

## PRIMARY SCREENING OF ANTIMICROBIAL ACTIVITY USING ENDOPHYTIC ACTINOMYCETES FROM *AVICENNIA MARINA* (FORSK). VIHERH

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### ABSTRACT

During the present study, actinomycetes were isolated from endodermal regions of the leaves of mangrove plant, *Avicennia marina* by serial dilution and agar plate method. All the twenty one isolated actinomycetes were curtailed for their antagonistic properties against pathogenic bacterial strains procured from IMTECH, Chandigarh, India. Initially the actinomycetes were primarily screened by cross streak method and only two potential actinomycetes were tested subjected to secondary screening which was done by agar well diffusion method. Seven isolates were found to prevent the pathogenic bacteria but Avp-5 isolate was established as active against *S. aureus*, *S. epidermis*, *K. pneumoniae*, *P. vulgaris*, *B. subtilis*, *E. coli*, *S. pyogenes* and MRSA but Avp-2 showed its potential against all pathogens other than *S. epidermis*, *K. pneumoniae*. Few of the endophytic actinomycetes (around 30%) showed good antagonistic effect against most of the tested pathogens and 70% of the total actinomycetes were found non-effective against the test pathogens.

**KEYWORDS:** Antimicrobial activity, Endophytic actinomycetes, agar well diffusion method, *Avicennia marina*, MTCC strains.

### INTRODUCTION

During 1866, de Bary described and introduced the name endophyte for the first time. The discovery of the endophytes came into sight in middle of the 19<sup>th</sup> Century. Moreover, Bacon and white projected widely accepted the definition, *endophytes* are the microbes that colonize living, internal tissues of the plants without carrying any immediate over negative effects.<sup>[1,2]</sup> Since then, many more definitions have been given by different researchers with few modifications. It has been proven that it is the richest source of bioactive natural products. Endophytes could be better protected from biotic and abiotic stresses than rhizosphere bacteria.<sup>[3]</sup> The name actinomycetes was derived from Greek atkis (a ray) and mykes (fungus) and share the features of both Bacteria and fungi.<sup>[4]</sup> Actinomycetes are Gram positive, filamentous, spore forming bacteria containing high G+C content (55-75%) in their DNA and L-L diaminopimelic acid in cell wall. Actinomycetes battered with bacteria in the same class of Schizomycetes but confined to the order Actinomycetales.<sup>[5,6]</sup> The mangrove forests are one among the world's most productive ecosystems with great economic and ecological significance. Mangroves are a group of highly specialized plants that have developed unusual adaptations to the unique environmental conditions in which they are found. Salt tolerant forest ecosystem of tropical and sub-tropical

intertidal regions of the world. Their primary production enhances numerous from of wild life and avifauna as well as estuarine and near shore fisheries. According to the forest survey report 4,872 sq.mts mangrove covers in India (0.15% of the total land area) globally a 69 plant species have been identified. However, mangroves exist under condition of high salinity, strong winds, extreme tides, high temperature and muddy, anaerobic soils. These type of plants and their associated microbes, fungi, plants and animals, constitute the mangrove forest community or mangal.<sup>[7,8,9]</sup> India is well known for its diverse flora. Around 20,000 medicinally important plants have been recorded recently, but only 800 plant species are being used by more than 500 traditional communities for remediation of several diseases.<sup>[10,11,12]</sup> The present demand of new drugs needs novelty, which requires exploration of the sites that would be exclusive and unique. One of such relatively unnoticed niche is the inner tissues of the plants.<sup>[13,14,15]</sup> It is hypothesized that these microorganisms can perform better than the epiphytes or soil microorganism because of the symbiotic relationship with plants.<sup>[16,17,18]</sup> The present study is an attempt in order to isolate Actinomycetes from endodermal regions of leaves of the mangrove plant, *Avicennia marina* (Forssk) and to screen out their antibiotic potential against pathogenic bacteria.

## MATERIALS AND METHODS

### Collection of mangrove plant samples

Leaves of *Avicennia marina* (Forssk) Vierh were collected from the back water river, Ariyankuppam region of Puducherry. Leaf samples were kept in sterile polythene bags and brought into the laboratory and kept in fridge at 4-8<sup>o</sup>c for further process.

### Pretreatment of leaf samples

Collected leaf samples were washed in running water to remove the soil and other debris. Leaf samples were surface sterilized by 70% ethanol for 5mins followed by sodium hypochlorite for 10mins and washed with sterile water to remove the sterilizing agent for 3 three times.

### Isolation of endophytic Actinomycetes

Sterilized leaf samples were cut into small fragments and plated in Starch Casein Agar medium and Potato Dextrose Agar medium which was supplemented with nystatin and cycloheximide (50µg/ml) to suppress the bacterial and fungal growth respectively. The plates were incubated at room temperature for 7-10 days. Plates were examined for appearance of actinomycetes colonies from 7<sup>th</sup> day onwards. Actinomycete colonies were isolated, cultured and maintained in SCA and PDA for further use.

### Preparation of Test Organisms

Bacterial pathogens were procured from Microbial Type Culture Collection (MTCC), Chandigarh. Selected pathogens such as *S. aureus*, *S. epidermis*, *K. pneumoniae*, *P. vulgaris*, *B. subtilis*, *E. coli*, *S. pyogenes* and MRSA. All the pathogens were inoculated in Nutrient broth and stored in refrigerators at 4-8<sup>o</sup>c. 24 hours culture was used for the screening process of the actinomycetes of antibiotic potential.

### Screening of endophytic actinomycetes for antibacterial activity

#### Agar well diffusion method

All the actinomycetes colonies were inoculated in Potato Dextrose Broth medium and incubated at room temperature for a fortnight. 15 Day PDB broth culture of the seven actinomycetes isolates of good antibiotic potential is given in Fig 1. After incubation, culture media were filtered using Whatman No.1 filter paper. Nutrient agar plates were prepared and 6mm wells were created using sterile cork borer. 100µl of culture filtrate was loaded into each wells and the plates were kept for overnight in BOD incubator at 37<sup>o</sup>c for 24 to 48 hours. After incubation period over, the antibacterial activity was determined based on the zone of inhibition developed surrounding the wells and the diameter of the zones were measured in mm.

## RESULTS AND DISCUSSION

### Isolation of Endophytic actinomycetes from mangrove leaf

During the study period, a total of 21 actinomycetes were isolated from the endophytic regions of the leaves of the

mangrove plant, *Avicennia marina* collected from back water river, Ariyankuppam, Puducherry. Janaki et al (2014) worked on the soil actinomycetes to analyze their antibacterial properties from the rhizosphere soil of *Avicennia marina* collected from the same place, Ariyankuppam, Puducherry.

### Antibacterial assay of Endophytic actinomycetes

All the twenty one actinomycetes were isolated and subjected to antibacterial assay by agar well diffusion method and the results are given in Table 1. Out of the total twenty one actinomycetes isolates, 7 actinomycetes showed good activity against test bacterial pathogens (Fig 2). Of these 7 actinomycetes, two isolates Avm-2 and Avm-5 showed broad spectrum activities against all the pathogens (Fig 2 and Fig 3). Avp-5 isolate was recognized as vigorous against *S. aureus*, *S. epidermis*, *K. pneumoniae*, *P. vulgaris*, *B. subtilis*, *E. coli*, *S. pyogenes* and MRSA but Avp-2 exhibited its potential against all pathogens other than *S. epidermis*, *K. pneumoniae*. Janaki et al<sup>[12]</sup> elaborated in their work that the 25 isolated actinomycetes were subjected for primary screening against the ten gram negative and two gram positive bacteria. The total percentage of inhibition by actinomycetes against bacteria in primary screening was noted as *E. coli* (8%), *K. pneumoniae* (4%), *P. vulgaris* (4%), *P. aeruginosa* (24%), *S. typhi* (4%), *S. flexneri* (16%), *V. cholera* (4%), *B. bronchiseptica* (52%), *P. fluorescens* (0%), *E. faecalis* (8%), *B. subtilis* (40%), *S. aureus* (8%). They also explained that in total 20 (80%) of actinomycetes showed antibacterial activity towards any one of the tested bacteria, 5 (20%) actinomycetes showed no antagonistic activity and only 2 actinomycetes were selected from *A. marina* and that were subjected for secondary screening. Broad spectrum antibacterial activity was confirmed by cross streak method for selected antagonistic actinomycetes.<sup>[10,11,12,13]</sup> Nayak et al<sup>[8]</sup> worked on the antimicrobial potency of the mangrove plant leaf extracts of *Avicennia marina* against selected bacteria and fungi. Among the extracts, Ethyl alcohol extract (50µl) showed wide inhibition against all the fungi used for the susceptibility test and showed inhibition against *P. aeruginosa*<sup>[18]</sup> and *Bacillus subtilis*.<sup>[6]</sup> *Staphylococcus aureus* found to be resistant against all the 4 solvent extracts used in the study. Ethyl Methyl Ketone showed inhibition against *Proteus vulgaris*.<sup>[12]</sup> The Ethyl acetate and Chloroform extracts showed no inhibition against the test bacteria. All the leaf solvent extracts showed good inhibition against the test fungi; *R. solani*, *C. gleosporioides*, *C. lunata*, *F. oxysporum* except *C. albicans*. The growth of *C. albicans* was inhibited in Ethyl alcohol extract. Paper disc method showed less inhibitory effect than the well diffusion method.

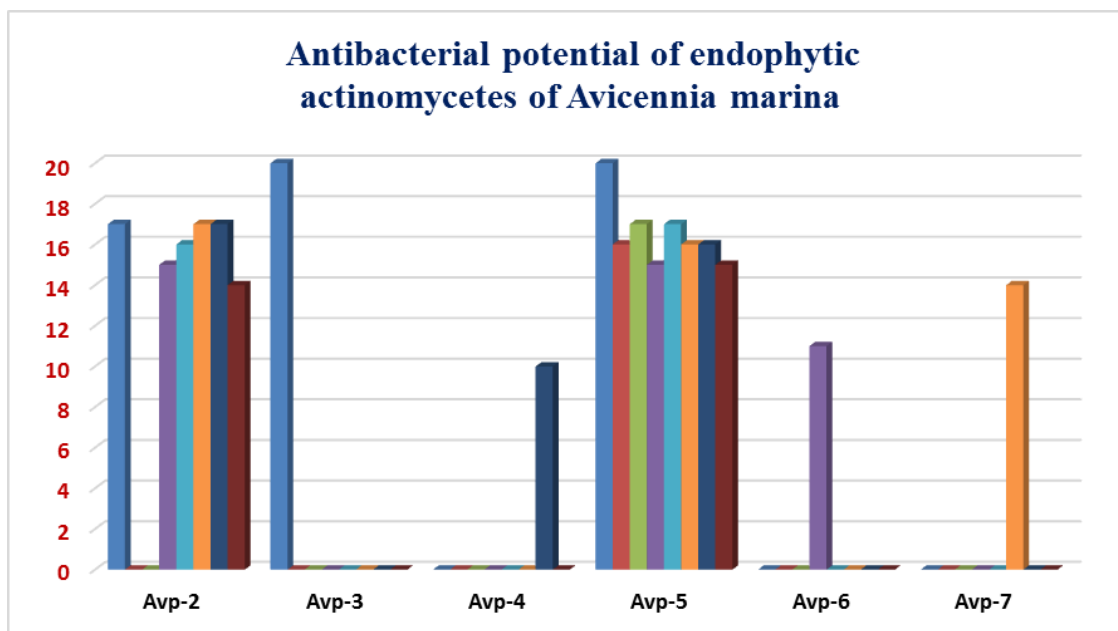
**Table 1: Primary screening of antibacterial activity of actinomycetes isolated from mangrove plant, *Avicennia marina* by well diffusion method.**

Sl. No.	Isolates of Endophytic Actinomycetes	Zone of inhibition in mm							
		<i>S. aureus</i>	<i>S. epidermis</i>	<i>K. pneumoniae</i>	<i>P. vulgaris</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>S. pyogenes</i>	MRSA
1.	Avp-1	-	-	11	-	-	-	-	-
2.	Avp-2	17	-	-	15	16	17	17	14
3.	Avp-3	20	-	-	-	-	-	-	-
4.	Avp-4	-	-	-	-	-	-	10	-
5.	Avp-5	20	16	17	15	17	16	16	15
6.	Avp-6	-	-	-	11	-	-	-	-
7.	Avp-7	-	-	-	-	-	14	-	-

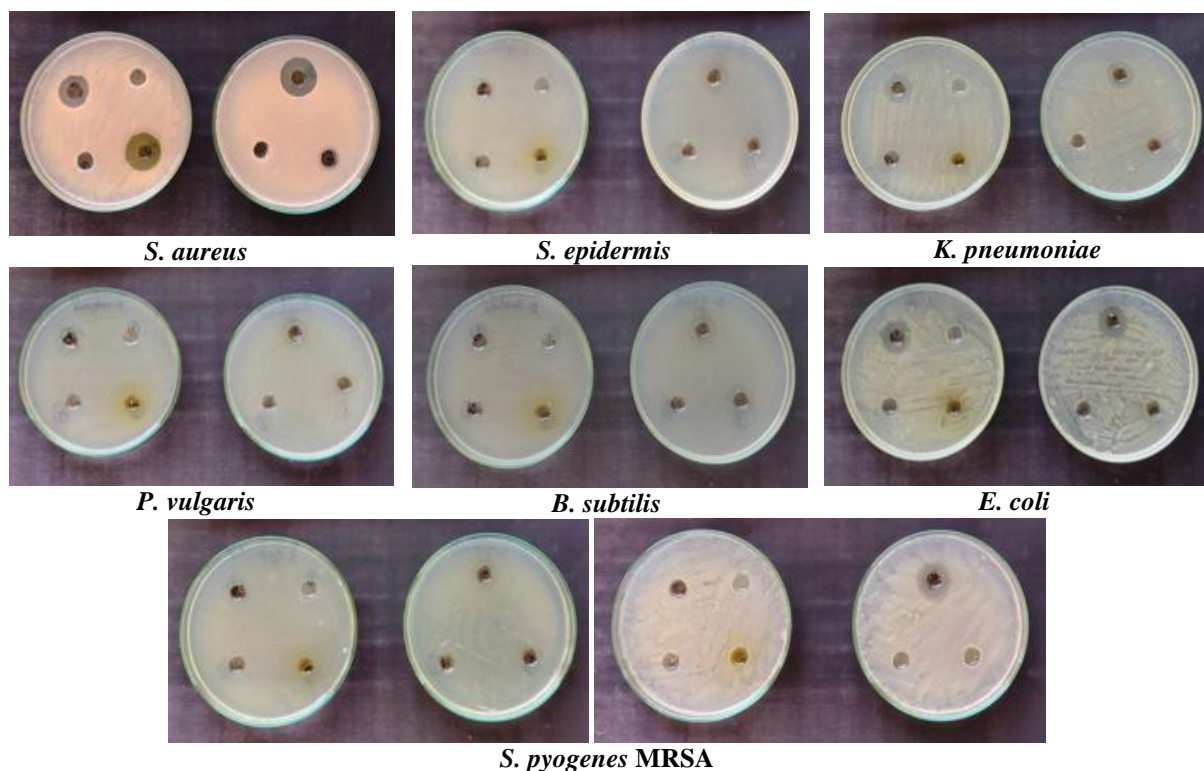


15 Day Broth culture of seven actinomycetes

**Fig. 1: 15 Day PDB broth culture of the isolated seven actinomycetes of good antibiotic potential.**



**Fig. 2: Antibacterial potential of endophytic actinomycetes of Avicennia marina.**



**Fig. 3: Antibacterial activity of Endophytic actinomycetes by Diffusion method by showing zone of inhibitions.**

### CONCLUSION

Arising of MDR pathogen and its' increasing scale day by day causing global problem in the treatment of infectious diseases and making the situation very dangerous and critical too. The present investigation aimed at overcome this circumstances by discovering new bioactive compounds from actinomycetes isolated from the endophytic region of the leaves of the mangrove plant, *Avicennia marina* which was found as a better source of actinomycetes producing bioactive metabolites having inhibitory activity against many pathogens. Since the Endophytic actinomycetes are potential source for producing antibiotics for pathogenic bacteria, these can be implemented in the pharmaceutical field to find novel drugs.

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