

## **ISOLATION OF ANTIBIOTIC PRODUCING MICROORGANISMS FROM DIFFERENT SOIL SAMPLES OF SATTENAPALLI, GUNTUR**

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### **ABSTRACT**

Antibiotics are chemicals that kill or inhibit the growth of bacteria and are used to treat bacterial infection. They are produced in nature by soil, bacteria and fungi. Soil is the outer region of earth crust consisting of loose material. In this soil samples from different areas were collected (soil from vegetable dump areas, normal street soil from different areas in sattenapalli, gutur) and analysed for different bacterial colonies from soil sample was carried out and tested for antibiotic producing activity. The isolated strains showed permanent zones of inhibition. The inhibitory activity of the organism was checked against important microbial flora. In the present study a trial

was done to find out a new antimicrobial agent producing bacteria from soil samples collected from different places.

**KEYWORDS:** Soil, Isolation, Antibiotics, Morphology.

### **INTRODUCTION**

Antibiotics are the chemicals that kill or inhibit the growth of bacteria and are used to treat bacterial infections. They are produced in nature by soil bacteria and fungi. This gives the microbe an advantage when competing for food and water, and other limited resources in a particular habitat as the antibiotic kills off their competition.

Antibiotics are a natural substance of biological, synthetic or semi-synthetic origin. In recent years several microorganisms that are able to produce antibiotics are grown on the artificial

media for the intense search for antibody producing microorganisms. Soil is the complex and very diverse environment producing versatile source of antibiotic producing organisms.<sup>[1]</sup>

Soil is the outer region of the earth crust consisting of loose material formed by gradual weathering of rock gives to plant both mechanical and nutritional support. Soil can be defined as the space- time continue forming the upper part of the earth crust. Soil is as important as water and air. Physicochemical properties of soil includes soil texture, water, air, inorganic chemicals and organic matter. Microorganisms which live in soil are algae, fungi, actinomycetes, bacteria, protozoa, nematodes etc. Structural components of soil and its major constituents determine soil quality. In 1904 L.HILTNER coined the term rhizosphere denote the area of intense microbiological activity. Therefore rhizosphere is a unique subterranean habitat for microorganisms. The microbial community of this zone can be examined by means of cultural, microscopic and manometric techniques.<sup>[2]</sup>

## HISTORY OF ANTIBIOTICS

Antibiotics are (anti=against, bios=life) chemical substances secreted by some microorganisms which inhibit the growth and development of other microbes. Most of them are produced by actinomycetes specially the genus streptomyces and filamentous fungi. The study of antibiotic began by discovery of penicillin in 1928, when ALEXANDER FLEMMING proved that the filtrate of a broth culture of penicillium notatum has antibacterial properties in relation to gram positive bacteria. The work of German physician PAUL EHRLICH (1854-1915) propounded that a chemical which pathogen but not human cells can be used for the control of disease. In 1944 SELMAN WAKSMAN discovered a new antibiotic called Streptomycin from streptomyces griseus which is a member of actinomycetes. He was awarded NOBLE PRIZE in 1952 for the discovery of streptomycin. Streptomycin helped the patient to screen about 10000 strains of soil bacteria and fungi later on chloramphenicol, neomycin, tetracycline and tetramycin were discovered during 1950's.<sup>[3]</sup>

## ANTIBIOTICS

Majority of antibiotic we consume are metabolised in our bodies. While consuming the organic waste, the bacteria encounters the antibiotics and expresses resistance genes that reduce effectiveness. As the bacteria eats, reproduces and grows, an excess is accumulated called biomass. SMITH said regardless of the influent antibiotics whether it's just one are really low concentrations there is likely a lot of multi drug resistance that is spreading one plasmid may carry resistance genes for several different types of antibiotics. The least

sensitive antibiotics were Ampicillin and the first generation cephalosporins. Third generation cephalosporins are commonly used as second line antibiotics.<sup>[4]</sup>

## SCREENING METHODS OF ANTIBIOTICS

There are two types of screening methods for antibiotic producing microorganisms. They are:

1. Primary screening
2. Secondary screening

### 1. Primary screening

It is a process for detection and isolation of microorganisms of our interest. It separate out only a few microorganisms, only few have commercial value while discards the value less microorganisms.

### 2. Secondary screening

It is use full in sorting of microorganisms that have real commercial value. The microorganisms having poor applicability in fermentation process are discarded. Provides information whether the product formed by microorganisms is new or not. This may be accomplished by paper, thin layer chromatographic technique.<sup>[5]</sup>

## AIM AND OBJECTIVE

To isolate the antibiotic producing microorganisms from soil sample.

To determine the pH of the collected soil sample.

To determine the capacity of soil.

## MATERIALS AND METHODS

### Chemicals and reagents

Trypticase soy broth (Hi-Media, India), Trypticase soy agar (Hi-Media, India), crystal violet, Gram's iodine, safranin, ethyl alcohol, commercial antibiotic disc (Hi-Media, India).

### Sample collection

Soil samples were collected from fish, vegetables and fruits, dump area from Guntur City.

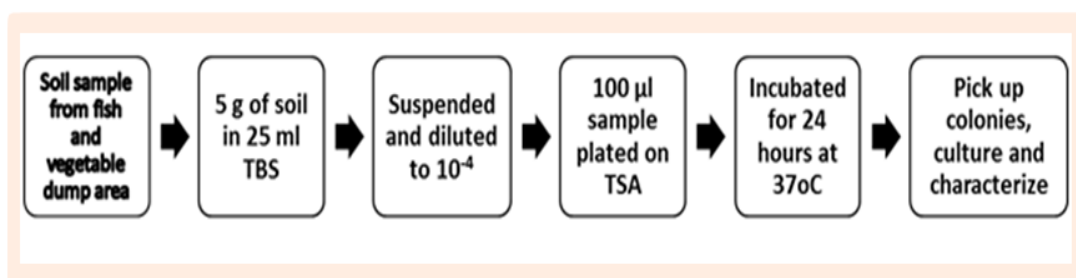
### Bacterial culture

About five grams of soil sample were suspended in TSB medium. The microbial suspension was further diluted ( $10^{-4}$ ) with the same medium. About 100  $\mu$ l of

microbial suspension was spread on TSA plate and incubated for 24 hours. The bacterial colonies were isolated and grown in TSB. Bacteria were characterized.<sup>[6]</sup>

### Gram staining

A smear of bacterial cells was prepared on a clean glass slide and the smear was then allowed to air-dry followed by a mild heat fixation. Crystal violet solution was added onto bacterial smear and incubated for one minute. The smear was washed with water. Mordant Gram's iodine solution was then added onto bacterial smear and incubated for one minute. The smear was decolorized by washing with 95% ethyl alcohol and rinsed with water. Finally safranin was used as counterstain for one minute and washed with water. Cells were then air dried and studied under microscope.<sup>[7]</sup>



A flow chart showing the summary of isolation of bacteria from soil samples. Bacterial colonies were picked up as shown in the figure above. Individual colonies were cultured and used for Characterization.<sup>[8]</sup>

**After production of Antibiotic producing microorganisms from different soil samples, the following steps are to be carried out**

- Identification of bacteria in soil samples
- Production of antibacterial factors by some bacterial isolates from soil samples of Guntur city area

### SUMMARY

This study describes isolation of three soil bacteria from Guntur city area. Bacterial isolates were tested that either they can produce antibacterial components against both gram positive and negative cells. Bacterial cell morphology was studied by scan electron microscopy and found to be short rod shaped. Antibacterial compound(s) was found to diffuse out of the cell. Further studies are in progress to determine chemical nature of the antimicrobial compound(s).

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