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Isolation of antibiotic producing Streptomyces sp. from soil of Perambalur district and a study on the antibacterial activity against clinical pathogens

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Abstract : Actinomycete are a group of microorganisms intermediate in properties between bacteria and fungi. They are gram-positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants. They produce slender, branched filaments that develop into mycelia. The filament may be long or short, depending on the species. They form aerial mycelia, much smaller than those of fungi and many species produce asexual spores called conidia. In fact the leathery or powdery appearance of actinomycetes colonies is due to the production of conidia. Actinomycetes were isolated from various soil samples collected from paddy field and Rice field of Perambalur district. The isolates were assayed for antibiotic activity against E.coli, Pseudomonas aeruginosa, Shigella flexneri, Staphyloccous aureus and Streptococcous pyogenes.
Keywords: Actinomycetes, Streptomyces, Antibacterial Activity, Antibiotic.

INTRODUCTION

Actinomycetes are gram-positive, free-living, saprophytic bacteria, widely distributed in soil, water, and colonizing plants. From the 22,500 biologically active compounds that have obtained form microbes, 45% are produced by actinomycetes, 38% by fungi, and 17% by unicellular bacteria[1]. The number and types of actinomycetes present in a particular soil would be greatly influenced by geographical location such as soil temperature, soil type, soil pH, organic matter content, cultivation, aeration and moisture content. The species belong to the genus *Streptomyces* constitute 50% of the total population of soil actinomycetes and are well known for producing a variety of bioactive secondary metabolites including antibiotics, immunomodulators, anticancer drugs, antiviral drugs, herbicides, and insecticides[2]. *Streptomyces* species are gram-positive, aerobic microorganisms with high DNA G+C contents and produce about half of all known antibiotics from microorganisms. In fact, *Streptomyces* species are the resource of 75% of commercially produced and medically useful antibiotics.

Actinomycetes resemble bacteria in which they contain peptidoglycan in their cell walls and possess flagella similar to that of bacterial flagella. In addition actinomycetes are sensitive to antibacterial antibiotics and not antifungal antibiotics. Actinomycetes are also sensitive to lysozyme. Actinomycetes differ from fungi in their cellular composition. They do not possess chitin and cellulose that is found in the cell wall of fungi. Actionmycetes will provide a valuable resource for novel products of industrial interest, including enzymes and antimicrobial agents[3,4]. The metabolic diversity of the actinomycetes family is due to their extremely large

genome, which has hundreds of transcription factors that control gene expression, allowing them to respond to specific needs[5]. They are of universal occurrence in nature, living and multiplying in both cold and tropical zones, and have been reported to occur even under the most extreme conditions of the desert[6]. The temperate zones are, however, generally most favorable for their development[7]. Many commercially important bioactive compounds and anti tumour agents has been produced using actinomycetes[8], Waksman and Lechevalier(1962) after the discovery of the antibiotic actinomycin the first antibiotic from actinomyete[9].

A number of actinomycetes species are human pathogens. They may be obligate pathogens. Actinomycete infections are acquired from natural wastes are however most likely to arise from exogenous species, particularly members of the genera Nocardia, Tetinomadara and Streptomyces. The ideal temperature for the growth of actinomycetes is in the range of 25 to 30^0C. It is anticipated that the isolation, characterization and the study on actinomycetes can be useful in the discovery of antibiotics and novel species of actinomycetes.

MATERIALS AND METHODS

Collection of soil samples : Soil samples were collected from the different places of Perambalur District . Samples were collected from various depth of the earth surface, ranging from layers just beneath the upper surface to 1 meter depth. They were collected in the sterile small plastic tubes and properly labeled with the date of collection. The collected soil samples were dried in a hot air oven at 60–65◦C for 3 hours and stored in 4◦C until examined.

Isolation of Actinomycetes

From each 1 gm of dried soil was suspended in 9mL sterile water, and successive serial dilutions were made by transferring 1mL of aliquots to 2nd test tube containing 9mL of sterile water, and in this way dilutions up to 10−4 were prepared. Each time the contents were vortexed to form uniform suspension. Numerous media have been used for the isolation of actinomycetes from soil and other natural materials. Glycerolarginine medium[10], starch casein agar medium[11]. An aliquot of 0.1mL of each dilution was taken and spread evenly over the surface of starch-casein nitrate agar[11] medium supplemented with cycloheximide on petridishes. Plates were incubated at 32◦C and monitored for 7 days. The colonies were carefully counted by visual observation and c.f.u per gram of soil was determined. Plates those gave 100–150 colonies were chosen for further isolation in pure culture. Suitable colonies those showed *Streptomyces* like appearance under light microscope were recultivated several times for purity. The purified actinomycetes were preserved on yeast-extract-glucose-agar slants at 4◦C for two months and at −20◦C in the presence of glycerol (15%v/v) for longer periods.

Identification and Characterization of Actinomycetes

The isolated actinomycetes which shows antimicrobial activity were characterized by

morphological and biochemical methods.The color of the aerial mycelia and pigment production by the isolates were determined on yeast-extract-glucose-agar plates after 7 days of incubation at 32◦C. The color of the substrate mycelia and those of the soluble pigment were determined according to the National Bureau of Standards Color Chart[12] . The microscopic characterization was done by cover slip culture method[13]. The mycelium structure, color and arrangement of conidiospores and arthrospore on the mycelium were observed through the oil immersion (100X). The observed structure was compared with Bergey's manual of determinative bacteriology, Ninth edition (2000) and the organism was identified.

Microorganisms:

In this study common human pathogenic bacteria against E.coli, Pseudomonas aeruginosa,Shigella flexneri, Staphyloccous aureus and Streptococcus pyogenes were used. These cultures were isolated from various clinical specimen and identified by morphological and biochemical characteristics. The organisms were maintained on agar slope at 4^0c and subcultured for 24 hours before use.

Screening of Antibacterial Activity

Screening for antibiotic activity of the three isolates were done by using streak-plating technique on yeast-extract-glucose- agar medium. Each pure isolates were streaked individually on different agar plates in a single line at the centre. The plates were then incubated at 32◦C for 5 days to allow the isolates to secrete antibiotics into the medium. After the incubation period, Standardized inoculum of 1 – 2 x107 CFU/ml with 0.5 Mc Farlaand was prepared for test bacteria. They were crossstreaked along the line at a 900 angle to of fully grown

actinomycetes strains.. Each streaking was started near the edge of the plates and streaked toward the *Streptomyces* growth line. The plates were then incubated for 12 hrs at 37◦C. *The plates were examined for the zone of inhibition after 24 hours.* Each zone of inhibition was measured with a ruler[14].

RESULT AND DISCUSSION

This study was performed to isolate actinomycete with antimicrobial activities using selective isolation media. Three different actinomycete strains were isolated from different soil samples collected from Perambalur district. These strains were isolated by using Starch-casein-nitrate-agar media supplemented with cycloheximide (100 μg/mL) to inhibit fungal growth. The colony forming units (c.f.u) were determined by counting the colonies on the dilution plates. Purified three isolates were grown on yeast-extract-glucose-agar media. They were slow growing,aerobic, glabrous or chalky, folded, and with aerial and substrate mycelia of different colors. They were gram positive and acid fast negative, Further they were identified as Streptomyces sp according to Bergeys manual of determinatve bacteriology by morphological and biochemical characteristics.

Three isolates antibiotic producing streptomyces sp (APS1.ASP2,ASP3) were tested for antibacterial activity against five clinically important pathogens E.coli, Pseudomonas aeruginosa,Shigella flexneri, Staphyloccous aureus and Streptococcous pyogenes (Table 1). Isolate APS 2 shows highest antibacterial activity against staphylococcus aureus 23mm.Similarly among the five organisms tested isolate APS1 shows highest activity against staphylococcus aureus 21mm and lowest against Shigella flexneri 9mm.Isolate APS 2 shows highest antibacterial activity against staphylococcus aureus 23mm and lowest against Streptococcous pyogenes 12mm. Isolate APS3 Isolate APS 2 shows highest antibacterial activity against Shigella flexneri 17mm and lowest against E.coli 9mm.

Actinomycetes exhibit a high degree of resistance at low temperature, a property which doubtless account for the occurrence in soils[15]. Although actinomycetes may be found in both cultivated and virgin soils, that are especially abundant under alkaline conditions and soils of high organic matter content[16]. Morphological and biochemical properties of the promising strain were found to be similar to those described by Williams and Cross (1971)[17]. *Streptomyces* is the most common saprophytic bacteria exist in the soil. Gottlieb (1973) described the existence of this organism in the soil is very important due to their roles in decomposing organic matter, and its ability to produce antibiotics. Dehand et al.,2010 reported that 13% of actinomycetes isolated from soil exhibit antimicrobial activity[18].

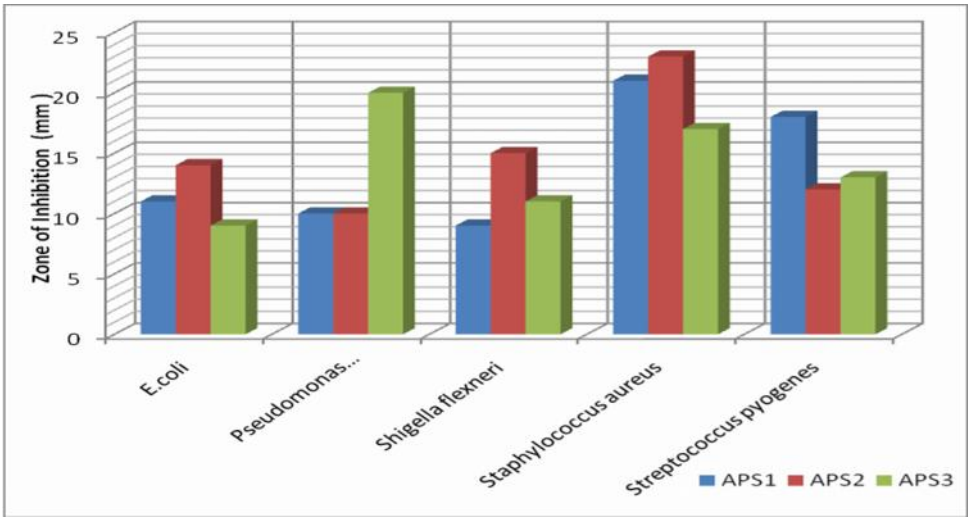
Streptomyces are well known group of *Actinomycetes* which have a great ability to produce most important secondary metabolites such as antibiotics, anti tumors, anti viral, anti fungal and etc. since some of the most important antibiotics that used in medicine obtain from *Streptomyces* resources, the investigation on the production of this kind of secondary metabolites from

Streptomyces species is already outspread.The present study shows that streptomyces sp APS2 shows antibaterial activity against staphylococcus aureus and Pseudomonas aeruginosa.It can be concluded that the soil samples of Perambalur district are rich source of actinomycetes which produce metabolites inhibitory to bacterial pathogens. Future studies will be done to identify the active isolates up to the species level. The type of antimicrobial agents produced by these isolates will be investigated as well.

Table. 1. Antimicrobial activity of Streptomyces isolate

Isolate	Zone of Inhibition (mm)				
	E.coli	Pseudomonas aeruginosa	Shigella flexneri	Staphyloccous aureus	Streptococcous pyogenes
APS 1	11	10	9	21	18
APS2	14	20	15	23	12
APS3	9	12	11	17	13

Figure. 1. Antimicrobial activity of Streptomyces isolate



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