2PO_4$, 0.2g $MgSO_4.7H_2O$, 0.04g $CaCl_2.2H_2O$, 2g glucose, 3g yeast extract in 10ml of distilled water, pH 7.3). The incubation period was 6 days at 28ºC with normal aeration and agitation (25).

**Isolation of antibacterial metabolites**

The mycelium was extracted from the fermented broth by filtration, and the clear filtrate was utilized for further isolation. The antimicrobial component was then extracted from the filtrate using a solvent extraction technique (26). The antibacterial component was extracted from the filtrate using ethyl acetate as a solvent. Ethyl acetate was added to the filtrate in a 1:1 (v/v) ratio and vigorously agitated for 1 hour to complete extraction. The antibiotic-containing ethyl acetate phase was isolated from the aqueous phase. It was dried in a water bath at 80°C – 90°C, and the resulting residue was employed in secondary screening.

**Determination of antimicrobial activity**

The antibacterial activity was tested using the paper disc technique (26). The ethyl acetate extract was evaporated to get the pure extract. The solution was then put onto a sterile disc, and test organisms (0.5 McFarland turbidity standards) were swabbed onto Muller Hinton agar plates. The plates were tested after being incubated at 37°C for 18 - 24 hours. The diameters of the full inhibitory zones were measured to the closest whole millimetre. Actinomycetes isolates produce secondary metabolites, which cause an inhibitory zone to develop surrounding pathogenic strains.

## RESULTS

**Description of sediments and sampling sites**

Samples were collected from six different sites. The sampling site, location, depth and sediment type are given in the Table1.

**Table 1:Description of sediments and sampling sites**

| S.No | Sampling Sites | Location | Depth (cm) | Sediment types |
|---|---|---|---|---|
| 1 | R-1 | ***Avicennia marina Rhizosphere*** | 5 | Fine mud with coarse sand |
| 2 | R-2 | ***Avicennia marina Rhizosphere*** | 10 | Clay with coarse sand |
| 3 | R-3 | ***Avicennia marina Rhizosphere*** | 15 | Clay with fine sand |
| 4 | NR-1 | ***Non rhizosphere*** | 5 | Fine mud with coarse sand |
| 5 | NR-2 | ***Non rhizosphere*** | 10 | Clay with coarse sand |
| 6 | NR-2 | ***Non rhizosphere*** | 15 | Clay with fine sand |

All of the samples were subjected to physiochemical analysis. The samples ranged in temperature from 23.8 to 25.2°C, pH from 6.2 to 6.8, and electrical conductivity from 3.1 to 4.8. The microbial population in the *Avicennia marina* rhizosphere was reported as 66.67 x $10^6$ bacteria at 5 cm depth, 32.68 x$10^6$ at 10 cm depth, and 21.14 x$10^6$ at 15 cm depth. At 5, 10, and 15 cm depths, the fungal populations were 33.92, 21.64, and 16.90 x $10^3$, respectively. At 5, 10, and 15 cm depths, the actinomycetes populations were 23.16, 52.81, and 32.99 x$10^4$, respectively. In the non-rhizosphere, 38.69, 21.21, and 14.58 x $10^6$ bacteria, 22.18, 18.21, and 11.26 x $10^3$ fungi, and 16.61, 37.04, and 26.68 x $10^4$actinomycetes were found 5, 10 and 15 cm respectively.

*The Avicennia marina* rhizosphere had the higher actinomycetes population than non-rhizosphere region. The highest bacterial and fungal population was obtained up to the depth of 5 cm and the population decreased in samples from further depths. In addition the maximum actinomycetes population was recorded at the depth of 10 cm whereas, low population was exhibited at 5 and 15 cm depths and the results are presented in Table2.

**Table2:Enumeration of actinobacterial population (x$10^4$) from different soil samples**

| S. No | Sampling sites | Temperature (°C) | pH | EC (ds/m) | Total bacterial population (x$10^6$) | Total fungal population (x$10^3$) | Total actinomycetes population (x$10^4$) |
|---|---|---|---|---|---|---|---|
| 1 | R-1 | 25.2 | 6.4 | 4.8 | 66.67±6.9217$^c$ | 33.92±3.0701$^c$ | 23.16±2.4040$^b$ |
| 2 | R-2 | 24.4 | 6.3 | 4.5 | 32.68±2.0743$^b$ | 21.64±1.9042$^c$ | 52.81±2.1585$^c$ |
| 3 | R-3 | 24.2 | 6.2 | 4.3 | 21.14±2.4110$^a$ | 16.90±2.7307$^b$ | 32.99±2.6951$^c$ |
| 4 | NR-1 | 24.8 | 6.9 | 3.6 | 38.69±3.9421$^b$ | 22.18±1.9605$^c$ | 16.61±2.5643$^a$ |
| 5 | NR-2 | 24.2 | 6.8 | 3.3 | 21.21±1.9588$^a$ | 18.21±2.3257$^{b,c}$ | 37.04±1.5803$^d$ |
| 6 | NR-2 | 23.8 | 6.8 | 3.1 | 14.58±1.7157$^a$ | 11.26±1.0717$^a$ | 26.68±2.0583$^b$ |

(Values are means ± standard deviation of three replicates. Different superscript in the same column indicates significant difference by Duncan's Multiple Range Test at the P ≤ 0.05 levels)

**Isolation of marine actinomycetes**

After a week of development on starch casein nitrate media, colony characteristics such as colour, consistency, and growth pattern were detected (Fig.1). In all, 31 actinobacterial isolates with varying colony shape were isolated and analysed as SDMRI 1 to 31. White, grey, greyish white, yellow, and brownish red were the colony colours. Among the 31 isolates, rough or leathery, powdery, smooth, spotted cottony, and cottony colony consistency were detected. Table 3 shows the colony features and growth trends.

**Fig 1: Isolation of marine actinomycetes**

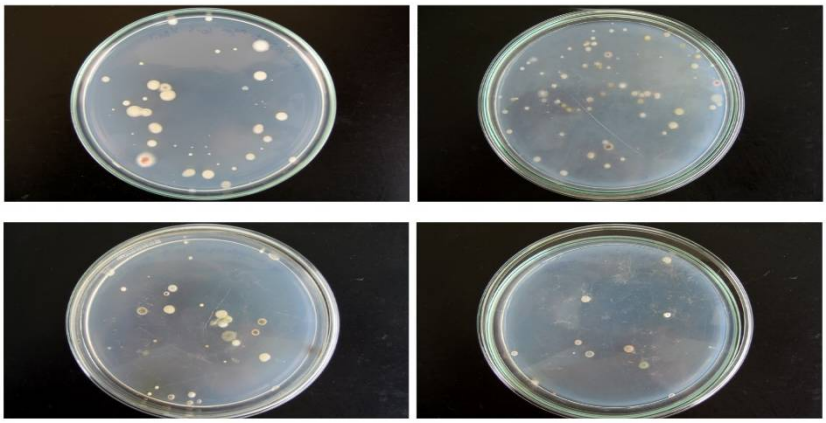

**Table 3: Morphological characteristics of marine *Actinomycetes* isolates**

| S.No | Name of isolates | Colony Colour on | Growth | Colony Consistency |
|---|---|---|---|---|
| 1 | SDMRI-1 | Greyish white | +++ | Powdery |
| 2 | SDMRI -2 | White | +++ | Powdery |
| 3 | SDMRI -3 | Grey | +++ | Leathery |
| 4 | SDMRI -4 | Yellow | +++ | Powdery |
| 5 | SDMRI -5 | Grey | +++ | Powdery |
| 6 | SDMRI -6 | White | +++ | Rough or leathery |
| 7 | SDMRI -7 | Grey | +++ | Leathery |
| 8 | SDMRI -8 | White | +++ | Powdery |
| 9 | SDMRI -9 | Greyish white | +++ | Leathery |
| 10 | SDMRI -10 | White | +++ | Powdery |
| 11 | SDMRI -11 | Greyish white | +++ | Leathery |
| 12 | SDMRI -12 | White | +++ | Leathery |
| 13 | SDMRI -13 | Grey | +++ | Dotted Cottony |
| 14 | SDMRI -14 | White | +++ | Cottony |
| 15 | SDMRI -15 | White | +++ | Leathery |
| 16 | SDMRI -16 | Yellow | +++ | Cottony |
| 17 | SDMRI -17 | Brownish Red | +++ | Powdery |
| 18 | SDMRI -18 | White | +++ | Powdery |
| 19 | SDMRI -19 | White | +++ | Powdery |
| 20 | SDMRI -20 | Brownish Red | +++ | Powdery |
| 21 | SDMRI -21 | Greyish white | +++ | Leathery |
| 22 | SDMRI -22 | Greyish White | +++ | Cottony |
| 23 | SDMRI -23 | Brownish Red | +++ | Cottony |
| 24 | SDMRI -24 | White | +++ | Powdery |
| 25 | SDMRI -25 | Grey | +++ | Powdery |
| 26 | SDMRI -26 | White | +++ | Powdery |
| 27 | SDMRI -27 | Yellow | +++ | Powdery |
| 28 | SDMRI -28 | White | +++ | Powdery |
| 29 | SDMRI -29 | Grey | +++ | Powdery |
| 30 | SDMRI -30 | Brownish Red | +++ | Powdery |
| 31 | SDMRI -31 | White | +++ | Powdery |

## Screening of actinobacteria for antibiotic production

### Primary Screening

Only 22 isolates demonstrated action against test organisms out of 31 actinomycetes exposed to first screening. Six isolates were active solely against gram negative organisms, five against gram positive organisms, seventeen against both gram positive and negative species, and three isolates exhibited no inhibitory effect against both gram positive and negative organisms (Fig.2). 22 of the isolates tested positive for *Bacillus cereus* and *Staphylococcus aureus*, 14 for *Escherichia coli*, 12 for *Pseudomonas aeruginosa*, and 11 for *Enterobacter aerogenes* and *Klebsiella pneumoniae*. SDMRI -3, SDMRI -6, SDMRI -7, SDMRI -8, SDMRI

-17, SDMRI -18, SDMRI -23, SDMRI -27, and SDMRI -31 isolates were chosen for secondary screening among the 31 isolates (Table 4).

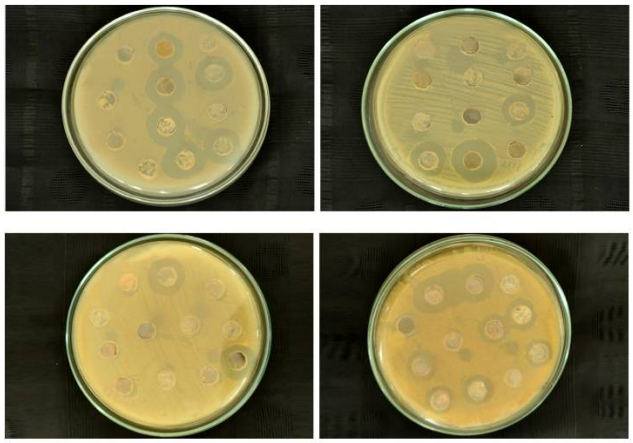

**Fig 2 :Primary screening of isolated actinomycetes for antimicrobial activity**

**Table 4: Primary Screening of isolated actinomycetes for antimicrobial activity**

| S.No | Isolates Name | *Bacillus cereus* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Enterobacter aerogenes* | *Klebsiella pneumoniae* |
|---|---|---|---|---|---|---|---|
| **1** | **SDMRI-1** | **10** | **8** | **0** | **9** | **0** | **0** |
| 2 | SDMRI -2 | 0 | 0 | 12 | 13 | 0 | 11 |
| **3** | **SDMRI -3** | **22** | **18** | **20** | **16** | **18** | **11** |
| 4 | SDMRI -4 | **10** | **9** | **13** | **0** | **0** | **0** |
| 5 | SDMRI -5 | 0 | 0 | 11 | 0 | 0 | 0 |
| **6** | **SDMRI -6** | **22** | **21** | **18** | **16** | **17** | **16** |
| **7** | **SDMRI -7** | **16** | **22** | **15** | **12** | **0** | **10** |
| **8** | **SDMRI -8** | **19** | **18** | **13** | **0** | **0** | **18** |
| 9 | SDMRI -9 | 12 | 11 | 0 | 0 | 0 | 0 |
| 10 | SDMRI -10 | 18 | 11 | 0 | 0 | 0 | 0 |
| 11 | SDMRI -11 | 0 | 0 | 13 | 12 | 11 | 15 |
| **12** | **SDMRI -12** | **10** | **9** | **0** | **0** | **10** | **0** |
| 13 | SDMRI -13 | 12 | 13 | 0 | 0 | 0 | 9 |
| 14 | SDMRI -14 | 11 | 13 | 10 | 0 | 0 | 0 |
| 15 | SDMRI -15 | 0 | 0 | 0 | 0 | 0 | 0 |
| 16 | SDMRI -16 | 0 | 0 | 14 | 0 | 0 | 0 |
| **17** | **SDMRI -17** | **15** | **12** | **16** | **18** | **13** | **16** |
| **18** | **SDMRI -18** | **21** | **19** | **15** | **13** | **14** | **10** |
| 19 | SDMRI -19 | 12 | 13 | 0 | 0 | 0 | 0 |
| 20 | SDMRI -20 | 13 | 12 | 0 | 0 | 0 | 0 |
| **21** | **SDMRI -21** | **9** | **10** | **0** | **0** | **10** | **0** |
| 22 | SDMRI -22 | 13 | 13 | 10 | 0 | 0 | 9 |
| **23** | **SDMRI -23** | **18** | **17** | **0** | **12** | **13** | **12** |
| 24 | SDMRI -24 | 15 | 14 | 0 | 12 | 0 | 0 |
| 25 | SDMRI -25 | 21 | 18 | 0 | 0 | 0 | 0 |
| **26** | **SDMRI -26** | **10** | **9** | **0** | **11** | **0** | **9** |
| **27** | **SDMRI -27** | **19** | **11** | **0** | **0** | **18** | **12** |
| 28 | SDMRI -28 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | SDMRI -29 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | SDMRI -30 | 0 | 0 | 0 | 0 | 0 | 0 |
| **31** | **SDMRI -31** | **20** | **15** | **16** | **18** | **11** | **0** |

**Secondary Screening**

Secondary screening by disc diffusion assay was performed on nine isolates, and the isolates SDMRI-3, SDMRI-6, SDMRI-17, and SDMRI-18 demonstrated broad spectrum activity against all test standard pathogens (Table 5). Except for *Enterobacter aerogenes* and *Escherichia coli*, the isolates SDMRI-7 and SDMRI-23 are active against all of the test pathogens. Among the nine isolates, SDMRI-3 marine actinomycetes isolate was discovered to be a superior antagoniser and was used for future research (Fig.3).

**Fig 3 : Morphological appearance of Streptomyces parvulus SDMRI-3**

**Table 5: Secondary Screening of isolated actinomycetes for antimicrobial activity**

| S.No | Isolates Name | *Bacillus cereus* | *Staphylococcus aureus* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Enterobacter* | *Klebsiella pneumonia* |
|---|---|---|---|---|---|---|---|
| **1** | **SDMRI -3** | **22** | **17** | **20** | **17** | **15** | **11** |
| 2 | SDMRI -6 | 20 | 14 | 15 | 12 | 10 | 13 |
| 3 | SDMRI -7 | 14 | 20 | 15 | 12 | 0 | 14 |
| 4 | SDMRI -8 | 19 | 17 | 13 | 7 | 9 | 15 |
| 5 | SDMRI -17 | 16 | 14 | 16 | 18 | 14 | 18 |
| 6 | SDMRI -18 | 21 | 19 | 16 | 12 | 14 | 12 |
| 7 | SDMRI -23 | 16 | 14 | 0 | 11 | 14 | 18 |
| 8 | SDMRI -27 | 18 | 11 | 12 | 0 | 17 | 12 |
| 9 | SDMRI -31 | 18 | 15 | 16 | 17 | 0 | 0 |

**DISCUSSION**

Actinomycetes continue to be a major target for biotechnological techniques and a source of lead compounds with innovative chemical structures in the modernistic era of medical brainstorming (27). Many prominent medications on the market today were initially created to mimic the mechanism of chemicals found in nature (28). The mangrove ecosystem is a peculiar habitat that is home to several kinds of microorganisms that play a significant role in nutrient cycling and manage the chemical environment of

the ecosystem, allowing actinobacteria to create a variety of metabolic compounds (29). Six distinct locations with varying depths ranging from 5 to 15 cm were sampled. The physio-chemical properties of the samples were examined, and the temperature varied from 23.8 to 25.2°C, the pH ranged from 6.2 to 6.8, and the electrical conductivity ranged from 3.1 to 4.8.

Bacteria in the mangrove ecosystem have dominating microorganisms that are quite well adapted to the harsh circumstances. Recent bacteriological investigations of mangrove environments focused on their sanitation, contamination, and bacterial population (30). The organisation of microbial communities is greatly influenced by sediment depth and salt levels (31), and as depth increases, bacteria and fungi are impacted by increasing hydrostatic pressure, low temperature, and nutrient deficiency.

The bacterial, fungal, and actinomycetes populations ranged from 66.67 to 14.58 x $10^6$, 33.92 to 11.26 x $10^3$, and 52.81 to 16.61 x $10^4$cfu/g of material, respectively. The findings are consistent with those of the rhizosphere population was 2.84 x $10^9$ for bacteria, 2.50 x $10^7$ for fungi, and 1.12 x$10^8$ for actinomycetes, and in non-rhizosphere 1.86 x $10^9$, 1.65 x $10^7$, and 1.00 x $10^8$ for bacteria, fungus, and actinomycetes, respectively, (32) in *Rhizophora apiculata* and *Rhizophora mucronata* plant rhizosphere and sediment in Pitchavaram mangrove ecosystem.

The discovery of multiple incipient marine actinomycetes species, their shown metabolic activity in their native surroundings, and their capacity to establish stable populations in various habitats suggest unequivocally that indigenous marine actinomycetes do exist in the oceans. Another noteworthy finding is the isolation of new compounds with biological activity from these marine actinomycetes (33), indicating that marine actinomycetes are a valuable source for the discovery of novel secondary metabolites.(34).

In India, little progress has been achieved in antibiotic research, with just a few marine sediment samples submitted to extensive examinations for the isolation of novel Streptomyces or the exercise of antibiotic discovery. According to the above assertion, comprehensive investigations on Indian mangrove sediments are required for the identification of novel Streptomyces species and newer antibiotics. As a result, it was considered that systematic screening of such samples was critical.

Actinomycetes were detected in greater abundance in *Avicennia marina* rhizosphere soil samples than in non-rhizosphere soil samples. The data clearly show that actinomycetes were most abundant in the rhizosphere soil. The highest numbers found in plant species may be attributed to the extensive root system of mangrove companions, which provides more area for actinomycetes to colonize, as well as the availability of maximum levels of aminoacids, sugars, and tannins in the rhizosphere soil (35).

The largest actinomycetes population was found at a depth of 10 cm, while the lowest populations were found at 5 and 15 cm below the sea surface. This might be because key nutrients like nitrogen and potassium were discovered in abundance between soil depths of 0 -10 cm in the mangrove environment on India's southwest coast.

There were many isolates isolated from mangrove soils that outnumbered those obtained from the terrestrial location. The prevalence of actinomycetes in mangrove soil may be attributed to the higher quality of the sediments, as measured by structure, pH, and humic substances (36). Actinomycetes diversity may be impacted by the diversity of cultivated plant species, because these bacteria thrive abundantly in the humus and leaf litter layer (37). Furthermore, various plants create a variety of secondary metabolites, some of which are harmful to soil microbes such as actinomycetes. Actinomycetes, on the other hand, have evolved to manufacture their own secondary metabolites as a result of adaptation. Thirty-one actinomycetes were isolated from the research location in this investigation.

SDMRI-1 to 31 were 31 actinobacterial isolates with distinct colony shape that were isolated and examined. White, grey, greyish white, yellow, and brownish red were the colony colours. Among the 31 isolates, rough or leathery, powdery, smooth, spotted cottony, and cottony colony consistency were detected.

Identified white-colored actinomycetes as the dominating forms in mollusks from the Porto Novo area of South India (38).

Actinomycetes have a noteworthy proven capacity for the development of novel antibiotics (39), and the practice of screening many species for new bioactive chemicals is ongoing. Screening for microbial species is critical because there is a rich supply of structurally varied secondary metabolites with pharmaceutically relevant biological activity (40). In the main screening, 22 of the 31 isolates were active against *Bacillus cereus* and *Staphylococcus aureus*, 14 against *Escherichia coli*, 12 against *Pseudomonas aeruginosa*, and 11 against *Enterobacter aerogenes* and *Klebsiellapneumoniae*.

The primary screening results show that the majority of active isolates were more active against gram positive bacteria than gram negative bacteria. The morphological variations between these microorganisms and gram negative bacteria with an outer polysaccharide membrane bearing the structural lipopolysaccharide components might be linked to the reasonableness for contrastive sensitiveness between these microorganisms. Because the cell wall is impermeable to lipophilic solutes,

gram positive bacteria should be more vulnerable, as they have just an exterior peptidoglycan layer, which is ineffective as a permeability barrier (41) SDMRI-3, SDMRI-6, SDMRI-7, SDMRI-8, SDMRI-17, SDMRI-18, SDMRI-23, SDMRI-27, and SDMRI-31 isolates were chosen for secondary screening, with the isolates SDMRI-3, SDMRI-6, SDMRI-17, and SDMRI-18 demonstrating wide spectrum activity against all test standard strains. When the putative isolates from primary screening were submitted to secondary screening, their activity differed from that of primary screening; some of the active isolates did not show activity in secondary screening, while others showed minimal activity and some showed better activity. Actinomycetes isolates that demonstrate antibiotic action on agar but not in liquid culture are frequently identified during the screening of the new secondary metabolite.(42). Among the 9 isolates, SDMRI-3 marine actinomycetes isolate found as superior antagoniser which was used in further studies

## CONCLUSION

The results of the present study revealed that the unexplored Punnakayal mangrove ecosystems a potential source for antagonistic actinomycetes which deserves for bioprospecting. Actinobacterial strain SDMRI-3 will be a good candidate for the isolation of antibiotic molecule since it showed good activity in the preliminary and secondary screening experiments.

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## COMPETING INTEREST

The authors have declared that no competing interest exists.

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