Research Article

WORLD JOURNAL OF CURRENT MEDICAL AND PHARMACEUTICAL RESEARCH

www.wjcmpr.com ISSN: 2582-0222

Isolation of Antibiotic Producing Microorganisms from Two Soil Samples of Phirangipuram,Guntur

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ABSTRACT

Antibiotics are 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. Soil is the outer region of earth crust consisting of loose material. In this, soil samples from two areas were collected (vegetable dump area(A), street soil(B)) and analysed, different bacterial colonies from soil samples was formed and tested for antimicrobial activity. The isolated and subcultured strains showed permanent zones of inhibition. The inhibitory activity of the organism was checked against important microbial flora. In the present study a trail was done to find out a new antimicrobial agent, producing bacteria from soil samples collected from two places.

Key words:
Soil, Isolation, Antibiotics, Subculturing, Morphology, Staining.

Article History:
Received On:18.2.02.2020
Revised On: 21.04.2020
Accepted On: 27.04.2020

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DOI: https://doi.org/10.37022/WJCMPR.2020.2224

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 perticular habitat as the antibiotic kills of their competition. Majority of antibiotics we consume are metabolized in our bodies. Antibiotics are a natural substances of biological, synthetic or semi synthetic origin[1-9]. In recent years several microorganisams that are able to produce antibiotics, and 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. 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 is as important as water and air. Physicochemical properties of soil includes soil texture, water, air, inorganic chemicals and organic matter [10]. Microorganisms which live in soil are algae, fungi, actinomycetes, bacteria, protozoa, nematodes etc.. Antibiotics are low molecular weight (non protein) molecules produced as secondary metabolites, mainly by microorganisms that live in the soil. Each year nearly 500 antibiotics were found, in which 60% of antibiotics are obtained from the soil. It is agreed that there are many species of bacteria in soil yet to be discovered.

The presence of antibiotic species in soil can rarely be dectected. The different species of microorganisms occupy the same environment with out effecting each other. Microbial population in soil depends of various factors such as temperature ,pH, carbon sources etc. Antibiotics are (anti=against, bios=life) chemical substances secreted by some microorganisms which inhibit the growth and development of other microbes [10 26]. The least sensitive antibiotics were Ampicillin and the first generation cephalosporins. Third generation cephalosporins are commonly used as second line antibiotics.

MATERIALS AND METHODS

Soil collection

Soil samples were collected from the two places in Phirangipuram, Guntur (Vegetable dump areas(A), Street soil(B)).

Sieving

Collected soil samples (A and B) were pass through the different sieves(sieve number 32,60 and 100) to obtain the fine soil sample, by the removal of unwanted rocks and soil debris.

Serial dilution

A serial dilution is a series of sequential dilutions used to reduce a dense culture of cells to a more usable concentrations. Each dilutions will reduce the concentration of bacteria by a specific amount. As per instance, the number and size of bacterial colonies that grow on an agar plate in a given time is concentration-dependent. 1gm of soil sample (A), was weighed and transfered into a beaker containing 10 ml of distilled water. Mixed throughly with a stirrer until the soil sample was dissolved. After certain time period (60-90min), the above soil solution was filtered.

9Test tubes were taken each containing 9ml of sterile water. The test tubes were labelled with numbers. 1ml of the soil solution was taken from the above filtrate solution with the help of micropipette and added to the 1st test tube and mixed well. Again 1 ml sample was taken from the 1st test tube and added to the 2nd test tube and mixed well. The procedure continued till the 9th test tube. Sample B is also diluted as same as sample A. From the above, last four dilutions(i.e 6,7,8and9), from both the samples(A and B) were taken for inoculation. 1ml sample from each testtube is inoculated in to petriplates containing agar media by pour plate technique, under aseptic conditions. The petriplates were marked as, sample A(A6, A7, A8and A9), sample B(B6, B7, B8 and B9).

PREPARATION OF NUTRIENT AGAR MEDIUM

COMPOSITION

Peptone - 0.5gm
Beef extract - 0.3gm
Agar - 2gm
Sodium chloride - 0.5gm
Distilled water - 100ml
pH - 7.1 to 7.2

PREPARATION

- Weigh all ingredients separately using physical balance.
- Weighed ingredients were taken in 250ml beaker and dissolved, by adding 2/3rd quantity of distilled water.
- Now agar was added and dissolve it by placing it in a water bath.
- Then sterilize the medium in an autoclave at 121^{0}C at 15lb pressure for 15-20 min.
- After sterilization pour it in to petriplates A6,A7,A8, and A9(sample A), B6,B7,B8and B9(sampleB).
- Incubate the petriplates for 24hours, at 37^{0}C in an incubator.
- After 24hours colonies were formed.
- From all the petriplates, select one petriplate which shows countable number of colonies, from both the samples(A, B).

SUBCULTURING OF MICROORGANISM

Bacterial colonies with clear margins was picked and subcultured on fresh nutrient agar plates using sterile loop using streakplate method in laminor air flow, followed by incubation for 24hours at 37^{0}C temperature.
After incubation colonies were formed, and are stained to observe the morphology.

GRAM STAINING

The gram staining procedure distinguish between gram positive and gram negative groups, by colouring the cells.
There are four basic steps in the gram staining :

- Place a loop full of subcultured sample on the glass slides, from the both samples(A&B).
- Applying a primary stain (crystal violet) to a heat fixed smear of a bacterial culture to both the slides A and B.
- Rapid decolourization with ethanol or acetone. Then counter staining with saffranin.
- Observe both the slides under microscope.
  Results are shown in the table **1.1.**

RESULT

In this two different soil samples were collected from two places in Phirangipuram (vegetable dump area(A), street soil(B)) colonies were observed in the crowded plate colonies showing clear margins were subcultured on the fresh medium plates. After incubation colonial morphology was observed. From both the samples (A and B) bacterial strains were selected. The selected culture strains were subjected to gram staining and biochemical characterization tests and their results are shown in the table **1.2**. The selected isolated culture strains of bacteria were identified as Bacillus subtilis and Bacillus cerus. The identified culture of bacteria were then checked for antibiotic production activity using agar well diffusion method. The zone of inhibition were observed against the test bacteria S.aureus(Stephylococcus aureus).

Tab 1.1 Assessment two different soil samples

Sl.no	Sample	Colour	Gram stain
1.	Sample - 1	Purple colour	Gram positive
2.	Sample - 2	Purple colour	Gram positive

ASSESSMENT OF ANTIBACTERIAL ACTIVITY

For the assessment of anti bacterial activity S.aureus culture was obtained from local private clinical laboratory. In S.aureus culture wells were made using sterile borer. Isolated and subcultured bacterial strains were centrifused at 6000rpm for 10min. Different concentrations of centrifused samples A and B were poured in wells, and kept in incubater for 48hours. After 48hours zones were observed around the wells[27]. Presence of zones indicates the antibacterial activity to the isolated organisms (A and B).

Table 1.2 : Assessment of Anti Bacterial Activity

Sl.no	Sample	Whole colony	Surface texture	Edge	Pigment	Gram stain	Indole test	Catalase test	Organism
1.	A	Irregular	Dry or rough	Undulate(wavy)	White dull	+	_	+	Bacillus subtilis
2.	B	Irregular	Dry	Undulate	Whitish to cream	+	_	+	Bacillus cerus

CONCLUSION

The present study was an attempt to identify and characterize versatile strains of bacteria and to check their ability for antibiotic production. The isolated bacterial strains were found to produce antibiotics.

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