RAMA UNIVERSITY

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FACULTY OF ENGINEERING & TECHNOLOGY

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Introduction

Application of scientific and engineering principles to the processing of materials by microorganisms to create useful products or processes.

Microorganisms utilized may be natural isolates, laboratory selected mutants or microbes that have been genetically engineered using recombinant DNA methods.

Deals with the prevention of deterioration of processed or manufactured goods, environmental protection and with waste disposal system.

Production of antibiotics, organic acids and enzymes by fermentation of natural microbes, laboratory selected mutants or microbes genetically engineered using recombinant DNA methods.

Microbial Biotechnology in Agriculture and Food

Development of genetically engineered plants with internal resistance to drought, frost, insect pests and infestation reduction in dependency of plants on chemical fertilizers and identification of alternatives to expensive fertilizers replacement of dangerous chemical pesticides with microbial pesticides to manage and control the problem of pests.

Microbial Biotechnology in Agriculture and Food

Reduction in the reliance on chemical treatments to control weeds by engineering herbicide tolerance into crops production of products that have high yield and enhanced nutritional value development of novel biomass products as foodstuffs, using organisms such as algae, fungi, bacteria and yeast.

Microbial Biotechnology in Food and Agriculture

Food improved and preserved by fermentation. Fermentation: Any process that produces alcoholic beverages or acidic dairy products. Any spoilage of food by microorganisms Any large-scale microbial process occurring with or without air. All metabolic processes that release energy from a sugar or other inorganic molecule.

Microbial Biotechnology in health care

Production of insulin: For many years, insulin was obtained by purifying it from the pancreas of cows & pigs slaughtered for food. This was expensive, difficult and the insulin could cause allergic reactions.

SCOPE OF MICROBIAL BIOTECNOLOGY

•Microorganisms are present everywhere on earth which includes humans, animals, plants and other living creatures, soil, water and atmosphere.

•Microbes can multiply in all three habitats except in the atmosphere. Together their numbers far exceed all other living cells on this planet.

•Microorganisms are relevant to all of us in a multitude of ways. The influence of microorganism in human life is both beneficial as well as detrimental also.

•For example microorganisms are required for the production of bread, cheese, yogurt, alcohol, wine, beer, antibiotics (e.g. penicillin, streptomycin, chloromycetin), vaccines, vitamins, enzymes and many more important products.

•There is vast scope in the field of microbiology due to the advancement in the field of science and technology.

•The scope in this field is immense due to the involvement of microbiology in many fields like medicine, pharmacy, diary, industry, clinical research, water industry, agriculture, chemical technology and nanotechnology.

•The study of microbiology contributes greatly to the understanding of life through enhancements and intervention of microorganisms. There is an increase in demand for microbiologists globally.

•**Genetics:** Mainly involves engineered microbes to make hormones, vaccine, antibiotics and many other useful products for human being.

•**Agriculture:** The influence of microbes on agriculture; the prevention of the diseases that mainly damage the useful crops.

•**Food science:** It involves the prevention of spoilage of food and food borne diseases and the uses of microbes to produce cheese, yoghurt, pickles and beer.

•**Immunology:** The study of immune system which protect the body from pathogens.

•**Medicine:** deals with the identification of plans and measures to cure diseases of human and animals which are infectious to them.

•**Industry:** it involves use of microbes to produce antibiotics, steroids, alcohol, vitamins and amino acids etc.

Agricultural microbiology – try to combat plant diseases that attack important food crops, work on methods to increase soil fertility and crop yields etc. Currently there is a great interest in using bacterial or viral insect pathogens as substitute for chemical pesticides.

Microbial ecology – biogeochemical cycles – bioremediation to reduce pollution effects

Food and dairy microbiology – try to prevent microbial spoilage of food and transmission of food borne diseases such as botulism and salmonellolis. Use microorganisms to make foods such as cheese, yogurt, pickles and beers.

Industrial microbiology – used to make products such as antibiotics, vaccines, steroids, alcohols and other solvents, vitamins, amino acids and enzymes.

Microbial physiology and Biochemistry – study the synthesis of antibiotics and toxins, microbial energy production, microbial nitrogen fixation, effects of chemical and physical agents on microbial growth and survival etc.

Microbial genetics and Molecular biology – nature of genetic information and how it regulated the development and function of cells and organisms. Development of new microbial strains that are more efficient in synthesizing useful products.

Genetic engineering – arisen from work of microbial genetics and molecular biology. Engineered microorganisms are used to make hormones, antibiotics, vaccines and other products. New genes can be inserted into plants and animals

Applications of Microbial Biotechnology

•Microbiology is one of the largest and most complex of the biological sciences as it deals with many diverse biological disciplines.

•In addition to studying the natural history of microbes, it deals with every aspects of microbe-human and environmental interaction. These interactions include: ecology, genetics, metabolism, infection, disease, chemotherapy, immunology, genetic engineering, industry and agriculture.

The environment:

•Microbes are responsible for the cycling of carbon, nitrogen phosphorus (geochemical cycles)

•Maintain ecological balance on earth

•They are found in association with plants in symbiotic relationships, maintain soil fertility and may also be used to clean up the environment of toxic compounds (bio-remediation).

•Some are devasting plant pathogens, but others act as biological control agents against these diseases.

Medicine:

•Commercial applications include the synthesis of acetone, organic acids, enzymes, alcohols and many drugs.

•Genetic engineering – bacteria can produce important therapeutic substances such as insulin, human growth hormone, and interferon.

Food:

•Microorganisms have been used to produce food, from brewing and wine making, through cheese production and bread making, to manufacture of soy sauce.

•Microbes are also responsible for food spoilage.

Isolation and Preservation of Pure Bacterial Culture

Pure Culture Technique

• **Culture** : Act of cultivating microorganisms or the microorganisms that are cultivated

Mixed culture : more than one microorganism

Pure culture : containing a single species of organism.

• A pure culture is usually derived from a mixed culture (one containing many species) by transferring a small sample into new, sterile growth medium in such a manner as to disperse the individual cells across the medium surface or by thinning the sample many times before inoculating the new medium.

Why important ?

Pure cultures are important in microbiology for the following reasons-

1.Once purified, the isolated species can then be cultivated with the knowledge that only the desired microorganism is being grown.

2. A pure culture can be correctly identified for accurate studying and testing, and diagnosis in a clinical environment.

3. Testing/experimenting with a pure culture ensures that the same results can be achieved regardless of how many time the test is repeated.

ISOLATION TECHNIQUE OF PURE CULTURE

1.Cultures composed of cells arising from a single progenitor

2.Progenitor is termed a CFU

3.Aseptic technique prevents contamination of sterile substances or objects

4.Common isolation techniques

-Streak plate method

-Pour plate method

-Spread plate method

Special methods of isolation on of pure culture

Single cell isolation methods

Capillary pipette method

Micromanipulator method

Enrichment culture method

The enrichment culture strategy provides a specially designed cultural environment by incorporating a specific nutrient in the medium and by modifying the physical conditions of the incubation.

1.Streak plate method

• Streaking is the process of spreading the microbial culture with an inoculating needle on the surface of the media.

• Sterilize the inoculating needle by flame to make red hot and allow it to cool for 30 seconds.

• The sample is streaked in such a way to provide series of dilution.

• purpose-thin out inoculum to get separate colonies.

•subculturing can be done by streaking well isolated colonies from streak plate to new plate.

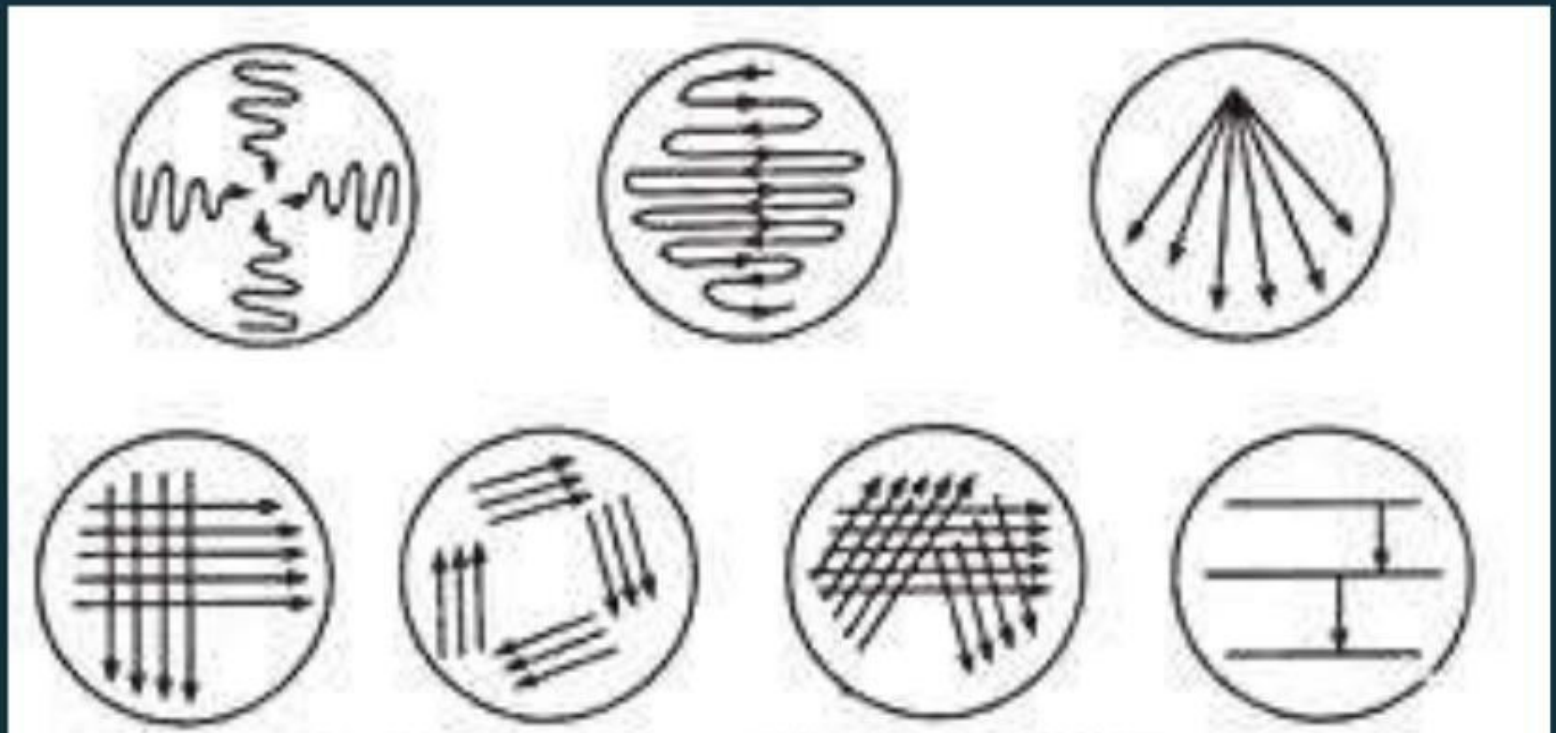


FIG. 16.13. Various methods of streaking.

2. Pour plate method

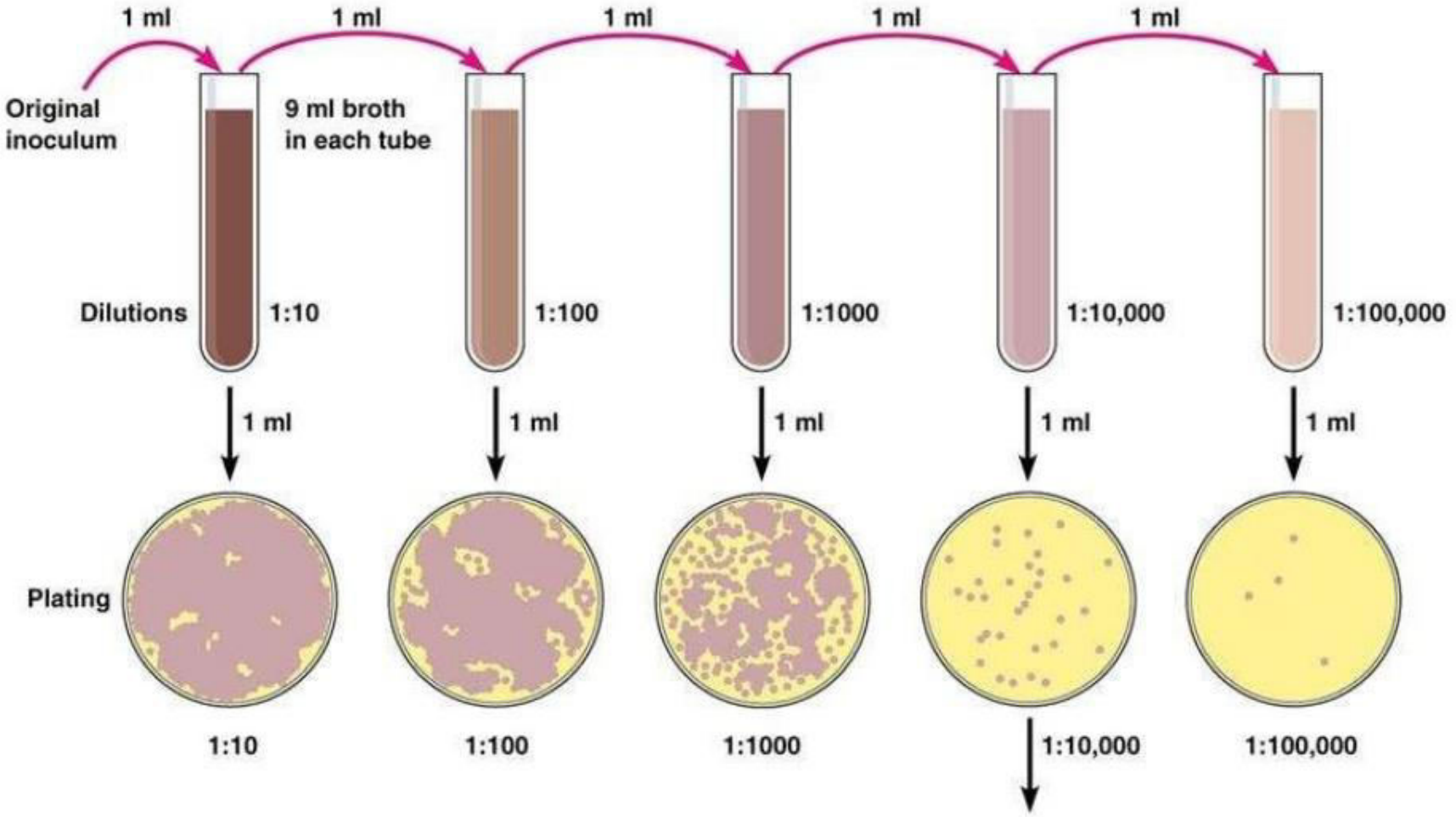
• The bacterial culture and liquid agar medium are mixed together.

• After mixing the medium, the medium containing the culture poured into sterilized petridishes (petriplates), allowed solidifying and then incubated.

• After incubation colonies appear on the surface.

DISADVANTAGES-

1. Microorganism trapped beneath the surface of medium hence surface as well as subsurface colonies are developed which makes the difficulties in counting the bacterial colony.
2. Tedious and time consuming method, microbes are subjected to heat shock because liquid medium maintained at 45℃.
3. Unsuitable- Psychrophile



Calculation: Number of colonies on plate × reciprocal of dilution of sample = number of bacteria/ml
(For example, if 32 colonies are on a plate of $^{1}/_{10,000}$ dilution, then the count is 32 × 10,000 = 320,000 bacteria/ml in sample.)

3. Spread plate method

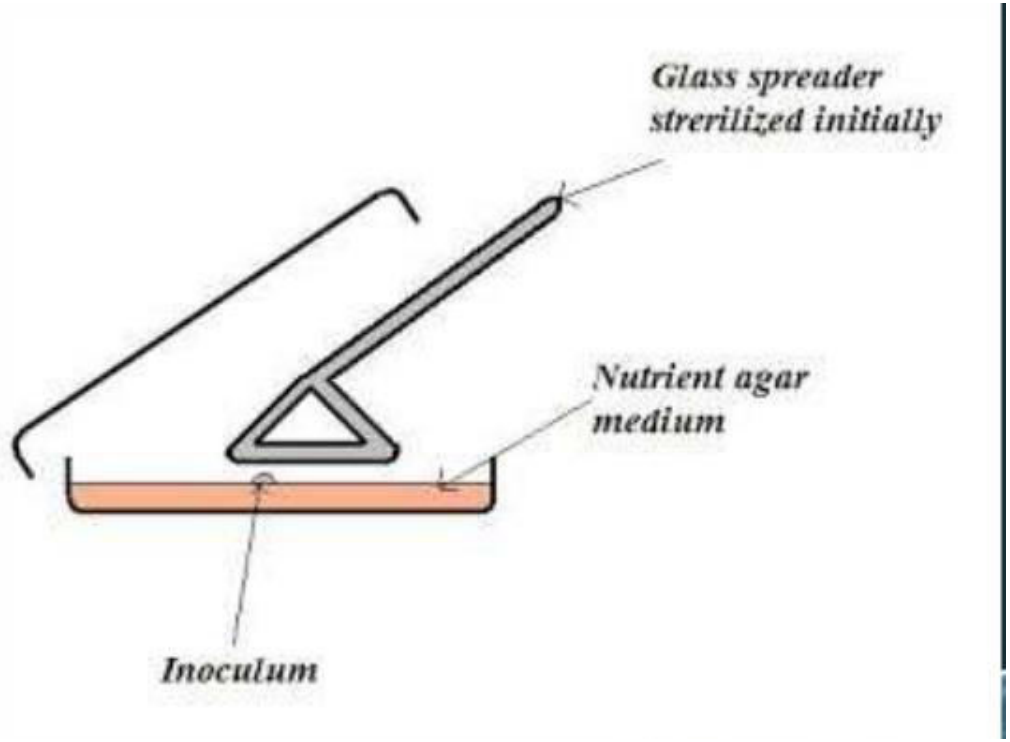
• This is the best method to isolate the pure colonies.

• In this technique, the culture is not mixed with the agar medium. Instead it is mixed with normal saline and serially diluted.

• 0.1 ml of sample taken from diluted mixture, which is placed on the surface of the agar plate and spread evenly over the surface by using L shaped glass rod called spreader.

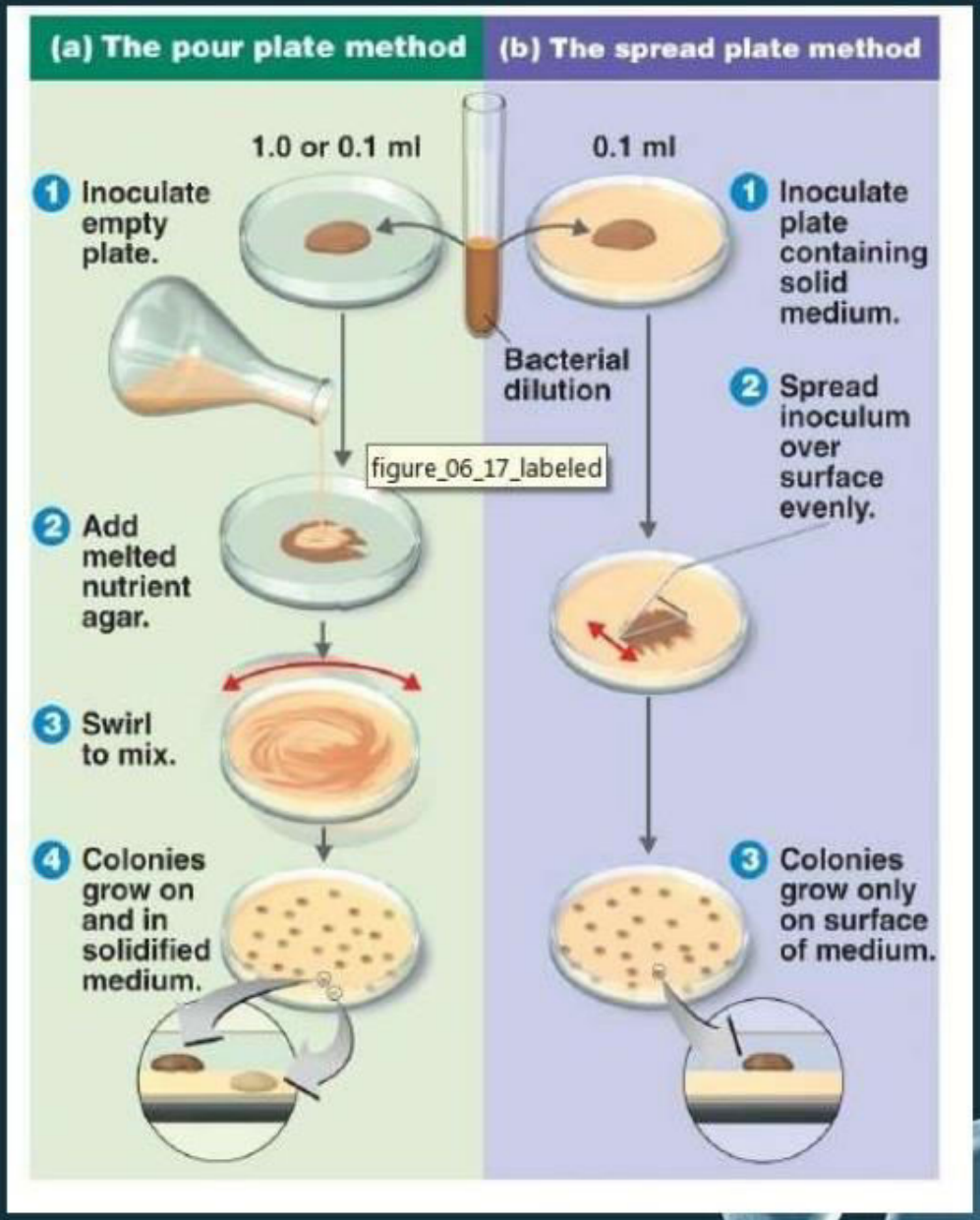
• Incubate the plates

• After incubation, colonies are observed on the agar surface.

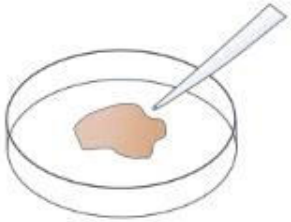

Glass spreader
strerilized initially
Nutrient agar
medium
Inoculum

DISADVANTAGES

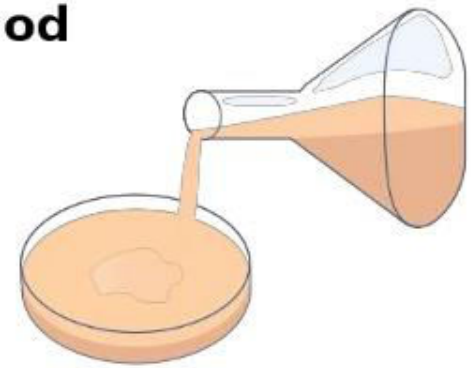
1. It is a simple method.
2. In this method only surface colonies are formed.
3. Micro-organisms are not exposed to higher temperature.


(a) The pour plate method
(b) The spread plate method
1.0 or 0.1 ml
0.1 ml
1 Inoculate empty plate.
1 Inoculate plate containing solid medium.
Bacterial dilution
2 Spread inoculum over surface evenly.
figure_06_17_labeled
2 Add melted nutrient agar.
3 Swirl to mix.
4 Colonies grow on and in solidified medium.
3 Colonies grow only on surface of medium.

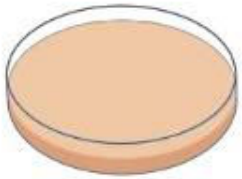
Pour plate method



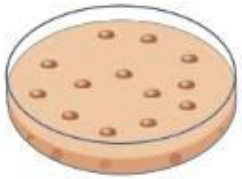
pipette inoculum onto sterile plate



add sterile medium



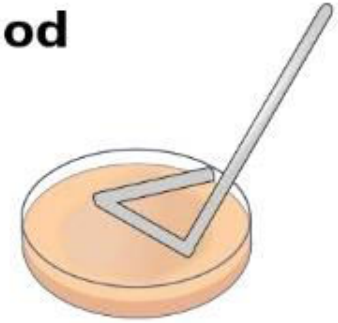
swirl to mix and incubate



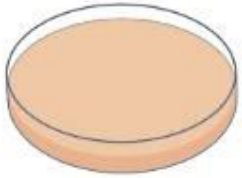
colonies grow
in and on medium

Spread plate method

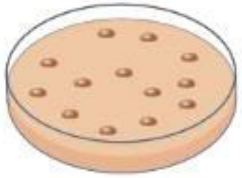
pipette inoculum onto the surface
of agar plate



spread evenly over the agar surface



incubate



colonies grow only
on the surface of medium

5. Micromanipulator method

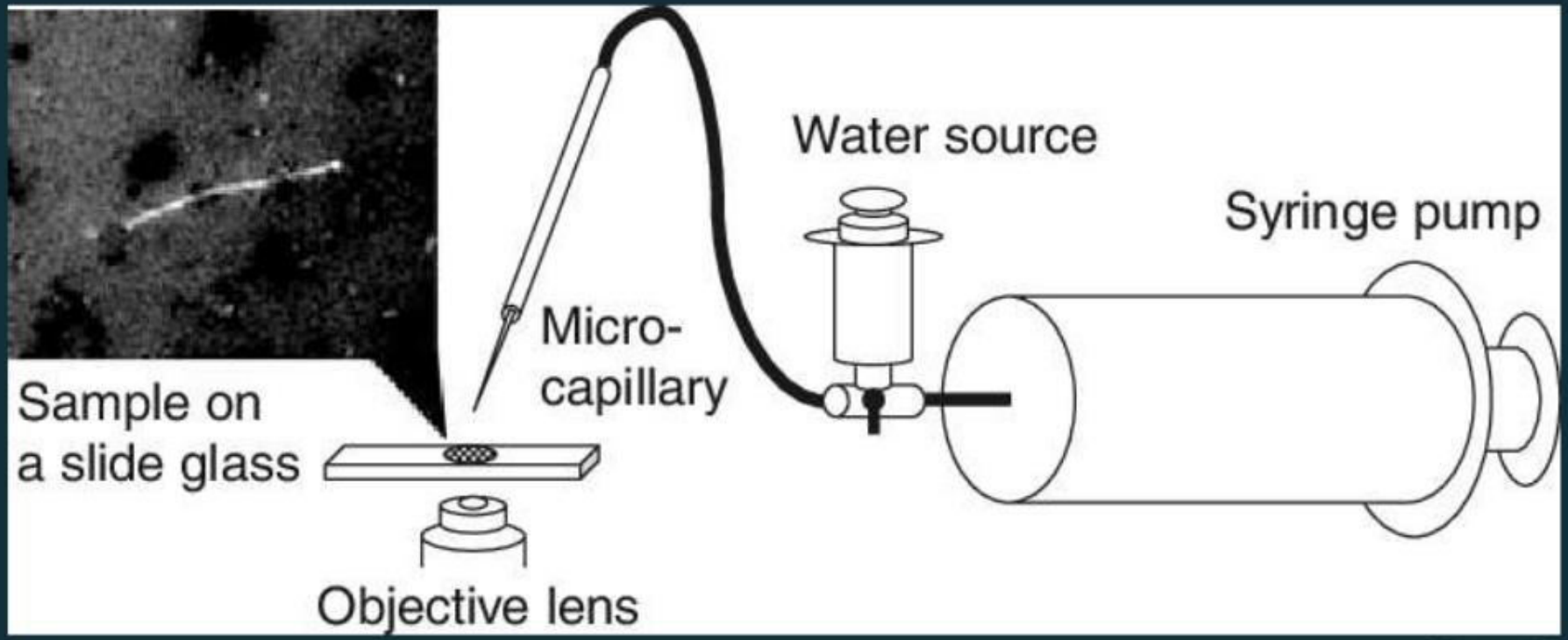
Micromanipulators have been built, which permit one to pick out a single cell from a mixed culture. This instrument is used in conjunction with a microscope to pick a single cell (particularly bacterial cell) from a hanging drop preparation. the single cell of microbe sucked into micropipette and transferred to large amount of sterile medium.

ADVANTAGES OF MICROMANIPULATOR METHOD-

The advantages of this method are that one can be reasonably sure that the cultures come from a single cell and one can obtain strains with in the species.

DISADVANTAGES-

The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled person.


Water source
Syringe pump
Micro-
capillary
Sample on
a slide glass
Objective lens

PRESERVATION OF PURE CULTURE

- To maintain pure culture for extended periods in viable condition without any genetic change is referred as Preservation.
- During preservation most important factor is to stop microbial growth or at least lower the growth rate.
- Due to this toxic chemicals are not accumulated and hence viability of microorganism is not affected.

Objectives of preservation

1. To maintain isolated pure culture for extended periods in a viable conditions.
2. To avoid contamination
3. To restrict Genetic Mutation

Why to Preserve Bacteria?

- In nature there are only 1% bacteria which is pathogenic and harmful to Animalia and Plantae.
- 99% of bacterial populations are of economic importance for human beings and plants.
- In soil for nutrient uptake in food industry, in sewage treatment, in medical industry.
- So the preservation of bacteria is one of the most profitable practice economically as well as environmentally.

1. Academic purpose
2. Research Purpose
3. Biotechnology field
4. Fermentation Industry

Preservation methods of bacteria

1. Periodic transfer to fresh medium
2. Storage at low temperature
3. Preservation by overlaying culture with mineral oil
4. Lyophilization or freeze drying

1. Periodic transfer to fresh medium

• Strains can be maintained by periodically preparing a fresh culture from the previous stock culture.

• The culture medium, the storage temperature, and the time interval at which the transfers are made vary with the species .

• The temperature and the type of medium chosen should support a slow rather than a rapid rate of growth so that the time interval between transfers can be as long as possible.

• Many of the more common heterotrophs remain viable for several weeks or months on a medium like Nutrient Agar.

• The transfer method has the disadvantage of failing to prevent changes in the characteristics of a strain due to the development of variants and mutants

2. Storage at low temperature

- REFRIGERATION
- CRYOPRESERVATION

REFRIGERATION

Pure cultures can be successfully stored at 0-4°C either in refrigerators or in cold-rooms.

This method is applied for short duration (2-3 weeks for bacteria and 3-4 months for fungi) because the metabolic activities of the microorganisms are greatly slowed down but not stopped.

Thus their growth continue slowly, nutrients are utilized and waste products released in medium.

This results in finally the death of the microbes.

CRYOPRESERVATION

- Cryopreservation (i.e., freezing in liquid nitrogen at -196°C or in the gas phase above the liquid nitrogen at -150°C) helps survival of pure cultures for long storage times.
- In this method, the microorganisms of culture are rapidly frozen in liquid nitrogen at - 196°C in the presence of stabilizing agents such as glycerol or Dimethyl Sulfoxide (DMSO) that prevent the cell damage due to formation of ice crystals and promote cell survival.
- This liquid nitrogen method has been successful with many species that cannot be preserved by lyophilization and most species can remain viable under these conditions for 10 to 30 years without undergoing change in their characteristics, however this method is expensive.

3. Preservation by overlaying culture with mineral oil

- This is a simple and most economical method of maintaining pure cultures.
- In this method, sterile liquid paraffin is poured over the slant (slope) of culture and stored upright at room temperature. The layer of paraffin ensures anaerobic conditions and prevents dehydration of the medium.
- This condition helps microorganisms or pure culture to remain in a dormant state and, therefore, the culture can be preserved form months to years (varies with species).

ADVANTAGES

1. We can remove some of the growth under the oil with a transfer needle, inoculate a fresh medium, and still preserve the original culture.
2. The simplicity of the method makes it attractive, but changes in the characteristics of a strain can still occur.

4. Lyophillization or freeze drying

• Freeze drying is a stabilizing process in which a substance is first frozen and then the quantity of the solvent is reduced, first by sublimation (primary drying stage) and then desorption (secondary drying stage)

• Better preservation occurs with freeze-drying than with other methods because freeze-drying reduces the risk of intracellular ice crystallization that compromises viability

• Removal of water from the specimen effectively prevents this damage

• Lyophilization is greatest with gram-positive bacteria (spore formers) and decrease with gram -negative bacteria but viability can be maintained as long as 30 years.

• Large numbers of vials of dried microorganisms can be stored with limited space, and organisms can be easily transported long distances at room temperature

• The process combines freezing and dehydration- Organisms are initially frozen and then dried by lowering the atmospheric pressure with a vacuum apparatus

• Specimens can be connected individually to the condenser (manifold method) or can be placed (in a chamber) where they are dehydrated in one larger airspace

ADVANTAGES

- Removal of water at low temperature
- Thermo labile materials can be dried.
- Sterility can be maintained.
- Reconstitution is easy

DISADVANTAGES

- Many biological molecules are damaged by the stress associated with freezing, freeze- drying, or both.
- E.g. the process of drying causes extensive damage to molds, protozoa, and most viruses.
- Hence, these microorganisms can not be stored by this method.
- The product is prone to oxidation, due to high porosity and large surface area.
- Therefore the product should be packed in vacuum or using inert gas.
- Cost may be an issue, depending on the product