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Study of antibiotic activities of some actinomycetes

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Antibiotics are the secondary metabolites that are produced in low concentration by one type of microorganisms that selectively inhibit or kill other microorganisms. The actinomycetes are good antibiotic producers. The ability of these microorganisms to produce useful antibiotics has focused attention on their isolation from their natural habitat, the soil.

The present investigation to isolate and study the antibiotic activities of actinomycetes against the specified bacterial strains was done by serially diluting the soil sample collected from the campus of Patna Women's College and plating it on the Casein Starch Peptone Yeast Malt Extract (CSPY-ME) medium. Among the isolates, five strains of actinomycetes were selected and were subjected to primary screening by perpendicular streak method against Gram-positive (Staphylococcus aureus) and Gram-negative (Pseudomonas spp., and Shigella flexneri) bacteria, obtained from the Department of Industrial Microbiology of Patna Women's College. The actinomycetes were subjected to secondary screening by agar well method to further test their strength and spectrum of the antibiotic properties against the specified bacterial strains. The results of screening revealed that majority of the selected actinomycetes were active against Gram positive bacteria. The reason for different sensitivity between Gram positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms. The Gram negative bacteria have an outer polysaccharide membrane carrying the structural lipopolysaccharide components.

Key words : *Antibiotics, actinomycetes, bacteria.*

Introduction: In the 20th century it was demonstrated that the growth inhibition of one microorganism was mediated by secretion of toxic metabolites by the other. This toxic metabolite was termed as “antibiotic” and the phenomenon of act of growth inhibition by antibiotics as “antibiosis” (R.C.Dubey, 2006). Antibiotics are the secondary metabolites which are produced by microorganisms and act against other microorganisms. Since the 1940's a vast array of antibiotics have been isolated and synthesized from moulds and actinomycetes, the latter remain as an important source of antibiotic substances. The actinomycetes are a diverse group of gram positive eubacteria which are often unicellular and irregular in shape. They are non-motile but some of them bear flagella, most useful in drug industry as they produce most of the antibiotics used in medicine (Singh *et. al.*, 2009). The actinomycetes are noteworthy an antibiotic producers. *Streptomyces* is the largest antibiotic-producing genus in the microbial world discovered so far. The number of antibiotic compounds reported from the species of this genus per year has increased almost exponentially for about two decades. Recent reports show that this group of microorganisms still remains as an important source of antibiotics (Sahin & Ugur, 2002). The popularity of antibiotics is due to their ability to destroy many kinds of pathogens and to their relatively non-toxic properties to the host when given systemically. Gram-positive bacteria are an important cause of serious infections, particularly those originating in hospitals.

The present study was carried out to test the antibiotic activity of some selected actinomycetes against *Staphylococcus aureus*, *Pseudomonas* spp. and *Shigella flexneri*.

Methodology:

The soil sample was collected from the campus of Patna Women's College, serially diluted and plated on Casien Starch Peptone Yeast Malt Extract medium in triplicates in aseptic condition. The plates were incubated at 37°C for 3-5 days. The different colonies of

actinomycetes were obtained. Five strains of the actinomycetes were purified by the method of single colony isolation and labeled as St-I, St-II, St-III, St-IV and St-V.

Morphological and microscopic characteristics of the selected strains of actinomycetes:

The actinomycetes were examined for their morphology and Gram stained. The results are tabulated in Table-1.

Table-1 Culture characteristics of the selected strains of actinomycetes

Colony Morpho-logy	St – I	St – II	St – III	St – IV	St – V
Colour	White	White	White	Grey	White
Margin	Radiating	Smooth	Smooth	Smooth	Smooth
Elevation	Flat	Slight	Slight	Slight	Convex
Texture	Powdery	Powdery	Powdery	Powdery	Powdery
Gram's reaction	(+)ve	(+)ve	(+)ve	(+)ve	(+)ve
Shape	Filamen-tous	Filamen-tous	Filamen-tous	Filamen-tous	Filamen-tous

The strains of the actinomycetes were screened for their antibiotic activities against the selected bacterial strains.

Subculturing of the selected bacterial strains provided by the Department of Industrial Microbiology:

Strains of *Staphylococcus aureus*, *Pseudomonas* spp. and *Shigella flexneri* obtained from the Department of Industrial Microbiology, Patna Women's College, Patna, were streaked in duplicates on sterilized Nutrient Agar slants and incubated at 37°C for 24-48 h.

Screening of the antibiotic activities of the actinomycetes:

Using sterile technique, a single line streak inoculation of each strain of the selected strains of actinomycetes was made on the surface of the GS (Glucose Soyabean meal) Agar medium so as to divide the plate in half. The plates were incubated at 37°C for 3-5 days. Following incubation, on the bottom of each plate three lines perpendicular were drawn to the growth of the antibiotic-producing strain. A single line streak inoculation of each of the three test cultures was made aseptically on the drawn lines. It was started close to, but not touching the growth of the antibiotic producing strains and it was streaked towards the edge of the plate. The plates were incubated in an inverted position at 37°C for 24 h.

It was observed that St-II showed antibiotic activity against all the three selected bacterial strains whereas St-I had antibiotic activity against *Pseudomonas* spp. and *Shigella flexneri* only. St-III and St-V showed antibiotic activities against *Staphylococcus aureus* and *Shigella flexneri* respectively. St-IV did not show antibiotic activity against any of the selected bacterial strains. The observations are tabulated in Table-2.

Table-2 Antibiotic activities of the selected strains of actinomycetes against *Staphylococcus aureus*, *Pseudomonas* spp. and *Shigella flexneri*

Actinomycetes	Antibiotic activity against the bacteria
St – I	*Psedomonas* spp., *Shigella flexneri*
St – II	*Staphylococcus aureus, Shigella flexneri* and *Psedomonas* (moderate)
St – III	*Staphylococcus aureus*
St – IV	Nil
St – V	*Shigella flexneri*

Assessment of the strength and spectrum of the antibiotic property of the selected strains of actinomycetes:

The bacterial strains were grown by pour plate method. Nutrient Agar medium was prepared, autoclaved and cooled to 45°C. The sterilized and cooled Nutrient Agar medium was separately inoculated with the three selected bacterial strains and poured in five sterilized plates each. The plates were incubated in an inverted position at 37°C for 24-48 h.

Following incubation, the strength and spectrum of the antibiotic property of the selected strains of actinomycetes was determined by Agar well diffusion method. Three bores were made on the Nutrient Agar plates with the help of sterilized cork borer.

Casein Starch Peptone Yeast Malt Extract (CSPY-ME) broth medium was prepared, autoclaved and cooled to 45 °C. The sterilized and cooled medium was inoculated separately with the five selected strains of actinomycetes.

Under aseptic conditions, 1 ml of the broth containing each of the actinomycetes was transferred in the wells of the Nutrient Agar medium of the three bacterial strains separately. The plates were incubated at 37°C for 3-5 days.

Following the incubation, areas of clear zone were observed around the wells containing the actinomycetes which showed their antibiotic activities against the selected bacterial strains.

The clear zones were measured with the help of scale and the results are tabulated in Table-3.

Table-3 showing zones of inhibition

Strains of actinomycetes	Clear zone in the plate containing *Staphylococcus aureus*	Clear zone in the plate containing *Pseudomonas* spp.	Clear zone in the plate containing *Shigella flexneri*
St – I	–	5 mm	3 mm
St – II	1 mm	2 mm	2 mm
St – III	2 mm	–	–
St – IV	–	–	–
St – V	–	–	3 mm

Results and Discussion:

A study has been conducted to assess the production of antibiotics by a number of unidentified strains of actinomycetes capable of inhibiting the growth of a variety of test organisms *in vitro*. Out of a number of different colonies of actinomycetes isolated from the soil sample, a total of five actinomycetes were selected to study their antibiotic activities against three bacterial strains namely *Staphylococcus aureus*, *Pseudomonas* spp. and *Shigella flexneri*.

In actinomycete-inoculated CSPY-ME (Casein Starch Peptone Yeast extract Malt extract) medium, antibiotic production was demonstrated by streaking the bacterial test organisms perpendicular to the single line streak of the strain of actinomycete.

Among the five selected strains of actinomycetes, the St-II was found to have antibiotic activity against all the three selected bacterial strains followed by St-I having antibiotic activity against two bacterial strains namely *Psedomonas* spp. and *Shigella flexneri* whereas St-III and St-V were active against only one bacterial strain each namely *Staphylococcus aureus* and *Shigella flexneri* respectively as shown in Table-2. St-IV was not active against any of the selected bacterial species. From Table-3, it is clear that St-II has the widest spectrum of antibiotic activity followed by St-I. St-III and St-V had narrow spectrum of antibiotic activity. St-I had the maximum antibiotic strength against *Pseudomonas* spp.

In general, the mechanism of biological action of the antibiotics is quite specific. They may exert their antibiotic activity by inhibiting the synthesis of the cell wall, by upsetting the function of the membrane, by inhibiting synthesis of nucleic acids selectively, by inhibiting the synthesis of pyrimidines, by inhibiting the synthesis of protein, by inhibiting respiration or by inhibiting oxidative phosphorylation (Singh et. al., 2009).

It may be seen that antibacterials act at different sites of the pathogenic bacterium to which they are applied. They may act as cell wall inhibitors, protein synthesis inhibitors. Antibiotics are used for Gram- negative bacteria. They may cause the leakage of cell contents of Gram-negative bacteria. *Pseudomonas* spp. and the other Gram-negative bacteria may be controlled due to this activity of the antibiotic.

The results of screening reveal that most of the active isolates were active against Gram positive bacteria (*Staphylococcus aureus*) as compared to Gram negative bacteria. The reason for different sensitivity between Gram positive and Gram negative bacteria could be ascribed to the morphological differences between these microorganisms, Gram negative bacteria having an outer polysaccharide membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to lipophilic solutes. The Gram positive bacteria may be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherrer & Gerhardt, 1971).

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