

**ANTIMICROBIAL POTENTIAL OF ACTINOMYCETES AGAINST MICROBES ISOLATED FROM AYURVEDIC DRUGS****Padma Singh^{1*}**, Bhavya Trivedi & Soma¹Microbiology Department, Kanya Gurukul Mahavidyalaya, Gurukul Kangri University, Uttarakhand, India**ABSTRACT**

Total seven microbes were isolated out of which one of the actinomycetes which was isolated from soil and then evaluated for antimicrobial potential against bacteria and fungi isolated from herbal drug sample. Out of six microbes, three bacteria are *Bacillus* sp, *E.coli*, *Staphylococcus* sp. Three fungi isolated were *Penicillium*, *Aspergillus*, *Fusarium*. Actinomycetes was evaluated for its inhibitory activities on these six test microorganisms. The results indicated that Actinomycetes was active against *Bacillus* and *E.coli* (5mm) and also inhibit the growth of *Aspergillus* (14mm). Seven isolates were highly active with an inhibition zone more than 20 mm in diameter. Antimicrobial activity present in Actinomycetes culture may lead to discovery of new antifungal drugs as it shows maximum inhibition against *Aspergillus* sp isolated from ayurvedic drugs. These microorganisms may have capability to produce some of the most important medicines ever developed.

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INTRODUCTION

Antibiotic or drugs are commonly used for the treatment of infectious disease, which are known as chemotherapeutic agents and its application in curing microbial disease is known as chemotherapy. Sometimes if proper quality control conditions of drugs are not maintained or aseptic conditions are not prevailing then, there are chances that microbes like bacteria or fungi are present in that drug sample before expiry date and after expiry date chances of microbes being present greatly increased. These microbes are pathogenic. Actinomycetes are the most widely distributed group of microorganisms in nature which primarily inhabit the soil. (Oskay et al, 2004).

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They have provided many important bioactive compounds of high commercial value and continue to be routinely screened for new bioactive compounds. These searches have been remarkably successful and approximately two thirds of naturally occurring antibiotics, including many of medical importance, have been isolated from actinomycetes (Okami and Hotta 1988). Almost 80% of the world's antibiotics are known to come from actinomycetes, mostly from the genera *Streptomyces* and *Micromonospora* [Pandey et al, 2004]. According to the World Health Organization, over-prescription and the improper use of antibiotics has led to the generation of antibiotic resistance in many bacterial pathogens.

Nowadays, the drug resistant strains of pathogen emerge more quickly than the rate of discovery of new drugs and antibiotics. Because of this, many scientists and pharmaceutical industry have actively involved in isolation and screening of actinomycetes from different untouched habitats, for their production of antibiotics [oskay et al,2004]. Serious infections caused by bacteria have become resistant to commonly used antibiotics and become a major global healthcare problem in the 21st century [Alanis, 2005]. Majority of the actinomycetes in soil that are potential drug sources remain uncultivable, and therefore inaccessible for novel antibiotic discovery. Goodfellow and Haynes reviewed the literature on isolation of actinomycetes and suggested that only 10% of the actinomycetes are isolated from nature [Goodfellow and Haynes ,1984]. Most of the antibiotics in use today are derivatives of natural products of actinomycetes and fungi [Butler and Buss, 2006 and Newman and Cragg 2007]. Actinomycetes can be isolated from soil and marine sediments. Although soils have been screened by the pharmaceutical industry for about 50 years, only a small fraction of the surface of the globe has been sampled, and only a small fraction of actinomycetes taxa has been discovered [Baltz ,2005 and baltz, 2007]. The present study was undertaken to isolate actinomycetes from the soil samples of Kanya Gurukul College garden , Hardwar and to assess their anti-bacterial properties. The resistance problem demands that to discover new antibacterial agents effective against pathogenic bacteria resistant to antibiotics. So we need to screen more and more actinomycetes from different habitats for antimicrobial activity in hope of getting some actinomycetes strains that produce antibiotics that have not been discovered yet and active against drug resistant pathogens.

MATERIALS AND METHODS

Soil sample collection:

Isolation and screening of soil *Actinomycetes* as source of antibiotics active against bacteria. Soil samples were collected from Kanya Gurukul Available online on www.ijprd.com

College garden, Hardwar . These were air-dried for 1 week , crushed and sieved. The sieved soils were then used for actinomycete isolation. . Bacteria and Fungi isolated from drugs.

Isolation and culture condition:

Microorganisms were isolated by serial dilution method. Added 1g of the soil were suspended in 10 ml of sterile distilled water . Now from tube 1 transferred 1 ml dilution into tube 2 to make it 10^{-2} dilution. This process was repeated to make 10^{-6} and vortex for 10-15 mins. Same in case of isolation of microorganism from drugs. now specific media for isolation of bacteria ,fungi and actinomycetes were poured into their respective labeled plates . Now 0.1 ml of aliquots were tranfered from each dilution to their respective sterile plates. Spread the inoculum with the help of sterile spreader for proper dispense of the given sample i.e. soil and drugs throughout the medium . After solidification, the plates were incubated in an inverted position under the following conditions bacteria 24-48 hrs at 30 35°C, fungi 4-5 days 25 -28°C and actinomycetes 7 -10 days at 28-30°C. After incubation plates were selected which contained colonies in the range of 30- 300, counted by using colony counter. The isolated strains are preserved at 4°C and maintained for longer period by serial subculture.

Gram staining

A smear of culture was taken in a clean glass slide and heated gently over a flame. The smear was covered with a thin film of crystal violet for 1 min and washed gently in slow running tap water. Gram's iodine solution was flooded over the smear for 1 min and washed with tap water. Alcohol was used to decolorize the smear until the violet color ceased to flow away. The slide was washed with water and counter stain safranin was flooded over the smear for 2 min, then the slide was washed, drained, air dried, and viewed under microscope. The presence of red coloured rods indicate Gram negative bacilli and violet coloured cocci and rods indicate Gram positive cocci and bacilli.

Fungal staining

AT first one drop of lactophenol cotton blue was taken on the clean slide . A small tuft of fungal

mycelium was taken with the help of forceps , kept on the drop. The mycelium was teased by the needles . covered the drop by the cover slip .observed under microscope.

Identification of actinomycetes

Actinomycetes can be identified either by simple observing the colonies with naked eyes or by performing Gram staining.

Biochemical test were also performed like catalase test, starch hydrolysis, methyl red test.

Screening of isolates for anti-bacterial activity

Petriplates were seeded with the agar on antibiotic medium . three holes were punched on agar by mean of cork borer or disc may be dipped on the broth of culture . Each hole is filled with culture broth of actinomycetes grown in tween 20 saline solution . observed for the zone of inhibition around each well or disc and screened out the antibacterial microbes.

Testing of antimicrobial activity

Paper disc diffusion assay (for the bacteria)

sterile molten cooled antibiotic medium was added in sterile plates . allowed it solidify then Actinomycetes culture was centrifuged for 10-15 mins at speed 10,000- 20,000 rpm. 0.1 ml of bacterial growth was transferred aseptically in the plates and spread the inoculum . now centrifuged actinomycetes culture was applied onto sterile filter paper disc and placed on lawn of bacterial culture on plates.for comparative study place commercially antibiotic discs were placed on agar surface . now plates were incubated overnight in inverted position at 37°C. plates were observed the zone of inhibition around the disc.

Well diffusion assay(for fungi)

In 150 ml flask sterile tween 20 saline solution were taken and a piece] of fungal mycelial mat was suspended and vortexed.The tubes were kept in shaker at 28°C for 24 hrs. pour sterile SDA on sterile plate and allow it to solidify. After solidification 1ml tween 20 saline was transferred to plate. Wells were prepared by cork borer. Now 0.8ml actinomycetes culture and antifungal were added

and kept it for diffusion of solution at normal room temperature for 30 min. plates were incubated at 28°C for 2-4 days. Plates were examined and measure the zone of inhibition around wells.

RESULTS AND DISCUSSION

In the present study actinomycetes was isolated from 1 gm of soil sample which was collected from kanga Gurukul college campus and inoculated on starch nitrate agar medium for purification and pure colonies were maintained in starch nitrate agar slant at 4°C . Isolates grew on starch casein agar media showing morphology typical of actinomycetes, colonies were chalky granular and heaped . rudimentary to extensively branched vegetative hyphae, growing on the agar surface . the hyphae fragments into bacteroids , rod shaped to coccoid elements . shprrt to long chains of well to poorly formed conidia found on the aerial hyphae, stain Gram positive to Gram variable , partially acid fast.. Along with that three fungus and three bacteria were isolated from drug Gumtone powder , manufactured by Charak pharma) using nutrient agar medium for bacteria and fungus for fungi . further the actinomycetes which were isolated from soil were evaluated for antimicrobial potential against bacteria and fungi isolated from herbal drug sample . it was found that isolated Actinomycetes was active against *Bacillus* and *E. coli*(zone of inhibition 5 mm) using chloramphenicol as reference antibiotic for comparative results showing maximum zone of inhibition of 39 mm. a culture was also found to inhibit the growth of *Aspergillus* (zone of diameter 14mm) using griesiofulvin as reference antibiotic for comparative results showing maximum zone of inhibition of 25mm. After observing the characteristic shown by actinomycetes culture , we can conclude that the antimicrobial culture may lead to the discovery of new antifungal drugs as it show maximum inhibition against *Aspergillus sp* isolated from ayurvedic drugs.

Table :1 antifungal assay of actinomycetes culture in comparison with griseofulvin

| S.N. | Antimicrobials | Zone of inhibition(mm) | | |
|------|-----------------------|-------------------------|--------------------|-----------------|
| | | <i>Penicillium</i> | <i>Aspergillus</i> | <i>Fusarium</i> |
| 1 | Actinomycetes culture | - | 14 | - |
| 2 | Griseofulvin | 25 | 25 | 5 |

Table :2 antimicrobial activity of actinomycetes against bacteria with other antibiotic

| S.N. | Antimicrobials | Zone of inhibition (mm) | | |
|------|-----------------------|-------------------------|-----------------|-----------------------|
| | | <i>E.coli</i> | <i>Bacillus</i> | <i>Staphylococcus</i> |
| 1 | ciprofloxacin | 18 | - | - |
| 2 | chloramphenicol | - | 35 | 39 |
| 3 | Actinomycetes culture | 5 | 3 | - |

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