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Isolation of Microorganisms from the Barren Soil of Gorakhpur, Uttarpradesh, India

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A B S T R A C T

This article clarifies the presented content regarding the isolation of microorganisms from the barren soil and elucidate their presence in soil. This content is focused to isolate microbiota from the soil which have not capacity to yield properly and cannot be suitable for agriculture. The microbial community plays their role in plant growth same as inorganic and organic materials. The purpose is to focus on the idea that microbes are present or absent in soil when it is considered as barren which means lack of productivity. To know the type of biota and to find out their role in soil, isolation is required. To isolate biota, various methods; as serial dilution, media prepration (LB media-Luria Bertani media and PDA media-Potato Dextrose Agar media), streaking and spreading methods, Gram staining, Antibiotic susceptibility test, DNA extraction, gel electrophoresis are performed. Passing through the process of isolation, there is bacterial and fungal colony observed but there are very few numbers of colony and less productive microbes are isolated. Therefore, it can be concluded that barren soil also contains microbes but in less quantity and with low viability.

Introduction

Soil is a thin layer of materials covering the earth's surface. It is formed by weathering of rocks. Soil is a basic resource which supporting life and provides service for benefits of man (Laishram *et al.,* 2012). The soil which is poor in nutritional quantity and not suitable for plant growth, is considered as *barren soil.* Barrenness can be caused by factors like soil degradation, lack of water and nutrient defficiency (Revitalizing Barren land, 2024). In general, barren land has thin soil, sand or rocks. Barren lands include desert, dry salt flats, beaches, sand dunes, exposed rocks, strip mines, queries and gravel pits. In this type of soil, one-third part of land has vegetation.

Areas that are dependent on soil and having few numbers of plants as well as sometimes without plants, also can consider as barrren while they are able to host photoautotrophic microbial communities as deserts, polar regions etc (Freeman and Monte, 2009). Environmental conditions such as toxic or infertile soil, high winds,

costal salt-spray and climatic conditions are often key factors in poor plant growth and development. There are some vegetations which can grow in barren soil i.e. sandwood, amla, mahogany, moringa and various citrus fruit trees.

As such plants can grow in barren land that means the barren land may contain nutrients and microbes. Plant growth promoting (PGP) bacteria could be isolated from the active volcano site and this site represent an ecological niche which contains microbial populations (Amresan *et al.,* 2014).

Microflora of soil

Soil microflora are major parts of soil and show their importance in plant growth. Soil fertility depends not only on the presence of inorganic and organic substances but also on the presence of various species of microorganisms which influence quantitative composition of soil.

All organisms in the biosphere depend on microbial activities (Pace, 1997). The number of microbes in 1kg of soil is 200 million bacteria and 5000 million bacteria in clay soil and black soil respectively. It influence the above ground surface via paying their attention to plant growth and development (Foster, 1998). There are various microbes are found in a fertile soil as bacteria, fungi, actinomycetes and cyanobacteria etc.

These microbial species are fixed and can found in- situ in laboratory (Satya and Vijaya Marathi, 2016). Microorganisms can also produce phytohormones, enzymes and are capable to prevent phyto pathogens and insects and by performing this role, they promote plant growth (Shira *et al.,* 2005). Microbiota perform nutrient cycling, plant symbiosis, decomposition and many other ecological processes and by doing this, they participate in vegetative road cuts (Verma and Kuala, 2019).

Bacillus has some tendencies to work like biofertilizers and biopesticides (Aurelia Orliz and Estibaliz Sansinanea, 2022). Microbes are important elements of bioremediation of heavy metals in polluted lands. Genetically modified organisms can reduce different polycyclic hydrocarbons (PAHs). *Flavobacterium, Pseudomonas, Bacillus, Arthrobacter, Corynebacterium, Methosinus, Rhodococcus, Mycobacterium, Stereum* are some microbial species that help in bioremediation of heavy metals (Atif Khan and Rao, 2019).

Bacteria

Bacteria are small microscopic organisms and most abundant organisms on earth. These are single celled and their presence in soil is based on soil type (Trankin *et al.,* 2021). A tea spoon of productive soil generally contains between 100 million and 1 billion bacteria. Members of the phyla Proteobacteria and Archaebacteria are the most abundant soil bacteria. Some bacteria like *Bacillus, Actinomycetes, Rhizobium, and Pseudomonas etc* are examples of soil bacteria. Soil bacteria is also considered as a base for the development of surrounding environment (Van der Heijden *et al.,* 1998).

Fungi

There are four groups of soil fungi. They are *zygomycota, ascomycota, basidiomycota* and *Deutromycota*. Bacteria and fungi participate in biogeochemical cycling and these are responsible for cycling of organic compounds in atmosphere (Kyrpides and Olsen, 1999).

Actinomycetes

This is a group of common soil microorganisms present in soil and sometimes called "thread or ray of bacteria."

Archaebacteria

These are primitive prokaryotes. They possess some distinctive features as they are capable of surviving in extreme environments. They can grow in the absence of oxygen (Woese *et al.,* 1978). The archaebacteria have some similarities with eukaryotic organisms (School, 2002).

Cyanobacteria

Cyanobacteria are important microbe for rock associated communities and are considered as endolithic communities of dolomite rocks. Cyanobacteria are one of the main primary producers of organic matters in cryptographic barrens of the oases. Cyanobacteria are considered as the first organisms on earth.

Isolation of microorganisms from barren soil is crucial to find out their presence and role in soil. In this work, very few colonies are observed and this observation shows that their habitat is available in the soil sample.

The purpose of this review is to ensure microbial presence and to observe their activities and growth. We must find out the way to enhance microbial habitat in soil and increase their quantity to make soil suitable for vegetations.

Materials and Methods

Sample collection

The sample of barren soil was collected from Deoria road, Gorakhpur (Uttar Pradesh) in month of April (April 4, 2022) from the purpose of isolation of microbiota. The sample was taken from 10-15 cm depth. The soil sample was stored in a bag and brought it to the laboratory for the research purpose. At that time, 34ºC temperature was observed at that place.

Preparation of media

Both LB Media as well as PDA media were prepared. LB Media (Luria- Bertaini media), also known as lysogeny broth, is a commonly used medium. It was prepared by mixing LB broth (8 gm) and agar (6 gm) with 400 ml distilled water and then autoclaved this mixture for 20 minutes at 121ºC temperature and 15 lbs pressure.

PDA media (Potato Dextrose Agar) media was made up of potato infusion and Dextrose. The media was prepared with 5 gm Dextrose, 3.75 gm Agar and potato infusion and maintained 250 ml by adding distilled water. Sterilized it for 20 minutes in autoclave.

Serial dilution

The procedure was followed by serial dilution. Sample was serially diluted and 10 diluted samples were prepared.

Pure culturing

Spreading and streaking ware performed for the pure culturing, identification and other further studies.

Isolation

Sample was isolated on LB media and PDA media via streaking method. and incubated it for 24 hrs at 37ºC.

Gram Staining (For Identification)

Procedure

Step 1. Staining with Crystal violet.

Step 2. Added gram's Iodine to reduce solubility

Step 3. Washed with alcohol to decolourise gram (-) cell wall

Step 4. Added safranin for counterstaining.

Result of gram staining

As a result of Gram staining, a purple coloured colony was observed with microscope. Figures 5 and 6 are showing the result of Gram staining. Here purple and diffused white colonies are observed.

Antibiotic Susceptibility Test

Agar Well Diffusion Method

Bacterial culture showed different susceptibility for different antibiotics. In this test, antibiotics like, Streptomycin, Cefotaxime, Ceftriaxone are used and one well was filled with dH_2O.

Agar Disc Diffusion Method

Here culture was poured in a liquid agar media. The wells were fixed with antibiotics i.e. Streptomycin and Cefotaxime of different concentrations. The bacteria is more susceptible to Cefotaxime.

Results and Discussion

Very less quantity of microorganisms are isolated. After that we can consider that there are few number of microbes are present in soil. They can't survive because of lack of nutrients. Although isolate's growth is not appropriate so it can be considered that they are not active means not enough viable. The colony was observed after slightly a long time. The work of isolation was go through culturing, Gram staining, antibiotic test etc. and finally such colonies which are mentioned above, are obtained. Bacterial colonies as well fungal colonies are isolated together. Purple coloured bacillus are identified after gram staining.

Table.1 Showing reagents used in staining

Reagents	***Uses of reagents***
Crystal violet	*Staining*
Gram's Iodine	*Make dye less soluble i.e. moderant*
Alcohol	*Decolourise gram negative cell wall*
Safranine	*Counterstain*

Table.2 Showing antibiotic zone of inhibition

S.N.	Antibiotics	Conc. of Antibiotic	Culture conc.	Zone of inhibition
1	Streptomycin	5mg/ml	0.1ml	2.5 cm
2	Cefotaxime	5mg/ml	0.1ml	3.0cm
3	Ceftriaxone	5mg/ml	0.1ml	2.7cm
4	Distilled water	-	0.1ml	-

Table.3 Showing zones of inhibition by culture mix method

S.N.	Antibiotics	Antibiotic conc.	Culture conc.	Zone of inhibition
1	Streptomycin	5mg/ml	0.1ml	2.7cm
2	Cefotaxime	2.5mg/ml	0.1ml	2.9cm
3	Distilled water	-	0.1ml	-

Figure.1 Mixed culture colonies

Figure.2 Pure culture colony by streaking

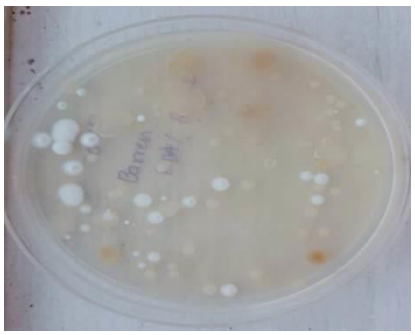


Figure.3 Pure culture on LB agar media

Figure.4 Pure culture on PDA agar media

Figure.5

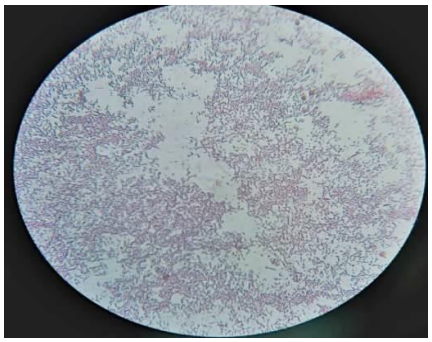


Figure.6

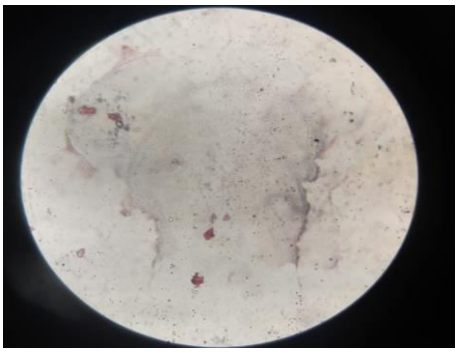
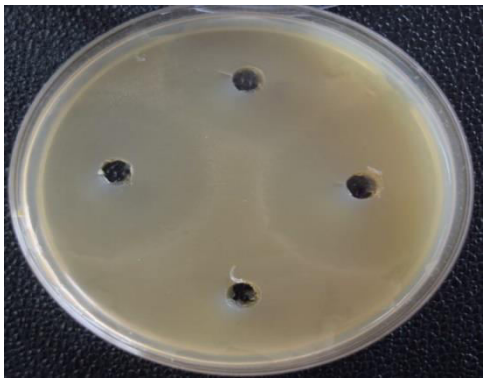


Figure.7 Showing inhibition of different antibiotics

Figure.8 Showing inhibition against antibiotics



The presence of microorganisms indicate that the soil is useful but the colonies are less developed and indicate that the soil is less productive. The soil is the base of ecological system. It's fertility and all other qualities perform their crucial role in sustainability of life and the microorganisms are also an important characters of this contribution. That's why it's necessary to improve their habitat in soil so that a barren soil can turn into the fertile and participate in susceptibility of ecosystem.

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Author Contributions

K. M. Kriti Mishra: Investigation, formal analysis, writing—original draft. Deepa Srivastava: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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