

ORIGINAL RESEARCH ARTICLE

Studies on Antimicrobial Activity of Marine Actinomycetes Isolated from Rameswaram

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ABSTRACT

An Actinomycetes Strain (MNR 5) was isolated from sediment sample screened for antimicrobial studies. The strain showed maximum inhibition against *B. subtilis* and *S. aureus* by cross streak assay and maximum growth was observed in ISP-1 ISP-2 and NA medium showed excellent growth, abundant aerial and sub merged mycelium formation at pH 7-9, temperature 28°C. Crude extract of benzene exhibited strong zone of inhibition against *B. subtilis* followed by *S. aureus* *P. aeruginosa*, and fungal pathogen *F. solain*. Crude extract from ethylacetate inhibited moderate against tested pathogens. The result of this study suggests that the Strain MNR 5 could be a promising source for potent antibacterial agents.

Key words: Actinomycetes, marine sediment, antimicrobial activity, human pathogen.

1. INTRODUCTION

Actinomycetes are ubiquitous in nature. They are primarily soil inhabitants and very widely distributed. It can degrade an enormous number and variety of organic compounds and are extremely important in the mineralization of organic matter. A comparative survey of all isolates demonstrated that the actinomycete population was the largest in soil of the surface layer and although the number of actinomycetes decreased gradually as depth increased, individual actinomycete strains were present in all soil layers [1].

Actinomycetes abundant in terrestrial soil, and produce bioactive compounds and provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compound. These searches have been remarkably successful and approximately two third of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes [2].

The need for new and safe bioactive compounds to provide aid and comfort in all aspect of mankind's life is ever increasing. Emergence of new diseases, development of drug resistant

pathogenic microorganisms, appearance of life threatening viruses, management of post operative complication in patient with organ transplantation, are some of the challenges in front of scientists. This situation has forced scientists to explore different natural sources for the safe and potent agents to meet the challenges of the twenty first century.

In India, the actinomycete research is still in its infancy. Pioneering work in this field was taken up by Hindustan Antibiotic Limited, Poona and they isolated 20 antibiotic substances from *Streptomyces*, *Streptovercillium* and *Chainia* species. All of them were isolated from soil samples near Poona.

Since the actinomycetes are a veritable source of valuable secondary metabolites, the net to trap them has to be cast wide by targeting a verity of habitats. Therefore, this study provides an actinomycetes strain MNR 5 isolated from Rameswaram pamban bridge sediment sample and displayed antagonistic activity and its characterization.

2. MATERIALS AND METHODS

Collection and Isolation of Sample:

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The bottom sediment mud was collected from the Pamban Bridge which is situated at the beginning of Rameswaram Town. Collected sediment was placed into a 250 ml conical flask containing 100 ml sterilized distilled water and shaken vigorously in rotary shaker at 250 rpm. This suspension was serially diluted by transferring 10 ml aliquots successively to six 50 ml conical flask containing 90 ml sterilized water. Up to seven dilutions were made the last two dilutions were used for inoculation.

One milliliter aliquots of each of the two dilutions ($10^{-6} \times 10^{-7}$) were aseptically transferred to sterilized petriplates. To this was added 20 ml molten (45°C) modified starch casein agar medium and swirled gently for uniform distribution of the inoculums. Five replicates were prepared for each dilution. This procedure was repeated for the sample. All plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 5 – 7 days^[3]. After incubation the selected colonies were subcultured on starch casein agar and PDA slants.

Antagonistic activity (Primary Screening):

The following organisms were used for antagonistic activity against bacteria pathogens *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and fungi pathogen such as *Candida albicans*, *Drechslera oryzae*, *Collectotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium solain*. The antagonistic activity was also tested by Cross streak assay^[4].

Physiological and Biochemical characters:

The Actinomycetes was characterized according to the convention of International Streptomyces Project (ISP). The standard differentiate culture media for morphological studies were (ISP-1, ISP-2, ISP-3, ISP-4, ISP-5, ISP-7, CZ, SCA, NA, and PDA). Morphological characters of the isolated were studied by inoculating a loopful of one week old cultures into 1.5% agar medium contained in test tubes at 37°C . The actinomycetes was suspended and thoroughly mixed in the semi-solid agar medium. One or two of the medium was aseptically pipette on to the cavity of a sterile glass slide. A drop of agar was spread well as the slide and allowed to solidify into a thin film so as to facilitate direct observation under microscope. The cultures were incubated at $28-30^{\circ}\text{C}$ and examined periodically for the formation of aerial mycelium, sporophore structure and spore morphology. The characteristics of the spore bearing hyphae and spore chains were observed

by direct microscopic examinations^[5]. Pigment production was observed on agar slants of peptone yeast extract iron agar (ISP – medium 5) and tyrosine agar^[6].

Culture condition on Different pH and temperature:

The growth of the isolate on ISP 4 media incubated at different temperatures (10, 15, 26, 37, 45, and 55°C) and at different pH (4 – 14) was tested to determine the optimal temperature and pH.

Fermentation and preparation of crude extract (Secondary Screening):

The selected antagonistic isolates (MNR 5) was inoculated into ISP 1 broth, and incubated at 28°C in a shaker (200-250 rpm) for seven days. After incubation the broths were centrifuged at 6000 rpm for 15 min and the cell free supernatant was filtered through Whatman No.1 filter paper. The filtrate was transferred aseptically into a conical flask and stored at 4°C for further assay. To the culture filtrate, equal volume of solvents, benzene, ethyl acetate, chloroform and ethanol were added and centrifuged at 5000 rpm for 10 min. The crude extract was then concentrated in rotary vacuum and lyophilized using a freeze drier for 5 hours at 5°C . The crude extract obtained from different solvents was tested for antimicrobial activity against selected pathogens by disc diffusion method^[7].

3. RESULT AND DISCUSSION

Microorganisms are miniature chemical factories as they produce a variety of valuable products or convert the raw-material into commercially important value-added compounds. Among the microorganisms, the filamentous bacteria actinomycetes are highly important as they are the major source of bioactive secondary metabolites, particularly antibiotics^[8]. Though they occur in every conservable habitat, hardly 10% of them been isolated so far within this group, the genus *Streptomyces* alone accounts for several novel secondary metabolites including 75% of commercially and medically useful antibiotics^[9, 10]. The sampling collected from s Pamban bridge, Mandapam post Ramananthapuram district, Tamilnadu, India [Latitude (N) $9^{\circ} 17' \text{N}$ and Longitude (E) $79^{\circ} 22' \text{E}$].

In the present study on of the promising isolates actinomycets strain MNR 5 is characterized partially and screened for bioactive potential. The antagonistic activity was also tested by Cross streak assay. MNR 5 strain inhibited *B. subtilis* and *S. aureus* and other pathogen resistant

illustrate in (Fig 1). Actinobacteria was active against *B.circulans*, *S. aureus*, *E. coli*, *S. cerevisiae* and *F. oxysporum* as well as 386 strains of actinobacteria from marine sources exhibited 75% exhibited antagonistic properties by cross streak assay [11, 12]. In this connection, present study also showed resembles report.

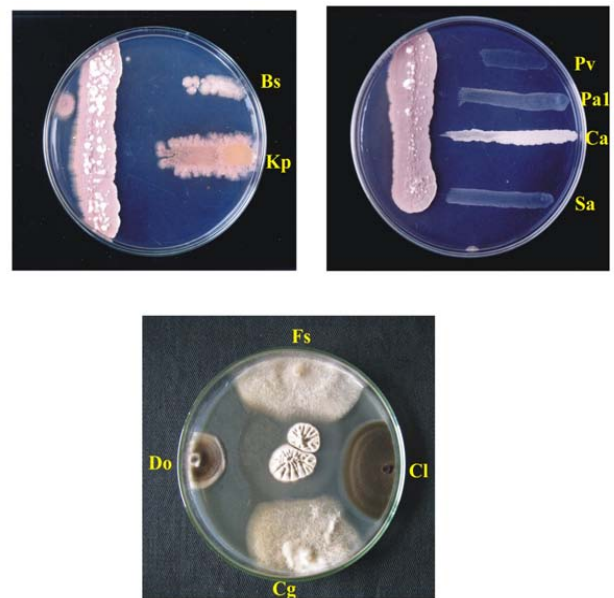
The Isolate MNR 5 was grown on ISP media for to 10 characterizations. In present study, maximum growth was observed in ISP-1 ISP-2 and NA and PDA it was poor in CZ. Both types of mycelia, aerial as well as basal mycelia were produced on all media. Whereas the basal mycelial filaments were always whitish or colorless, the aerial mycelium was white or grey. The pigmentation as viewed from the reverse side of the plate indicated that only ISP-4 did not favor pigment production showed in (Table 1). Thus the majority of streptomycete soil isolates, grouped by easily determines pigmentation characteristics, are identified to corresponding clusters in a probability matrix [13]. Similarly, acidophilic actinomycetes belonging to cluster 25 can be instantly detected as they produce characteristic pink pigmented colonies on starch casein agar supplemented with Rifampicin [14, 15].

The potential strain showed excellent growth, abundant aerial and sub merged mycelium formation at pH 7-9, temperature 28C and less growth and aerial mycelium formation at lower and higher on potato dextrose agar described in (Table 2).

Totally, 120 strains of marine actinobacteria from sediments of the Sandy Hook Bay, New Jersey by pretreatment methods among the 120 strains, 19 strains displayed significant antimicrobial activity. Most of the activity was directed against gram positive bacteria, but inhibition of gram negative species and yeasts was also evident [16]. Bioactive actinobacteria from marine sediment using starch casein agar supplemented with Rifampicin. Isolated *Streptomyces* and *Micromonospora* strains showed antimicrobial activity against both gram-positive and gram-negative bacteria [17].

Correspondingly, the solvent extract of benzene from MNR 5 strain was exhibited strong zone of inhibition against *B. subtilis* followed by *S. aureus*, *P. aeruginosa*, and fungal pathogen *F. solain*. Crude extract from ethyl acetate inhibited moderate against tested pathogens. Correspondingly crude extract of chloroform showed maximum zone of inhibition against *P. aeruginosa* and fungi pathogen *D. oryzae*, *C.*

gloeosporioides, *F. solain*. Antimicrobial activity was almost similar to that of the positive control, Ampicillin and Nystatin. An actinomycete strains of *Streptomyces* isolated from an intertidal sediment sample collected from the Key West, Florida, produced a series of new antibiotics, the bioxalomycins which showed excellent antimicrobial activity against *Staphylococcus* and *Enterococcus* spp [18]. Marine sediments were good sources for isolation of actinobacteria and M2 media good for isolation of marine actinobacteria [19]. In this study, that the isolate MNR 5 strain had broad spectrum antimicrobial activity as it suggested its potential against human pathogen.



Test Organisms : Bs = *Bacillus subtilis*; Kp = *Klebsiella pneumoniae*; Pv = *Proteus vulgaris*; Pa I = *Pseudomonas aeruginosa* I; Ca = *Candida albicans*; Sa = *Staphylococcus aureus*; Ec = *E.coli*; Fs = *Fusarium solani*; Cl = *Curvularia lunata*; Cg = *Colletotrichum gloeosporioides*; Do = *Drechslera oryzae*

Figure 1: Showing antagonistic activity of the isolate MNR 5

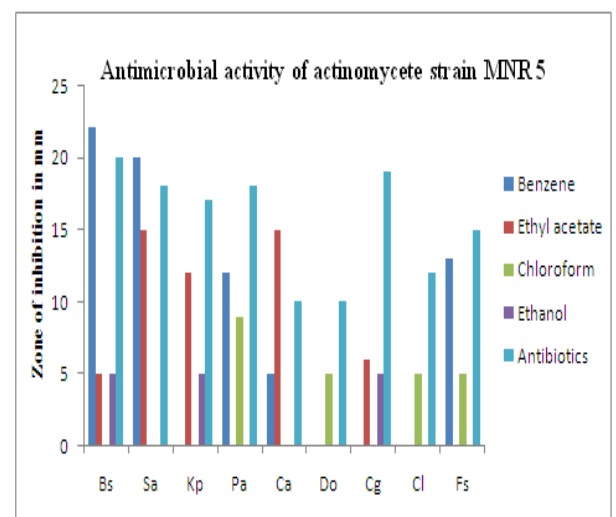


Figure 2: Antimicrobial activities of Actinomycetes isolate MNR 5 in different solvent extracts

Table 1: Colony Characteristics of Strain MNR 5 on different International Streptomyces Project (ISP) and other media

Medium	Culture Characters				
	Growth	Aerial mycelium	Basal mycelium	Colour on reverse side	Pigmentation
ISP-1	++++	White	Colourless	Black in the centre with brown margin	Pale brown
ISP-2	++++	Grey	Colourless	Black in the centre with brown margin	„
ISP-3	+++	Grey	Colourless	Brown	-
ISP-4	+++	Grey	Colourless	White	-
ISP-5	+++	Whitish	Colourless	Brown	-
ISP-7	++	Grey	Colourless	Dark Brown	-
CZ	+	White	Colourless	Brown	-
SCA	++	Grey	Colourless	Blackish	-
NA	++++	White	Colourless	Black in the centre with brown margin	Pale brown
PDA	++++	Grey	Colourless	Black in the centre with brown margin	Reddish brown

– (No growth), + (Less growth), ++ (Less to moderate), +++ (moderate), ++++ (Good), +++++ (very good)

Table 2: Growth of Actinomycetes [MNR 5] in Potato Dextrose broth (PDB) at different pH and temperature

S. No	Initial pH	Temperature °C	Initial Color of medium	Color of aerial mycelium	Growth		Final colour of filtrate
					aerial mycelium	sub-merged mycelium	
1	4	5	-	-	-	-	-
2	5	10	-	-	+	+	-
3	6	15	-	-	++	++	Brownish
4	7	20	Light Yellow	Pale grey	+++	+++	Brownish
5	8	28	Light Yellow	Light grey	++++	++++	Brownish
6	9	30	Golden brown	Grey	+++	+++	Brown
7	10	35	Dark grey	Dark grey	+	+	Dark Brown
8	12	40	Brown	-	-	-	Dark brown

4. CONCLUSION

It has been concluded that, the use of new screening approaches to identify potent actinomycetes from marine sediment is essential for the discovery of novel natural substances with valuable biological activities.

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