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Research Article

Isolation and Screening of Micro-Organisms from Soil Samples of Bilaspur (C.G.)

Pushpa Naik*, Shweta Sao and Varsha Gupta
Department of Microbiology, Dr C V Raman University, Kota, Bilaspur, (CG) - India

Abstract
Present investigation representing the isolation and identification of Bacteria and Fungi present in Industrial Soil sample, road side soil (Traffic area of Begumpet) and Garden soil. The bacteria identified from soil samples are *Staphylococcus aureus, Escherichia coli, Pseudomonas aerugionosa.* Fungi identified in Industrial soil were *Aspergillus niger, Penicillium spp.,* in road side soil are *Mucor and Rhizopus spp.,* in garden soil are *Penicillium spp., Mucor* and *Rhizopus.* The natural environment of micro flora is observed under laboratory conditions by setting Winogradsky column.
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Key words: Micro organism, Isolation, Soil, Bilaspur

Introduction
Soils normally contain low background levels of heavy metals. However, in areas where agricultural, industrial or municipal wastes are land-applied as fertilizer, concentrations may be much higher. Excessive levels of heavy metals can be hazardous to man, animals and plants. Although most organisms have detoxification abilities (i.e. mineralization, transformation and/or immobilization of pollutants), particularly bacteria, play a crucial role in biogeochemical cycles and in sustainable development of the biosphere (Diaz, 2004). Soil is the upper layer of most of the earth's surface and varies in depth from inches to over twenty feet. It is a product of weathered rock, but quite distinct in its characteristic. Soils are excellent cultural media for the growth of many types of organisms. This includes bacteria, fungi, algae, protozoa and viruses. A spoonful of soil contains billions of microorganisms. In general the majority of microbial population is found in the upper six to twelve inches of soil and the number decreases with depth. In the endogenous approach, plasmids are extracted from soil bacteria isolated on agar plates followed by a visualization of the plasmids on agarose gels (Campbell et al., 1995). The number and kinds of organisms found in soil depend upon the nature of soil, depth, season of the year, state of the cultivation, reaction, organic matter, temperature, moisture, aeration, etc.

*** Corresponding Author**

Microorganisms can be used to determine the bioavailability of a given chemical compound in soil. Specifically, measurement of plasmid- containing bacteria, using either an endogenous or exogenous approach, serves as a general indicator of environmental contaminants (Arias et al ., 2005).
Microbial survival in polluted soils depends on intrinsic biochemical and structural properties, physiological, and/or genetic adaptation including morphological changes of cells, as well as environmental modifications of metal speciation (Wuertz and Mergeay, 1997). For example, high levels of heavy metals can affect the qualitative as well as quantitative composition of microbial communities.

Material and Methods
Sampling
The samples were collected from different sites to identify soil micro flora present in different locations. The samples are collected from Industry, Road side soil of and Garden soil is collected . The samples are collected in sterile polythene bags.
Material
Soil Sample- From different place of soil collected at a depth of 6-10cm polythene bags by sterile method.
Laboratory media:- Dehydrated chemically defined media was used and prepared as per manufacture instructions.
I. *Nutrient agar media (NAM),* **II**. *Potato Dextrose Agar Media (PDA*), **III.** *Blood Agar Media,* **IV.** *Brain Heart Infusion Media* and V. Macconky Agar media:-
Materials
Petri plates, Test tube ,Test tube stand, Sprit lamp, Inoculation loop ,Spreader ,Conical flask ,Distilled water, Thread, Tape, Alcohal, Culture Tube, Cover slip, Cotton.
Method
To identify the bacterial unknowns in a mixed culture by morphological and biochemical methods. The identification of bacteria is a careful and systematic process that uses many different techniques.
Isolation
Sample preparation:
Soil Sample take From Different region of Bilaspur (C.G) of some plants at a depth of 6-10cm were collected in polythene begs and labelled. Prior to isolation soil

sample were allowed to air dry at room temperature for 3-4 Days, than made into fine powder for further use.

Isolation from sample (soil) by serial dilution Agar plating Method: Soil sample was performed by serial Dilution plate technique.

Principle

Micro-organism are abundant and ubiquitous in environmental. Soil is the principle habitat for different micro-organism. The quality and quantity of microbes depends on the soil's nature. The method is based upon the principle that when material containing micro-organism is cultured, each viable micro-organism will developed into a colony, hence the no. of colonies appering on the plates represent the no. of living organism present in the sample. Therefore, we can say that as the dilution increases, the no. of colonies decreases and the isolation of micro-organism from soil by serial dilution is made easy.

Procedure

- Normal saline solution was prepared and 10ml was taken into the test tube labelled as Stock and 9ml each into the remaining Ten test tubes and was sterilized by autoclaving at 121^0C, 15 lbs pressure for 15-20 minutes. 1 g of sample was weighed and added into the first tube, it was the stock solution.
- 1ml of suspension from stock solution was transferred into the test tube labelled as 10^{-1}. Then again 1ml was transferred from 10^{-1} to the tube labelled as 10^{-2}. Like this , up to 10^{-10} Serial dilution was done and 1ml from the last tube discarded to make the volume of all the tubes equal. This procedure was repeated for the sample.
- Required amount of Constituent of the NAM media was dissolved in distil water, and was autoclaved at 121^0C, 15 lbs pressure for 15-20 minutes.
- After Sterilization, the medium was poured into the sterile petri plates and was allowed to Solidify.
- After Solidification 0.1 ml of the suspension from the dilution 10-6 and 10-7 & 10-8 was spread on petri plates using spread plate technique.
- The inoculated plates were labelled and incubated at 28^0C for 5-7 days.
- Culture thus obtained are then purified and maintained in respective media for further Studies.

Identification of microorganisms

Staining (Gram Staining)

Principle: Staining is a simple basic technique that is used to identify microorganisms. Simple staining is used to study the morphology of all microorganisms.

This is a differential Staining method developed by Dr . Hans Christian Gram in 1884. It is a very useful stain for identifying and classifying bacteria into two major groups, the gram positive and the gram negative. The differences in staining process can be related to physical and chemical changes in their cell walls. The gram negative bacteria cell wall is thin, complex and multi-layered structure and contains relatively a high lipid contents in addition to protein and mucopeptides. The higher amount of lipid is readily dissolved by Alcohol, Resulting in the formation of large pore in the cell wall Which do not close appreciably on dehydration of cell wall protein thus facilitating the leakage of crystal violet(CV) Iodine complex and resulting in the decolorization of the bacterium Which later takes the counter stain and appears red in contrast gram positive cell wall are thick and chemically simple compound mainly protein and cross linked mucopeptides. When treated with alcohol, it causes dehydration and closure of cell wall pore. Therefore dose not loss CV-I complex and remain purple. The bacteria which retain the primary stain i.e. not decolourization when stained with grams method Gram positive. The bacteria which lose the crystal violet and counter stained by safranine Gram negative.

Gram Staining Requirments:

Cristal violet(CV), 2. Iodine, 3. Alcohal or Acetone, 4. Safranine.

Staining Procedure

A thin bacterial smear was prepared on a clean glass slide and air dried and heat fixed. The smear was flooded with crystal Violet (1min). The excess stain was removed by rinsing it under tap water. After that, the smear was fixed with Gram iodine (1 min). The excess stain was removed by rinsing it under tap water. Then it was decolorized with 95% alcohol (10-20 sec. or until the excess alcohol which flow off the slide is colourless.) Again it was rinsed under running tap water slowly. Finally, the smear was counter stained with Safranine (30 sec.). The excess stain was removed by rinsing it under tap water. The slide was blot dried with bibulous paper and observed under 10x, 40x and 100x objectives of microscope.

Biochemical test

1. Carbohydrate Fermentation Test
2. Antifungal Assay
3. Indol Acetic Acid (IAA)Production

Carbohydrate Fermentation test

Principle: Microorganism metabolize carbohydrates to meet their energy requirments and synthetic activity under anaerobic condition resulting in the production of acid or gases. The metabolic end products of carbohydrates fermentation can be either organic acid (ex:-lactic, formic or Acetic) or Organic acid and gas(example-hydrogen or carbon-di-oxide). The production of acids can be detected by incorporating pH

indicator in the medium (phenol red) which is red at netural and turn to yellow at or below pH 6.8.

Procedure

- Glucose/Lactose/Sucrose Agar was prepared and was autoclaved at 121^{0}C 15 lbs pressure for 15-20 minutes.
- Above three types of sugar Fermentation agar was inoculated with each isolates and one was kept un-inoculated control.
- Inoculated agar with the specified cultures was kept for incubation at 28^{o}C for 5 day.

Antifungal Assay

The actinomycetes isolates were evaluated for their activity against two fungi: *Alternaria* sp. These fungi were maintained on potato dextrose agar (PDA). Screening of actinomycetes for antifungal assay against these fungi was studied by dual culture technique. Fungal discs (6 mm diam.), 5 days old on (PDA) at 28^{o}C were placed at the center of PDA plates. Two discs of actinomyces (6mm) 5 days old, grown on yeast malt extract agar (YM) incubated at 28^{0}C were placed on opposite sides of the plates, 3 cm away from the fungal disc. Plates without the actinomycetes disc served as controls. All plates were incubated at 28^{o}C for 7-14 days and colony growth inhibition (%) was calculated by using the formula: C-T/C x 100, Where C is the colony growth of pathogen in control, and T is the Colony growth of pathogen in dual culture.

Table 1: Represents the List of Fungi identified in the study area soil sample

S/No.	Name of Microorganism Bacteria & Fungus	Biochemical Test					
		Glucose Fermentation Test	Casien Hydrolysis Test	H2S production	Ureast test	Indol Test	Staning
1.	*Clostridium Tetanae* (B.A media)	-	-	-	-	+	-
2.	*Clostridium Tetanae* (M.A media)	-	-	-	-	+	_
3.	*Actinomycetes* (Brain Heart infusion media)	_	_	-	_	_	+
4.	*Aspergillus sp.(NAM)*	_	_	_	_	_	+
5	P*enecillium (NAM)*	_	_	_	_	_	+
6	*Rhizopus*(B.A media)- Indian ink prepaieation	_	_	_	_	_	+
7.	*Mucor*(B.A) Indian ink prepairation	_	_	_	_	_	+
8.	*Candida sp*.(B.A media)	_	_	_	_	_	+

Indole Acetic Acid (IAA) Production

Indole acetic acid is a plant growth promoter that produced by microorganism in rhizospheric region. The production of IAA by the isolates was determined according to the method of Bano and Musarrat. The actinomycete disc (8 mm), grown on yeast malt extract agar (YM) incubated at 28^{0}C for 5 days, were inoculated into 5ml YM broth Containing 0.2% L-tryptophan and incubated 28^{0}C with shaking at 125 rev/min for 7 days. After incubation culture were centrifuged at 11,000 rev/min for 15 min. 1ml of supernatant was mixed with 2 ml of salkowski reagent (1ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) and incubated at room temperature for 30 min in the dark. Appearance of a pink color indicated IAA production.

Table 2: List of Bacteria identified in the study AREA SOIL SAMPLE

Sample sites	**Bacteria**	**Fungi**
Industrial Soil (NTPC Seepat Area)	*Staphylococcus aureus Escherichia coli, Cynobacteria, Actinomyces, Clostridium.*	*Aspergillus Niger Penicillium, Rhizopus.*
Road Site soil (CMD & Bus stand Area)	Staphylococcus aureus Escherichia coli, Clostridium, Actinomycetes.	*Mucor Rhizopus, Aspergillus.*
Garden Soil (Company garden of bilaspur)	*Staphylococcus aureus Escherichia coli, Clostridium, Streptomycetes, Cynobacteria, Pseudomonas aeruginosa.*	*Penicillium, Mucor Rhizopus, Candida, Aspergillus Niger.*

Results and Discussion

The soil samples were analysed with respect to different types of Bacteria and Fungi. The common bacteria, *Staphylococcus aureus, Escherichia coli, Pseudomonas aerugionosa, Cynobacteria, Actinomycetes, Clostridium,* are found in all the soil samples. But, in Industrial area, road side (Traffic area) samples, *Pseudomonas aerugionosa* was not observed (Table2)

Similarly, when the soil samples are tested different types of Fungi, *Penicillium* fungi are found in all the soil samples. *Mucor* and *Rhizopus, and Candida,*are found in Road side sample and Garden soil sample. *Aspergillus Niger* was observed in Industrial soil sample (Sugar Industry) because of easily and abundantly available carbon source by-product of sugar industries Ali (2004) (Table 1).

In addition, bacterial isolates recovered from both sites showed slightly difference in MICs for Cu, Cd, pb, Zn, Hg and Ni. Similar observation was reported by earlier researchers (Kunito et al ., 1986; Chaudhary and Kumar, 1996). However, Bacterial isolates recovered from contaminated soil showed relatively high MICs for Co, Cr, and As in comparison with those isolated from non-uncontaminated soil (Campbell et al ., 1995; Appanna et al., 1996). Bacterial isolates from soil irrigated with canal water were exhibited high MICs for tellurium (Te) in comparison with isolates isolated from contaminated soil. Previous results reported that Enterobacteriaceace resistance to potassium tellurite (K 2TeO 3) are found in urban sewage and were particularly prevalent in waste from a photographic processing plant (Taylor and Summers, 1979). In industrial area, Road side soil (traffic areas) soil samples, the minimum required nutritional conditions are not observed due to heavy metal pollution, such as Lead, Copper, Nickel. Nilufer Cevik, Ayten Karaca . Effect of Cadmium, Zinc , Copper and Fluoranthene on Soil bacteria. (Turkey**).** Even though oxygen is not pre requisite for the growth of *Pseudomonas aerugionosa,* at least NO3 must be available as respiratory electron acceptor which is minimum in these soil samples.

Some microbial strains possess genetic determinants that confer the resistance. In bacteria, these determinants are often found on plasmids, which have facilitated their study at the molecular level (Cervantes et al ., 1994). Monitoring of antibiotic-resistant bacteria in soil can be used as an indicator of industrial and urban pollution. Several studies have found that metals influence microorganisms by harmfully affecting their growth, morphology, and biochemical activities, resulting in decreased biomass and diversity (Baath, 1989; Reber; 1992; Malik and Ahmed, 2002).

From the present Study ,it Could be Concluded the

Conclusion

Different type of soils selected from the Different Sites & Isolates Different types of Microorganism (Bacteria, Fungi) etc. *Actinomycetes , Cynobacteria , Clostridium Tetani , Aspergillus , Penicilline, Mucor, Candida, Rhizopus*, etc.

Detail studies of some microorganism eg. Actinomycetes, Their more detail investigation is required to Demostrate the potential of these Organism for the Bio-Control of pathogenic fungi & in plant growth promotion Which may be useful in pharmacological and agriculture fields in the future.

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