MICROBIAL CULTURES

It is a method of multiplying microbial organisms by letting them reproduce in predetermined culture medium under controlled laboratory conditions. The term culture is usually employed for a deliberate growth in the laboratory. To culture microbes in laboratory we require the preparation of substance which they can use as food. Such nutrient preparations are called culture media. Different microorganisms require different nutrient materials hence they vary from species to species.

PURE CULTURE

A pure culture is one that contains only a single kind of microbes. A pure culture is usually derived from a mixed culture by transferring a small sample into a new sterile growth medium in such a manner to disperse the individual cells across the medium surface or by thinning the sample many fold before inoculating the new medium. Isolation of a pure culture may be enhanced by providing a mixed inoculum with a medium favouring the growth of one organism.

METHODS TO OBTAIN PURE CULTURE:

Various methods are used to obtain pure culture. They are:

1. **Streak Plate method:**
   This method is used most commonly to isolate pure cultures of bacteria. A small amount of mixed culture is placed on the tip of an inoculation loop/ needle and is streaked across the surface of the agar medium. The successive streaks thin out the inoculum sufficiently and the microorganisms are separated from each other. These plates are incubated to allow the growth of colonies. The key principle of this method is by streaking, a dilution gradient is established across the face of the Petri plate as bacterial cells are deposited on the agar surface. Isolated colonies are picked up separately using sterile inoculating loop/ needle and are restreaked onto fresh media to ensure purity.

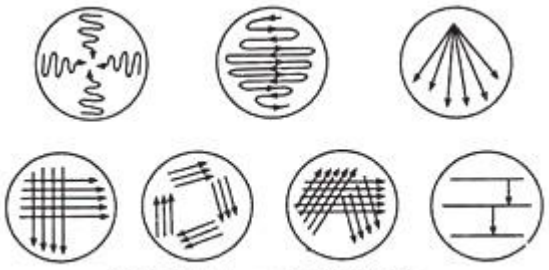

FIG. 16.13. Various methods of streaking.

2. **Spread plate method**
   In this method the mixed culture or microorganisms is diluted in a series of tubes containing sterile liquid. A drop of diluted liquid from each tube is placed on the centre of an agar plate and spread evenly over the surface by means of a sterilized bent- glass rod. The medium is incubated. The isolated colonies are picked up and transferred onto fresh medium to ensure purity.

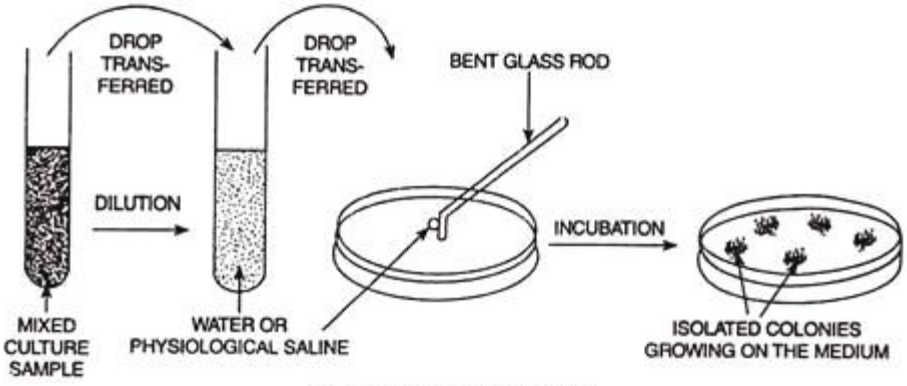

FIG. 16.15. Spread plate method.

3. **Pour plate method**

This method involves plating of diluted samples mixed with melted agar medium. The main principle is to dilute the inoculum in successive tubes containing liquefied agar medium so as to permit a thorough distribution of bacterial cells within the medium. The mixed culture of bacteria is diluted directly in tubes containing melted agar medium maintained in the liquid state at a temperature of 42-45 degree Celsius. The bacteria and the melted agar are mixed well. The content of the each tube are poured into separate Petri plate allowed to solidify and then incubated. When bacterial colonies develop both within the agar medium and on the medium. These isolated colonies are then picked up by inoculation loop and streaked onto another petriplate to ensure purity.

Disadvantages:

- Picking up of subsurface colonies needs digging them out of the agar medium thus interfering with other colonies.
- Microbes being isolated must be able to withstand temporary exposure to 42-45°C.

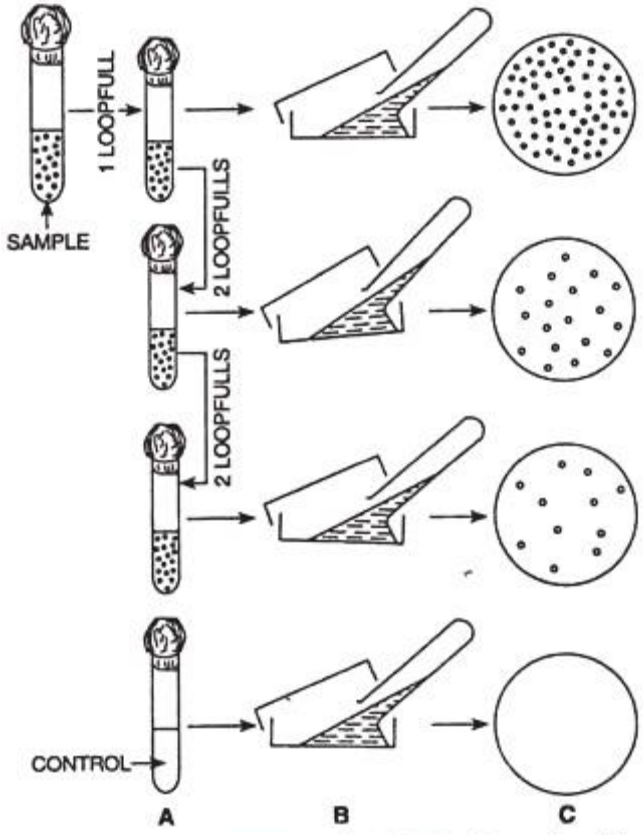

FIG. 16.14. Pour plate method. **A.** Media/dilution; **B.** pouring of the plate; and **C.** colony development after incubation. Control consists of the sterilized plating medium alone.

4. Serial Dilution

This method is commonly used to obtain pure cultures of those microorganisms that have not yet been successfully cultivated on solid media and grow only in liquid media. A microorganism that predominates in a mixed culture can be isolated in pure form by a series of dilutions. The inoculum is subjected to serial dilution in a sterile liquid medium, and a large number of tubes of sterile liquid medium are inoculated with aliquots of each successive dilution. The aim of this dilution is to inoculate a series of tubes with a microbial suspension so dilute that there are some tubes showing growth of only one individual microbe.

5. Single cell isolation method

An individual cell of the required kind is picked out by this method from the mixed culture and is permitted to grow.

- **Capillary pipette method**

  Several small drops of a suitably diluted culture medium are put on a sterile glass-coverslip by a sterile pipette drawn to a capillary. One then examines each drop under the microscope until one finds such a drop, which contains only one microorganism. This drop is removed with a sterile capillars pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture.

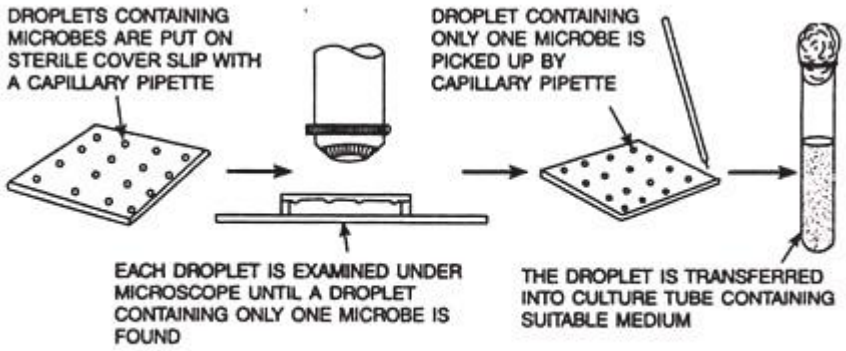

FIG. 16.17. Capillary method for obtaining a single macrobial cell.

- **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 micro-manipulator has micrometer adjustments by means of which its micropipette can be moved right and left, forward, and backward, and up and down.

  A series of hanging drops of a diluted culture are placed on a special sterile coverslip by a micropipette. Now a hanging drop is searched, which contains only a single microorganism cell.

  This cell is drawn into the micropipette by gentle suction and then transferred to a large drop of sterile medium on another sterile coverslip. When the number of cells increases in that drop as a result of multiplication, the drop is transferred to a culture tube having suitable medium. This yields a pure culture of the required microorganism.

- **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. The medium of known composition and, specific condition of incubation favours the growth of desired microorganisms but, is unsuitable for the growth of other types of microorganisms.

METHODS OF PRESERVATION OF PURE CUTURE

A number of methods are used for maintaining organisms in a viable condition over long period.

- **Agar Slant Culture:**

  The slants are incubated for 24 hrs or more. They are stored in a refrigerator. These cultures are periodically transferred to fresh media the time interval which transfers are made which varies with the organisms and the condition of growth.

- **Agar slant cultures covered with oil:**

  The agar slants are inoculated and incubated until good growth appears. They are then covered with sterile mineral oil to a depth of 1 cm above the tipoff the slanted surface. Transfers are made by removing a loopfull of the growth, touching the loop to the glass surface to drain off excess oil, inoculating a fresh medium and then preserving the initial stock culture. The method is very simple and special equipment not required.

- **Saline suspensions:**

Bacteria are suspended in 1% salt solution in screwcap tubes to prevent evaporation. The tubes are stored at room temperature whenever needed the transfers are made on agar slants and incubated.

- **Preservation at very low temperatures:**
  The organisms are suspended in a nutrient broth containing 15% glycerol or skim milk containing 7.5% glucose. The suspensions are frozen and stored at -15°C to -30°C. The cultures are frozen with a protective agent in sealed ampules. The frozen cultures are kept in liquid nitrogen refrigerators.
- **Preservation by Freeze drying:**
  The microbial suspension is placed in small vials. A thin film is frozen over the inside surface of the vials. A thin film is frozen over the inside surface of the vial by rotating it in a mixture of dry ice and alcohol, acetone at a temperature of -78°C. The vials are immediately connected to a high vacuum line. This dries the organisms while still frozen. Finally, the ampules are sealed off in a vacuum with a small flame. These cultures can be stored for several years at 4°C. This method is also employed for the preservation of sera, toxins, enzymes. To revive microbial cultures it is merely necessary to break open aseptically. Add a suitable, sterile medium and after incubation make further transfers.

ITCC- INDIAN TYPE CULTURE COLLECTIONS

The Indian Type Culture Collection (ITCC) was established in 1936 with a view to furnish the knowledge on living fungi. It is the largest fungal genetic resource centre in India. ITCC is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM, registration number 430). Ministry of environment and Forest, Government of India designated ITCC to act as biological repository during 2007 under Biological diversity Act, 2002.

The main objectives of ITCC are to act as repositories for fungal and bacterial cultures, to provide services viz., supply of authentic fungal and bacterial cultures and identification of fungi and bacteria to technocrats and scientists working in research Institutions, Universities and Industries.

ACTIVITIES

1. Conservation, Preservation and maintenance of fungal and bacterial cultures
2. Identification and Supply services of fungal and bacterial cultures
3. Deposition of authentic fungal and bacterial cultures
4. Taxonomic Investigations of fungi and bacteria
5. Documentation of the fungi and bacteria

MAINTENANCE

More than 3,800 cultures of fungi of all groups, viz., Oomycetes, Zygomycetes, Ascomycetes, Basidiomycetes and Deuteromycetes which includes plant pathogens, biocontrol agents, fungi for medical and industrial use including mushroom and yeast and 145 cultures of plant pathogenic bacteria are being conserved and maintained at ITCC.

PRESERVATION

All the ITCC cultures are preserved as actively growing cultures on agar slants, under mineral oil and isolates producing spores are being lyophilized (freeze drying).Bacteria are preserved in 50%glycerol stock and maintained at -80^0C.

SERVICES

- Identification of fungal and bacterial cultures Identification of different groups of fungi viz., Oomycetes, Zygomycetes, Ascomycetes and Deuteromycetes and plant pathogenic bacteria are done on payment basis for the cultures received from researchers and students of different parts of the country.

- Supply of fungal cultures Authentic fungal and bacterial cultures are supplied as an active culture on an agar and are dispatched by surface mail along with the report within the country.
- Deposition of cultures Fungal and bacterial cultures of Indian origin having some special features (producers of enzymes, toxins, and antibiotics), bio-control and newly reported taxa (including new genera, new species, and new records) will be deposited at ITCC to ensure ex-situ conservation of microbial diversity.

MTCC- MICROBIAL TYPE CULTURE COLLECTIONS

The Microbial Type Culture Collection and Gene Bank (MTCC), a national facility established in 1986 is funded jointly by the Department of Biotechnology (DBT) and the Council of Scientific and Industrial Research (CSIR), Government of India. It is an affiliate member of the World Federation for Culture Collections (WFCC) and is registered with the World Data Centre for Microorganisms (WDCM).The main objectives of this national facility are to act as a depository, to supply authentic microbial cultures and to provide related services to the scientists working in research institutions, universities and industries.
MTCC has about 20,000 microbial cultures (actinomycetes, bacteria, fungi, yeasts and plasmids) in its collections. The collections include the microbial type strains of several taxa, strains used for teaching purposes, genetic stock, cultures used for various testing.
Cultures can be ordered by accessing the online catalogue. Most of the cultures are supplied in freeze-dried form. Some cultures, which do not survive freeze drying, are supplied in active form.

ATCC- AMERICAN TYPE CULTURE COLLECTIONS:

The American Type Culture Collection (ATCC) is a private, nonprofit organization dedicated to the acquisition, preservation, authentication, and distribution—the "APAD" activities—of diverse biological materials. ATCC was founded by scientists in 1925 to serve as a national repository and distribution center for cultures of microorganisms. Since that time, viruses, animal and plant cell cultures, and recombinant DNA materials have been added. ATCC is now the largest general service culture collection in the world, with collections in six areas: Bacteriology, Cell Culture, Molecular Biology, Mycology, Protistology, and Virology.

The mission of ATCC is to serve as the world's leading repository for standard reference cultures, related biological materials, and associated data. ATCC provides for the permanent preservation and availability of these materials for use by qualified people engaged in science, industry, and education. In pursuit of its mission, ATCC's principal goals are

- to acquire, preserve, propagate, and distribute cell cultures, microorganisms, viruses, cellular products, and biological materials used in and derived from recombinant DNA technology;
- to maintain the highest standards of authentication, documentation, and maintenance of the characteristics and viability of the materials entrusted to the collections;
- to pursue research based on or related to the collections;
- to provide the highest-quality service to members of the scientific, commercial, and public sectors who work with collection materials;
- to educate scientists and the public about ATCC holdings and activities via training programs, lectures, publications, databases and other means; and
- To collect, manage, disseminate, and exchange information applicable to the materials in the collections.

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