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Short Communication

ISOLATION OF ANTAGONISTIC ACTINOMYCETES FROM MUTHUPET MANGROVE FOREST SOIL AND THEIR ANTIBACTERIAL ACTIVITY

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ABSTRACT

The development of new antimicrobial agents, preferably naturally occurring ones with novel mechanisms of action, is an urgent medical need. Soil in particular is an intensively exploited ecological niche, the inhabitants of which produce many useful biologically active natural products, including clinically important antibiotics. In the present study, actinomycetes species were isolated from Muthupet mangrove forest soil samples and analyzed their antagonistic effect. The positive antagonistic actinomycetes colony was identified and antibacterial activity of positive actinomycetes strains was analyzed against antibiotic resistant bacterial strains. Actinomycetes are prolific producers of antibiotics and important suppliers to the pharmaceutical industry, can produce a wide variety of secondary metabolites. *Streptomyces parvulus* fermentation antibiotic compound was extracted using ethyl acetate by centrifugation and antibacterial compound containing disc were prepared separately, the disc were used for assay of antibacterial activity against antibiotic resistant bacterial strains. Finally concluded that the isolated actinomycetes *Streptomyces parvulus* was highly recommended for antibiotic production in industrial level, it will create new sector in the pharmaceutical field.

Keywords: Actinomycetes, *Streptomyces parvulus,* Antagonistic activity.

1. INTRODUCTION

Now a days most important problem for clinical filed is antibiotic resistant bacteria. In the developing countries like India, this problem persists from last two decades. The reason of problem is overuse and misuse of antibiotics. Antibiotic resistant bacteria affect anyone, of any age in any country. The various studies on antibacterial resistance showed that extended spectrum β-lactamases (ESBL), Metallo- β- lactamases (MBL) and Methicillin Resistant *Staphylococci aureus* (MRSA) have become very common in India [1-5]. In this situation two courses of action are done, one is awareness of anti-biotic uses for inhabitants and another one is to discover new antibiotics against antibacterial resistance strain from natural sources because the resistant power usually not present in environmental habit of bacteria but that strain transfer or infected to human become antibacterial resistant.

2. MATERIAL AND METHODS

2.1. Sample collection

Soil samples were collected from Muthupet Mangrove forest (Latitude of 10°46'N Longitude of 79.51'E), Tamilnadu, India in sterile airlock polythene bags and transported to the laboratory according to a previously described method [6]. Collected samples were stored at 4°C until do the further use.

2.2. Preparation of soil samples

The collected soil samples were subjected to pre treatment of dry heat at 56°C for 10 minutes in order to increase the number of mycelium-forming actinomycetes relative to the non-actinomycetal heterotrophic microbial flora. After that one gram dried soil samples were added to 10 ml sterile water and further diluted up to 10^{-6} dilution in sterile water.

2.3. Isolation of actinomycetes

A 0.1 ml of each diluted sample was inoculated by spreading with a sterile glass rod on actinomycetes isolation agar medium separately [7]. The media were supplemented with antibiotics of cycloheximide (40 µg/ml), nystatin (30 µg/ml) and nalidixic acid (10 µg/ml) after autoclave to inhibit the fungal and

nonfilamentous bacterial growth. The inoculated plates were incubated at 30°C for 7 to 9 days or until appearance of colonies with a tough leathery texture, dry or folded appearance and branching filaments with or without aerial mycelia.

2.4. Test Antibiotic resistant bacterial Strains

Four antibiotic resistant bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus pyogenes* were used in this study and obtained from Microbial Type Culture Collection Centre at Chandigarh, India.

2.5. Primary Screening of antibacterial activity

2.5.1. Screening of Antagonistic effect

The log phase antibiotic resistant bacteria cultures were swabbed on prepared muller-hinton agar plates separately. The ESBL and AmpC detection Ezy MICTM Strip was placed on bacteria cultures swabbed plates. It is a unique phenotypic ESBL and AmpC detection strip which is coated with mixture of 4 different antibiotics with and without clavulanic acid on a single strip in a concentration gradient manner. The upper half has Ceftazidime, Cefotaxime, Cefepime and Cloxacillin (Mixture) + Clavulanic acid with highest concentration tapering downwards, whereas lower half is similarly coated with Ceftazidime, Cefotaxime, Cefepime and Cloxacillin (Mixture) in a concentration gradient in reverse direction. The bacteria cultures swabbed plates top corner were inoculated isolated actinomycetes. All plates were incubated and antagonistic effect was observed.

2.5.2. Identification of Actinomycetes

The positive antagonistic actinomycetes colonies were identified based on the cultural and morphological characteristics [8].

2.5.3. Preparation of Inoculum

The isolates actinomycetes were grown on starch casein agar slant at 30°C for 7 days for complete sporulation. Five (5)ml of sterile water was added to the slant and the spores were scraped and transferred into a 100ml Erlenmeyer flask containing 50ml of broth medium. After inoculation, the flask was incubated at 30°C in shaker for 48 hours. The microorganisms were harvested and washed with sterile saline solution and the cells were resuspended in 25ml sterile saline solution. This cell suspension was used as inoculums.

2.5.4. Fermentation and Extraction of Antibiotic Compounds

The selected antagonistic actinomycetes was inoculated into starch casein nitrate broth medium separately and incubated at 28°C on rotary shaker at 220 rpm for 7 days. After incubation, the broth was filtered through Whatmann no1 filter paper and then Millipore filter. The filter was transferred aseptically into a conical flask and stored at 4°C for further study. The culture filtrate mixed with equal value of ethyl acetate separately and centrifuged at 5000rpm for 10min to extract anti-microbial compounds.

2.5.5. Disc preparation

The 6 mm (diameter) discs were prepared from whatmann no.1 filter paper. The discs were sterilized by autoclave at 121°C. After sterilization the moisture discs were dried on hot air oven at 50°C. The discs were impregnated with each extracts and left to dry on hot air oven at 40°C.

2.6. Assay of antibacterial activity

The antibacterial activity of actinomycetes extracts were analyzed against antibiotic resistant bacteria by Disc diffusion assay method [9]. The sterile Muller Hinton agar plates were prepared and the test organisms such as *Escherichia coli, Staphylococcus aureus, Streptococcus pyogens* and *Pseudomonas aeruginosa* were spread over the Muller Hinton agar plates by using sterile cotton swaps separately. After the bacterial swaping, the prepared extract disk was placed on the each plate. All the plates were incubated at 37°C for 24hours. After incubation, the plates were observed for zone of inhibition.

3. RESULTS AND DISCUSSION

In the present study, actinomycetes species were isolated from Muthupet mangrove forest soil samples and analyzed their antagonistic effect. The positive antagonistic actinomycetes colonies were identified and antibacterial activity of positive actinomycetes strains were analyzed against clinical pathogen. In this study, totally six actinomycetes species were isolated from the mangrove forest soil sample. In this starch casein nitrate agar medium, actinomycetes colonies showed in powdery white colour colonies, which are named as ISA1, ISA2 upto ISA6. Sivakumar [10] reported that the characteristics can be used as markers by which an individual strain can be recognized. Particularly, chemotaxonomy plays a major role in identification of actinomycetes to generic level. West Coast of India,

especially from Ernakulam to Kannur has wide range of salinities and was selected as an ecosystem for studying the diversity of actinomycetes and their antimicrobial properties.

The isolated actinomycetes colonies antagonistic effects were analyzed against some antibiotic resistant bacterial strains such as *Escherichia coli, Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus pyogens*. Among the 6 Actinomycetes isolates positive antagonistic effect only noted in ISA1 (table 1). The positive antagonistic effect results were viewed in fig. 1. The present study revealed that among the isolates *Streptomyces* was the dominant genera. Frequency and dominance of *Streptomyces* among actinomycetes in various soil types were reported by several workers [11-12]. Alexander [13] reported that about 20-45% of marine actinomycetes exhibited antimicrobial activity; whereas actinomycetes isolated from marine sediments of Vishakapatnam, exhibited only 18% of antimicrobial activity as stated by Ellaiah and Reddy [14].

Table 1: Screening of Antagonistic effect

Isolated Actinomycetes	Antagonistic effect
ISA1	+
ISA2	-
ISA3	-
ISA4	-
ISA5	-
ISA6	-

+ Positive; - Negative

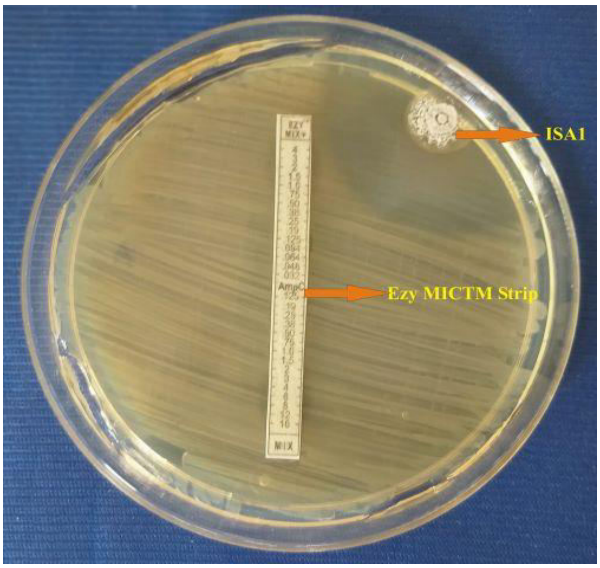

Fig. 1: Antagonistic effect against antibiotic resistant bacteria *Streptococcus pyogens*

After screening positive antagonistic isolates were identified at a generic level based on the cultural and morphological characteristics. Based on the observation, the positive antagonistic isolate ISA1 was confirmed as *Streptomyces parulus*. Tartora *et al.,* [15] reported normally, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella typhimurium* are even capable of growth in some antibiotics and their resistance to more antibiotics has also been medical concern. In this study maximum antibacterial activity were noted *Streptococcus pyogenes* (14mm) compare than other test organisms (table 2). Marine *Streptomyces sp* exhibited the highest antibacterial activity against *Pseudomonas aeruginosa* followed by *Staphylococcus aureus, Klebsiella pneumoniae* and *Salmonella typhimurium*. It is interesting to note that this response represents antibiotic potential competing microorganisms against *Pseudomonas aeruginosa, Salmonella typhimurium* and *Klebsiella pneumonia* in the environment.

Table 2: Antibacterial activities of *Streptomyces purvulus* fermented extracts against some clinical antibiotic resistant bacteria

Bacterial Strains	Zone of Inhibition (mm in diameter)
Escherichia coli	10±0.81
Pseudomonas aeruginosa	08±0.78
Staphylococcus aureus	12±0.29
Streptococcus pyogens	14±0.80

Values are expressed Mean ± Standard Deviation; n=3

4. CONCLUSION

In the isolated actinomycetes colonies, antagonistic effect was analyzed against some antibiotic resistant bacteria. The positive antagonistic actinomycetes colony ISA1 was identified based on the cultural and morphological characteristic and confirmed as *Streptomyces parvulus*. After fermentation, the antibiotic compound was extracted using ethyl acetate by centrifugation. Antibacterial compound containing disc were prepared separately, the disc were used for assay of antibacterial activity against clinical pathogens. Among various pathogens highest antibacterial activity recorded against *Streptococcus pyogenes* compared the other test bacteria. Finally concluded that the isolated *Streptomyces parvulus,* were highly recommended for antibiotic production in industrial level, it will create new sector in the pharmaceutical field. In the further study optimization of the productivity of antimicrobial compounds and analysis of the chemical nature of the product is proposed.

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