



ISOLATION, BIOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF EXTRACTED BIOACTIVE COMPOUNDS

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Over the years, Antibiotics have served in successful treatment of a number of microbial diseases. However in current times, there has been a great increase in resistant microorganisms that have adapted to the previous antibiotics. A World Health Organization report says that antimicrobial resistance is a "major global threat" to human health based on analysis of around 110 countries. The report covers seven pathogenic bacteria which cause common diseases like pneumonia, diarrhea and blood infections. It also emphasized that more antimicrobial compounds need to be discovered and individuals need to help slow the antibiotic resistance process. Drug resistant bacteria has facilitates the requirement of newer antimicrobial agents which can fight against MDR pathogens. Even though around 80% of antibiotics have been discovered and supplied to the Pharmaceutical industries and treated various disease caused by these bacterial pathogens. Present study was done on the primary and secondary screening of actinomycetes from Rhizospheric soil and antibiotics used to treat various diseases caused by MDR organisms. Main study was focussed on isolation and biochemical testing of isolates and extraction of compounds from broth of isolates. Samples taken were from different Rhizospheric regions of Rajasthan.

Keywords: Antibiotics; Actinomycetes; Infections; MDR; Pathogens; Pneumonia.

Introduction

The Rhizospheric is a region in soil highly influenced by plant roots, and has maximum microbial activity and a great microbial diversity, which in most cases is enriched with the by products of plant metabolism. Bacteria, fungi, Actinobacteria, and plants live in a constant process of symbiosis^{1,2}. The study of microorganisms from the Rhizospheric plants is really important, since they are a means by which low-

fertility soil can become a powerful source of biotechnologically essential metabolites. The search for products effective against multi-drug resistant bacteria pathogenic to humans is a central goal. The quick and aggressive development of antimicrobial-resistant bacterial species has motivated scientists to develop more Novel biomolecules. Microorganisms of the Rhizospheric in particular the actinomycetes are a promising source of new antibiotics

and various other products and are of enormous interest to the pharmaceutical industry and biotechnology^{3,4}. The demand for new antibiotics continues to grow due to the rapid spread of multi drug resistant pathogen causing life threatening diseases⁵. The actinomycetes are important in the field of Pharmaceutical industries as well as in agriculture. Antibiotics are the best known products of actinomycetes. For their fundamental success against various types of pathogenic microorganisms, antibiotics can be actually referred as the 'wonder drugs'⁶. This amazing group of compounds forms a heterogeneous assemblage of biologically more active molecules with different modes of action and structures to treat bacterial and other infections.

The actinobacteria constitute an essential group of bacteria commonly isolated from the Rhizospheric soil. They are Gram-positive and are known to produce several types of antibiotics, enzymes, pesticides, antibacterial, antifungal, antiviral etc with practical applications in industry, agriculture, medicine, and veterinary science. The genus *Streptomyces* is the finest known among the actinobacteria, and includes over 3000 identified species⁷. They are in greater abundance in the soil, but may also occur in various other environments. These filamentous bacteria are aerobic and can synthesize crucial volatile compounds, such as geosmin, which gives soil its characteristic "wet earth" odor. *Streptomyces* sp are very valuable, as they can produce several commercial antibiotics, including streptomycin^{8,9}.

Material and Methods

Collection of Soil Samples-

Four different soil samples were collected from different regions of Rajasthan State at depth (4 - 5 cm) in sterile polythene bags

with the help sterile spatula and were transported to laboratory for further processing.

Isolation of Actinobacteria-

Isolations were performed with Actinomycetes isolation agar media. Spread plate and pour plate technique were used to isolate Actinomycetes. Plates were incubated inverted at 35⁰c for 5-7 days.

Primary screening-

Primary screening was done by disc diffusion method¹⁰ with some of the test pathogens.

Test pathogens-

Antimicrobial Activity of Isolates was performed against standard cultures of imtech Chandigarh. 5 cultures were used as: *Proteus vulgaris*, (MTCC-*1771) *Pseudomonas aeruginosa*, *Bacillus subtilis*, (MTCC-*121) *E.coli* (MTCC-1084), *Staphylococcus aureus* (MTCC-7443).

Biochemical tests of isolates-

Some of tests were performed for isolates. Tests were Indole test, MR, VP test, Carbohydrate fermentation tests, Starch hydrolysis, etc.

Extraction of Compounds from broth-

After fermentation, from the fermented broth the mycelium was removed by filtration and then clear filtrate was used to check antimicrobial activity. Then the isolation of anti-microbial compound was done from the filtrate by solvent extraction method¹¹.

Antibacterial compounds were extracted from the filtrate by solvent extraction with ethyl acetate and chloroform. Solvent was mixed to the filtrate in 1:1 (v/v) ratio and shaken vigorously for at least half an hour for complete extraction of metabolites. Two layers separated and collected in different beakers. Solvent was evaporated by keeping them on water bath at 50- 60⁰C and crude remaining in beakers were used for

secondary screening.

Determination of activity of crude extract-
Antimicrobial activity was measured with disc diffusion method¹⁰ after Solvent

extraction against different pathogens.

Results and Discussion

There are huge diversity of organisms like bacteria, fungi etc in soil.

Table 1. Morphology observed of isolated strains

| Isolates no. | Color of colony | Shape of colony |
|--------------|-----------------|-----------------|
| H 4 | Creamy | Cocci (single) |
| NH 11 | Yellowish | Rods (single) |
| NH 13 | Creamy | Rods (single) |
| NH 15 | Pinkish | Rods (single) |
| NH 16 | Dark yellowish | Rods (chain) |
| NH 17 | Yellowish | Cocci (single) |
| NH 18 | Creamy | Cocci (single) |
| NH 20 | White creamy | Rods (chain) |
| NH 23 | Yellowish | Cocci (chain) |
| NH 26 | Light brown | Rods (chain) |
| NH 29 | Pink | Rods (chain) |



a) Indole test



b) MR test



c) VP Test



d) Carbohydrate test



e) Starch hydrolysis test



f) Liquid liquid solvent extraction



g) Isolate (NH26) Activity of crude extract

Soil of different sites of Rajasthan has been studied and different 11 isolates were first identified primarily on the basis of their Morphology. Different soil organisms give different results on different media and temperature conditions. Actinomycetes have provided many industrially important bioactive compounds¹². Actinomycetes underlined the significance of screening for antibiotics against specific pathogens, in addition to the search for active Novel antibiotic. Gram-negative pathogens are a main problem in the nosocomial environment¹³. Isolates were tested for their biochemical tests and antibiotic producing capability so we found isolate (NH26) was having higher antimicrobial activity against indicator organisms. The Gram positive bacteria was more active than Gram negative bacteria similarly to results reported^{14,15}. Study is continued on the Rhizospheric soil of Rajasthan because microorganisms of these soil having antimicrobial property.

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