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Production, Extraction and Assay of Antibiotics from Bacterial Isolates Using Soil as Source

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Abstract

Antibiotic is a type of antimicrobial substance active against bacteria. Most important type of antibacterial agent for fighting against infection. Antibiotics are commercially exploited soil metabolites produced by the bacteria. Bacteria are easy to isolate, culture, maintain and to improve the strain. *Bacillus* species are predominate soil bacteria because of resistant endospore formation and production of vital antibiotic. The objective of the present study is production, extraction and assay of antibiotics from bacterial isolates using soil as a source. Micro organisms are omnipresent and always exist in a competitive environment. Soil bacterium with antibiotic activity is screened for morphological characters which can provide about valuable information about the strain. The extracted substance was found effective against gram positive bacteria. Most of the antibiotics are extracted and isolated from soil.

INTRODUCTION

Antibiotics are a natural substance of biological, synthetic or semi synthetic in origin. In 1942 the term antibiotic was introduced by Waksman. The demand of new antibiotics growing day by day due to emergence of multiple pathogens those are resistant to antibiotics cures for life threatening diseases. In recent year several microorganisms that are able to produce antibiotics grown on the artificial media. Soil is a complex and very diverse environment providing versatile source of organism producing antibiotic. Soil contains many different types of bacteria. *Bacillus* species are gram positive, rod shaped, sporulating and aerobic bacteria that was most abundant bacterial strains found in the soil. *Bacillus* species produce antimicrobial compounds having pharmaceutical and biotechnology importance. According to food and drug administration approximately 80% of antibiotics produced from natural habitat are fed to animals and only 20% are used to treat infection in humans. Antibiotics are widely distributed in nature; they play an important role in regulating the microbial population of soil, water, sewage and compost. Antibiotics are low molecular weight molecules produced as secondary metabolites mainly by microorganism that live in the soil. Many species such as *Streptomyces*, *Penicillin* and *Bacillus* have ability to produce antibiotics. The objective of this study was to find antibiotic producing microorganism and to check their ability to inhibit the growth of *S.aureu* and *E.coli.* John Parkinson was first person used moulds to treat infection. Alexander Fleming (1881-1955) discovered modern day Penicillin. Antibiotics were once called as wonder drugs. In recent years several microorganism that are able to produce antibiotic are grown on the artificial media for the intensive search for antibody producing microorganism. The most important antibiotics include Amino-glycosides, Penicillin, Macrolides, Glycopeptides, Cephalosporin and Tetracycline. Soil is a complex and very diverse environment providing versatile source of antibiotic producing organisms. Each year nearly 500 antibiotics were found, in which 60%.

Tea Spoon of soil contains million to billion bacteria in each acre of soil. Treatment of Infectious disease caused by pathogenic bacteria and fungal strains was one of the traditional problems in clinical field.

The objective of present study is production, extraction and assay of antibiotics from bacterial isolates using soil as a source.

Isolation of antibiotic producers:

Methods of Preparation:

Material Required: Soil sample, Sterile Petri Plates, Sterile Test Tubes, Nutrient Agar media

Collection of Soil Sample: Soil Sample collected from two different states (250g from Punjab and 250g from

Himachal Pradesh).

Preparation of Soil Dilutions: Normal Saline (30ml) was prepared and distributed equally (10ml) each into 3 test tubes. Soil Sample (1g) was added into first test tube, shake it properly (1:10 dilution). Sample from first test tube mixed into second test tube and shake it properly (1:100 dilution).Take a Sample from second test tube mixed into third test tube and shake it properly (1:1000 dilution).

H.P sample

Punjab Sample

Nutrient Agar Composition:

Peptone – 1g Beef Extract-0.6 g

Sodium Chloride-1g Agar-3g

Distilled water-200ml pH-7.0

Preparation: Mixed all these ingredients in Distilled water to make a clear yellowish solution, after preparing this solution, Autoclaved at 121°C for 15 minutes. Nutrient Agar media is poured into test tubes and allow solidifying in slanting manner in the laminar air flow. Sample is streaked on the slant. Test tubes are closed by cotton plugs and kept in incubator at 37°C for 24 hrs. Poured nutrient agar media into Petri plates and allow solidifying .After solidifying spread samples from each dilution and incubate at 37°C for overnight.

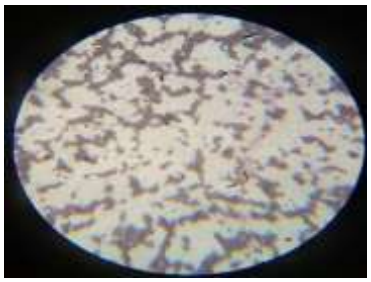
Screening: Inhibition Zone in competitive environment developed by organic acid producers and the antibiotic producing strain. Strains with organic acid production are eliminated by growing medium on calcium carbonate. Organic acid react with calcium carbonate and dissolved calcium oxide and carbon dioxide.

Procedure: Nutrient agar media and aqueous calcium carbonate solution was prepared and sterilized in autoclave. After sterilization poured media into Petri plates and allow to solidifying. After solidifying, isolated colonies from slant culture were selected with inoculating loop and streaked on solidifying media in Petri plates and incubate the Petri plates at 37°C overnight.



Morphological Observations (Gram Staining):

Take a Clean Glass slide. Colony was taken from the sample with the help of inoculating loop and put on clean slide and fixed with heat. Stain with crystal violet for one minute then rinse with water. Stain with Gram iodine for one minute then rinse with water. Decolorizer was added and waits for one minute. Safranin stain was added and waits for one minute .After one minute rinse with water. Airs dries the slide and observe under microscope

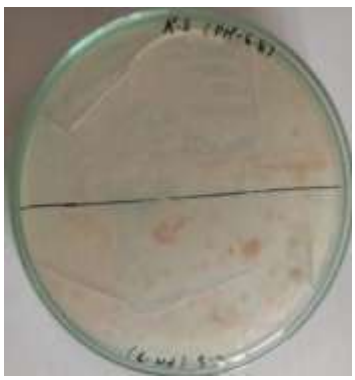




Streptococcus ***Micrococcus*** ***Bacillus***

Assay of Antibiotic activity of microorganism by streak plate method:

Composition of Bacterial Production media: Glucose(3g), Sodium nitrate(0.6g), Potassium Dihydrogen phosphate(1g), Poatssium chloride(0.5g), Magnesium Sulphate (0.02g), Ferous Sulphate (0.05g),Peptone(1g), Beef extract(3g), Distilled water(100ml).

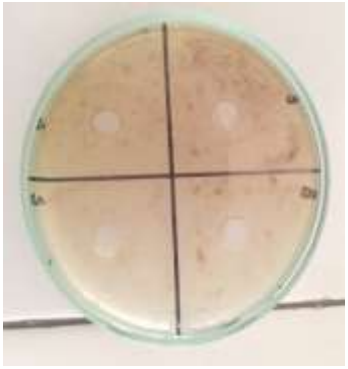
Procedure: Weigh all components and dissolved in distilled water made up to 50 ml, sterilized in autoclave at 121°C for 15 minutes. Sterilized broth is inoculated with test culture under aseptic condition, Incubate in Shaker for one day, after one day transfer the broth to normal incubation.



Extraction: Prepare Antibiotic production media and Sterilized broth culture. After two days sufficient amount of antibiotic produced. Secondary metabolite released into medium during stationary phase. Water soluble antibiotic broth was separated with the help of ion exchange method.

Preparation of Crude Extract: When optical density of culture broth was up to 0.4-0.6 ml. Inoculated production broth was taken and subjected to centrifugation at 6000rpm for 5-10 minutes. Cell pellet was discarded and supernatant stored at 4°C in refrigerator. Supernatant in crude extract was tested for antibiotic activity.

Well method of antibiotic assay: Nutrient agar media was prepared and poured into Petri plate and allow to solidifying. Wells (8-10mm) were made at selected areas in Petri plates. Test organism micrococcus and Bacillus strains was stabbed on Petri plates. Wait for some time. Cefixime trihydrate antibiotic dilution media prepared were taken with micropipette and loaded into wells. Incubate at 37°C for overnight. Strain showing more inhibition was selected and checked.



Results

Morphological Observations

Genera	Shape	Arrangement	Gram Nature
Bacillus	Bacilli	Singles and chains	Gram positive
Micrococcus	Cocci	Tetrads and pairs	Gram positive
Streptococcus	Cocci	Singles and pairs	Gram positive
Escherichia coli	Staright rods	Singles and pairs	Gram negative

Antibiotic Assay by Well method:

Inhibition zone for *Micrococcus* and *Bacillus* were observed around the region surrounding the well:

Optimization of Antibiotic Producers:

Concentration of bacterial production media (dilution%)	Zone of inhibition(cm)
1	1
1.2	1.1
1.4	1.3
1.6	1.5
Concentration of Antibiotic (dilution %)	**Zone of inhibition**
1	1.1
1.2	1.4
1.4	1.4
1.6	1.6

Conclusions: Bacteria isolated from soil showing antibiotic activity under normal condition and inhibit some gram positive and gram negative bacteria. Gram positive strain more resistant because of spores formation. This work provides helpful information on bacterial strain application in industrial production of antibiotic that can easily control the gram positive strains.

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