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ISOLATION OF PURE CULTURE BACTERIA FROM SOIL SAMPLES

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The isolation of soil bacteria from extreme environments represents a major challenge, but also an opportunity to characterize the metabolic potential of soil bacteria that could promote the growth of plants inhabiting these harsh conditions. The aim of this study was to isolate and identify bacteria from soilsoil Samples environments and characterize the beneficial traits for plants through a biochemical approach.

Keywords : Culture media ,Soil, Serial dilution technique , Pour Plate technique, Spreads Plate technique

1.INTRODUCTION

PURE CULTURE: A culture containing only one species of microbe is called pure culture.

Isolation of pure culture -

Microorganisms are generally found in nature (air, soil and water) as mixed populations. Even the diseased parts of plants and animals contain a great number of microorganisms, which differ markedly from the microorganisms of other environments. To study the specific role played by a specific microorganism in its environment, one must isolate the same in pure culture.

A pure culture theoretically contains a single bacterial species. There are a number of procedures available for the isolation of pure cultures from mixed populations. A pure culture may be isolated by the use of special media with specific chemical or physical agents that allow the enrichment or selection of one organism over another. The differential and selective procedures will be utilized later in this course.

Isolation of pure culture method

1. Serial dilution technique
2. Pour Plate technique
3. Spreads Plate technique

Culture media -

The food material or substances required for growing microorganisms in vitro (outside the body) is called culture medium.

Classification of culture media based on physical state:
a) solid medium
b) semi solid medium
c) liquid medium

Soil

Soil is the thin layer of material covering the earth's surface and is formed from the weathering of rocks. It is made up mainly of mineral particles, organic materials, air, water and living organisms—all of which interact slowly yet constantly. Agriculture soil is a dynamic medium in which a large number of pathogenic and non-patho-genic bacterial and fungal flora live in close association

Soil Samples

1. Brown soil
2. Black soil
3. Red soil

2. AIM AND OBJECTIVE

Aim- Isolation of pure culture bacteria from soil samples

Objective-

1. To demonstrate good aseptic technique in culture transfer or inoculation and in handling sterile materials.
2. To demonstrate skill in isolation of organisms from a mixed culture using selective and differential media.
3. To isolate microorganisms from a wide variety of soil and describe their colonial.

3. SELECTION OF SOILS

1 Brown soil

Selected soil from yeshodeep Institute of pharmacy college ground.

2 Black soil

Selected soil from farm

3 Red soil

Selected soil from play ground

Fig.1 Black Soil

Fig.2 Red Soil

Fig 3.Brown Soil

4. MATERIAL AND METHODS

Material

1. Eight 9ml dilution tubes of sterile saline and Distilled water
2. Eight nutrient agar plates
3. 1.0 ml and 0.1 ml pipets
4. Glass spreader aka (hockey stick)
5. 95% ethyl alcohol in glass beaker (WARNING: Keep alcohol away from flame!!)
6. Mixed overnight broth culture of Staphylococcus aureus and Serratia marcescens

Methods

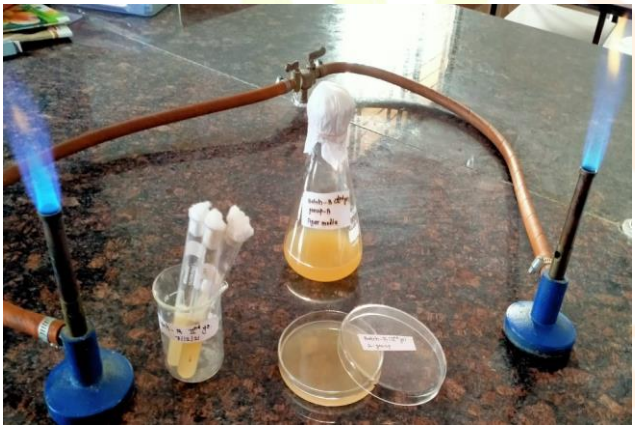
1. Serial Dilution technique
2. Pour Plate technique
3. Spreads Plate technique

Agar Media Preparation

Weigh all the ingredients as per given quantity add all ingredients in proper proportion into water in conical flask apply heat to dissolve and then cover its mouth. Place this conical flask for sterilization at autoclave for sterile of agar solution. After sterilization pour agar solution in petri plate make them solidify. All this process carried out in aseptic area.

Tab. 1: Composition of Agar Media

Ingredient	Amount (gm/ml)	Uses
Peptone	5gm	Growing Bacteria
Yeast extract	3gm	Additive for culture media.
Nacl	8gm	Preparing microbiological culture Media
Agar	15gm	For Growing microorganism
Distilled water	1000ml	Vehicle



Serial dilution Technique

1. Take 9 ml of sterile physiological saline or sterile distilled water in a sterile test tube numbering from 1 to 5 the number of dilution is not fix for any particular bacteria suspension / Specimen.

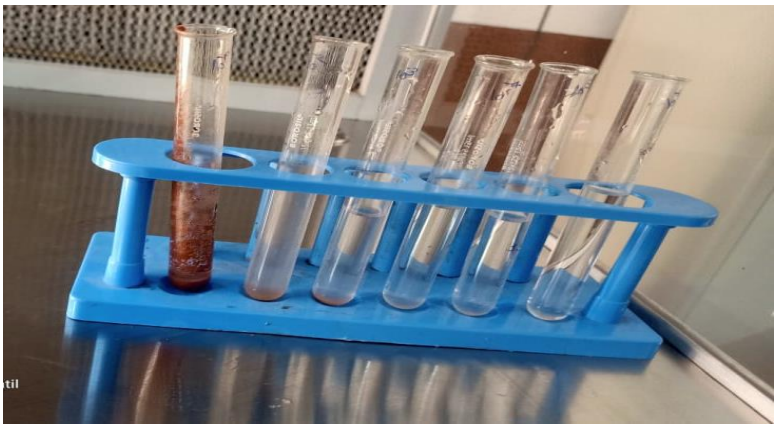
2. Transfer 1 ml of suspension of a given mixed culture to tast tube to shake the tube well.

3. Transfer 1 ml of suspension for test tube 1 to test tube to shake well.

4. Repeat this same process after the other test tube so has to get the desired diutions

5. Transfer 1 ml of the suspension for each dilution to sterile perti plates, separately to which melted and cooled Agar medium is poured.

6. The plates are rotated gently to solidity and incubated at 35°C for 25 to 48 hours to inverted positions.



2. Pour Plate Method

1. Take 9 ml of sterile physiological saline or sterile distilled water in a sterile test tube numbering from 1 to 5 the number of dilution is not fix for any particular bacteria suspension / Specimen.

2. Transfer 1 ml of suspension of a given mixed culture to tast tube to shake the tube well.

3. Transfer 1 ml of 10^1, 10^2, 10^3, 10^4, 10^5, 10^6, 10^7, 10^8 suspension for test tube 1 to test tube to shake well.

4. Repeat this same process after the other test tube so has to get the desired dilutions.

5. Transfer 1 ml of the suspension for each dilution to sterile perti plates, separately to which melted and cooled Agar medium is poured.

6. The plates are rotated gently to solidity and incubated at 35°C for 25 to 48 hours to inverted positions.

3. Spreads Plates Techniques

1. Take three nutrient Agar plates and label them with the name of the organism to be inoculated

2. Aseptically inoculate the plates with a loopful of the given organism.

3. Place plate 1 on the turn plate table.

4. Sterilize the spreader by putting it first in ethanol (95%) in a beaker, than on the flame of bunsen burner and cool the rod for the 3 second.

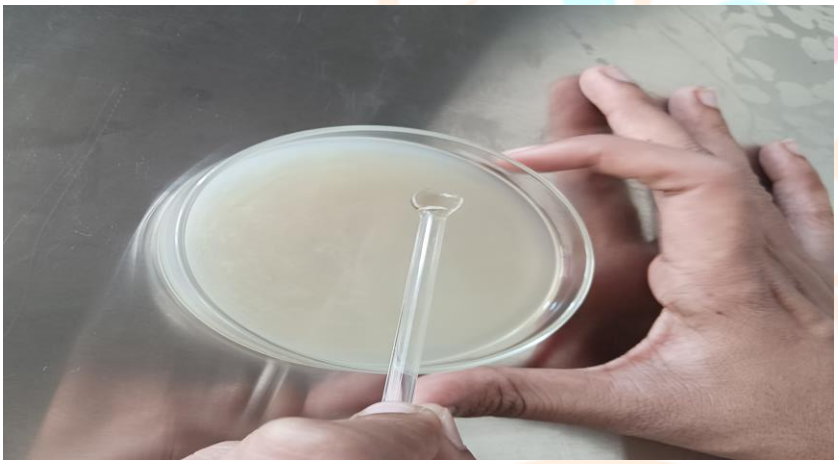
5 . Remove the lid of plate and Spin the turn table.

6 . Touch the Spreader gently on the surface ofi agar and move it for the and back to Spread bacterial cells on the agar surface when the turn table is stanning

7. When turn table stop spinning but the lid over the lower half of petri dish.

8. Sterilize the Spreader again and reapeat the same process for the other two platesplates.

9. Incubate all the plates at 31°c for 24 hours.



4. Colney Count

Turn ON the Instrument by pressing the On/Off Switch. Place the Petri Plate on the Glass grid. register a count, there will be a beep and an ink dot will be marked on the Petri Dish. Continue till all the colonies are thus counted.

Observation

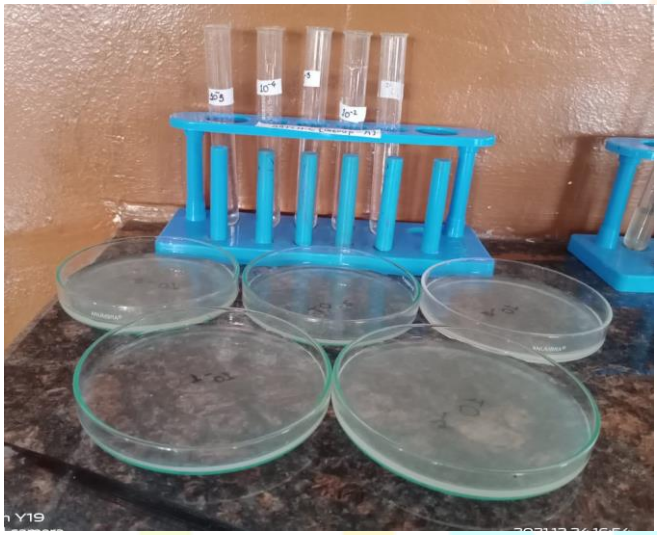


Fig Serial Dilution atechnique

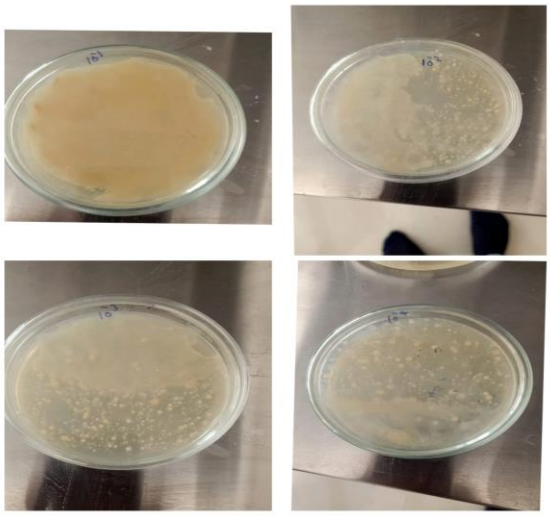


Fig Pour Plate technique

Fig Pour Plate technique

Fig Colney Count

Colney Count

	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8
S1	70	63	50	46	34	32	26	18
S2	61	58	47	40	38	36	29	20
S3	40	31	25	24	22	15	13	11

Identification Test

Soil Samples	**Bacteria**	**Gram Positive**	**Gram Negative**
Red Soil	Steptomycetaceae	+ve	-
Brown Soil	Actenomycetes	+ve	-
Black Soil	Azatobacter	-	-ve

Result:

Isolation of pure culture and identified bacteria from soil samples was performed.

Conclusion

Isolation of soil bacteria from different places were differnciate. The different soil having different range of bacteria some are useful and some were harmful. This is carried out by the method of Isolation of Pure culture, in this maintain all the sterility for growth of microorganisms in soil.

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