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Isolation of Antibiotic Producing Actinomycetes from Untapped Soils of Yarada Hills And Assessment of their Antimicrobial Activities

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ABSTRACT

The purpose of this study was to isolate the antibiotic producing actinomycetes from soil from different parts of Yarada hills and to access their antimicrobial activity against different pathogenic bacteria like *Staphylococcus aureus* (ATCC 25923), *Escherichia coli*(ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). This study also focuses on investigating the distribution of the antibiotic producing actinomycetes according to the texture and cultivation status of soil. Seventy four colonies of actinomycetes were isolated from the soil collected from five different parts of Yarad hills in the standard medium out of which 10 colonies produced antibiotics; all of them belonged to genus Streptomyces. One of the isolated colony largely inhibited the growth of *E. coli* and *Staphylococcus aureus* with zone of inhibition more than 20 mm for both. Antibiotic from this colony was produced by submerged fermentation and the minimum inhibitory concentration of the crude antibiotic was determined.

Keywords: Actinomycetes, Antibiotics, Streptomyces, Submerged Fermentation.

I. INTRODUCTION

Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil [1]. They have been noted to serve as rich reservoirs of medicinal antibiotics and are therefore extremely relevant to scientists, pharmaceutical industries and agricultural industries. They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. Approximately two thirds of naturally occurring antibiotics have been isolated from actinomycetes [2]. A huge number of currently used antibiotics including erythromycin, streptomycin, rifamycin and gentamycin, are all products isolated from soil actinomycetes [3]. The two major groups of soil actinomycetes that serve as important sources of antibiotics are Streptomyces and Micromonospora. It has been stated that Streptomyces account for about 80% of the total antibiotic products; while Micromonospora closely follows with less than one tenth as much as Streptomyces [4]. According to the World Health Organization, inappropriate prescription and the improper use of antibiotics has led to the development of antibiotic resistance in many bacterial pathogens. Due to this reason, the current leading problem of the world is that the emergence of drug resistant strains of pathogens is more rapid than the rate of discovery of new drugs and antibiotics. Serious infection causing bacteria have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century [5]. *Staphylococcus aureus*, for instance, a virulent pathogen that is responsible for a wide range of infections, has developed resistance to most classes of antibiotics [6]. In addition to that other opportunistic pathogens like *E. coli* and *Pseudomonas aeruginosa* are also rapidly developing resistance to a variety of classes of antibiotics and becoming multidrug resistant organisms. Today, many scientists and pharmaceutical industries concentrate on the isolation and screening of actinomycetes from different unexplored habitats, for the production of antibiotics. The increasing problem of antibiotic resistance demands the discovery of new antibacterial agents effective

against thoseresistant to currently available antibiotics. Actinomycetes are distributed widely, however only a small percentage of globe and a small proportion of actinomycetes have been screened [7]. So there is a need to screen actinomycetes, a potential antibiotic producer from different habitats for antimicrobial activity with the hope of discovering new strains capable to produce antibiotics against the multi-drug resistant pathogenic microorganisms. Thus, the present study was undertaken to isolate actinomycetes from the soil samples of Yarada hills and to assess their anti-bacterial activities.

II. METHODS AND MATERIAL

Isolation of Actinomycetes

During the study, Nine soil samples were collected aseptically from nine sites at different depth of 15 cm using standard methods. The collected samples were transferred to research laboratory of microbiology, Department of Microbiology, Andhra University, Visakhapatnam, where the entire research work was carried out. From each sample, 1g of soil sample was added in test tube containing 10 ml distilled sterile water and shaken well using vortex mixer. This was then serially diluted by using two fold serial dilution methods up to 10-6. These test tubes were considered as stock cultures for different soil sample sites. And from each tubes 0.1 ml of sample was taken and spread plate technique was performed and the plates were incubated at 37 °C for 3-4 days for the isolation of actinomycetes. The colonies of actinomycetes were identified and the basis of musty odour and powdery colony and simple staining was performed to visualize hyphae and mycelial structure under microscope.

Primary Screening

Actinomycetes isolated and identified from different soil samples were screened for antibiotic production. The test bacteria used for primary screening were *Escherichia coli* ATCC25922 (*E. coli*), *Staphylococcus aureus* ATCC2923 (*S. aureus*), *Pseudomonas aeruginosa* ATCC27857 (*P. aeruginosa*). Plate containing Mueller Hinton agar was streaked with isolated colonies of actinomycetes in the centre along the length and then, fresh sub cultured test organisms were streaked perpendicular to the actinomycetes isolates. Then the plates were incubated at 37°C for 24 hours. After incubation observation for zone of inhibition was made and recorded.

Table 1 : Total number of actinomycetes isolated and characterization of antibiotic producers after microscopic observation

S.No.	Soil type	No. of colonies isolated	Antibiotic producing colonies	Genus (Microscopic observation of hyphae)
1	Lacustrine	9	MB-1	Streptomyces
2	Sandy	4	MB-2	Streptomyces
3	Alluvial	11	MB-3	Streptomyces
4	Alluvial	23	MB-4	Streptomyces
5	Lacustrine	6	MB-5	Streptomyces
6	Alluvial	9	MB-6	Streptomyces
7	Sandy	4	---	Streptomyces
8	Lacustrine	3	---	Nocardia
9	Sandy	5	MB-7	Micromonospora

Table 2. Antimicrobial activity of the antibiotic producing actinomycetes

Test organism	Antibiotic producing colonies (Zone of inhibition in mm)						
	MB-1(white)	MB-2(red)	MB-3(grey)	MB-4(grey)	MB-5(brown)	MB-6(white)	MB-7(yellow)
Staphylococcus aureus (ATCC 25923)	15	9	14	11	16	20	4
Escherichia coli(ATCC 25922)	11	7	8	8	10	18	9
Pseudomonas aerugin	13	8	5	-	5	-	6

osa (ATCC 27853).							

Submerged Fermentation and Extraction of Crude Antibiotics

Based on the results of primary screening each isolates of actinomycetes were subjected to submerged fermentation in Yeast Malt Extract Agar (YMEB) with continuous shaking for about 4 days at 28°C. The extract was separated by centrifugation at 3000 rpm for 20 minutes. The pellets were discarded and supernatant was mixed with double volume of chilled acetone and left overnight. The mixture was centrifuged at 5000 rpm for 20 minutes. The crude extract of antibiotic was washed with tris-phosphate buffer.

Assessment of antimicrobial activity

Minimum inhibitory concentrations (MIC) of the antibiotics were determined by broth two fold serial dilution method. Different concentration of the test crude antimicrobial extract was taken along with nutrient broth and growth was observed after 24 hours of incubation at 37°C. The mixture tube with minimum growth was streaked on nutrient agar and again incubated at 37°C for 24 hours. Scarce or no growth in the plates confirms that concentration as MIC [8].

III. RESULTS AND DISCUSSION

Actinomycetes isolation

From all of the soil samples collected from different places which varied in their texture, a total of 74 isolates of actinomycetes were isolated. The distribution of actinomycetes varied with the texture and cultivation status of the soil, with the highest number found in alluvial soil. (Table 1). Each of the isolates were later categorized according to their morphology that includes colony colour ranging from dark grey, grey, dark brown, brownish, whitish and yellowish white and also microscopic appearance of colonies after simple staining.

Primary Screening

Three strains of pathogenic bacteria *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853) were chosen as the test strains for the study. Primary screening performed showed that 18 colonies were able to produce antibiotics. Out of those, seven colonies had great potential for antibiotic production with large zone of inhibition (Table 2). The distribution of antibiotic producers varied with the texture and cultivation status of the soil, with the highest number found in regularly cultivated alluvial soil. Among them three colonies (MB-1, MB-5, MB-6) produced antibiotic against *Staphylococcus aureus*, one of the colony (MB-3) against *Pseudomonas aeruginosa* and one of the colony (MB-6) was a broad spectrum antibiotic producer that largely inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* (Figure 2).

Minimum Inhibitory Concentration (MIC) The crude antibiotic from colony MB-6 was obtained by submerged fermentation and the MIC of the crude antibiotic was determined by turbid metric method. It was found that the MIC for *Staphylococcus aureus* was 70µl/ml and 90µl/ml for *Escherichia coli*. (Figure 3). The MIC was confirmed by streaking the suspension from respective test tubes from the turbid metric test tube in Nutrient Agar plates which showed very scarce growth after 24 hours of incubation at 37°C.

IV. CONCLUSION

Present study was an attempt to isolate different strains of actinomycetes from Yarada hills that possessed antimicrobial activity. The present investigation concludes that the availability of antibiotic producing actinomycetes varied depending on the texture and cultivation status of the soil, the highest number found in regularly cultivated alluvial soil. Out of the total isolates 18 colonies produced antibiotics; all of them belonged to genus Streptomyces. One of the isolated colony largely inhibited the growth of *E. coli* and *Staphylococcus aureus* with zone of inhibition more than 20 mm for both during primary screening. It was found that the MIC for *Staphylococcus aureus* was 70µl/ml and 90µl/ml for *Escherichia coli*. Further studies need to be carried out to discover potential antibiotic producers along with the detailed molecular study.

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