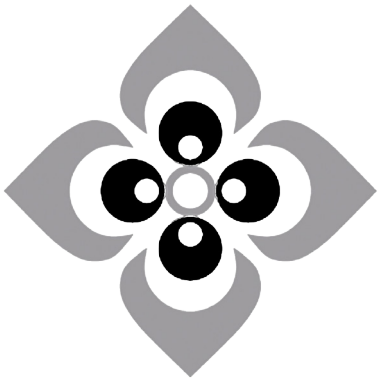

Consortium for Educational Communication

Module on

# Methods of Isolation of Pure Culture and Culture Characteristics


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# METHODS OF ISOLATION OF PURE CULTURE:

Microorganisms are generally found in nature (air, soil and water) as mixed populations. 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. Pure culture involves not only isolation of individual microorganisms from a mixed population, but also the maintenance of such individuals and their progenies in artificial media, where no other microorganisms find way to grow.

However, it is not easy to isolate the individual microorganisms from natural habitats and grow them under imposed laboratory conditions. For this, great deal of laboratory manipulation is required. It is necessary to make the colonies well-isolated from each other so that each appears distinct, large and shows characteristic growth forms. Such colonies may be picked up easily and grown separately for further study. Several methods for obtaining pure cultures are in use. Some common methods are in everyday use by a majority of microbiologists, while the others are methods used for special purposes.

1. Spread Plate Method:

In this method, the mixed culture or microorganisms is not diluted in the melted agar medium (unlike the pour plate method); it is rather diluted in a series of tubes containing sterile liquid, usually, water or physiological saline.

A drop of so diluted liquid from each tube is placed on the center of an agar plate and spread evenly over the surface by means of a sterilized bent-glass-rod. The medium is now incubated.

When the colonies develop on the agar medium plates, it is found that there are some plates in which well-isolated colonies grow. This happens as a result of separation of individual microorganisms by spreading over the drop of diluted liquid on the medium of the

plate.

The isolated colonies are picked up and transferred onto fresh medium to ensure purity. In contrast to pour plate method, only surface colonies develop in this method and the microorganisms are not required to withstand the temperature of the melted agar medium.

2. 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 micro-organisms are separated from each other.

It is usually advisable to streak out a second plate by the same loop/needle without reinoculation. These plates are incubated to allow the growth of colonies. The key principle of this method is that, by streaking, a dilution gradient is established across the face of the Petri plate as bacterial cells are deposited on the agar surface.

Because of this dilution gradient, confluent growth does not take place on that part of the medium where few bacterial cells are deposited. Presumably, each colony is the progeny of a single microbial cell thus representing a clone of pure culture. Such isolated colonies are picked up separately using sterile inoculating loop/needle and re-streaked onto fresh media to ensure purity.

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.

Here, 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°C (agar solidifies below 42°C). The bacteria and the melted medium are mixed well.

The contents of each tube are poured into separate Petri plates, allowed to solidify, and then incubated. When bacterial colonies develop, one finds that isolated colonies develop both within the agar medium (subsurface colonies) and on the medium (surface colonies). These isolated colonies are then picked up by inoculation loop and streaked onto another Petri plate to insure purity.

Pour plate method has certain disadvantages as follows:

(i) The picking up of subsurface colonies needs digging them out of the agar medium thus interfering with other colonies, and

(ii) The microbes being isolated must be able to withstand temporary exposure to the 42-45° temperature of the liquid agar medium; therefore this technique proves unsuitable for the isolation of psychrophilic microorganisms.

However, the pour plate method, in addition to its use in isolating pure cultures, is also used for determining the number of viable bacterial cells present in a culture.

4. Serial Dilution Method:

As stated earlier, 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. For convenience, suppose we have a culture containing 10 ml of liquid medium, containing 1,000 microorganisms, i.e., 100 microorganisms/ml of the liquid

medium.

If we take out 1 ml of this medium and mix it with 9 ml of fresh sterile liquid medium, we would then have 100 microorganisms in 10 ml or 10 microorganisms/ml. If we add 1 ml of this suspension to another 9 ml. of fresh sterile liquid medium, each ml would now contain a single microorganism.

If this tube shows any microbial growth, there is a very high probability that this growth has resulted from the introduction of a single microorganism in the medium and represents the pure culture of that microorganism.

5. Single Cell Isolation Methods:

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

The following two methods are in use:

i. 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 capillary pipette to fresh medium. The individual microorganism present in the drop starts multiplying to yield a pure culture.

ii. 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.

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 within the species. The disadvantages are that the equipment is expensive, its manipulation is very tedious, and it requires a skilled operator. This is the reason why this method is reserved for use in highly specialized studies.

6. Enrichment Culture Method:

Generally, it is used to isolate those microorganisms, which are present in relatively small numbers or that have slow growth rates compared to the other species present in the mixed culture.

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.

## CULTURE CHARACTERISTICS:

When a single bacterial cell is deposited on a solid or in a liquid medium, it begins to divide. These cells divide exponentially, eventually forming a colony. When grown on a variety of media, microorganisms will exhibit visible physical differences in appearance in their isolated colonies and their growth. These differences are called cultural characteristics or morphology. Cultural characteristics may be used as an aid in identifying and classifying some organisms. These physical characteristics are often specific for the type of bacteria and can be used as a means of recognition. The appearance of colonial growth on

agar media can be very distinctive for individual species. Some microorganisms have characteristic growth patterns but they aid in the identification of species only if they are distinctive. Although some bacteria grow in distinctive patterns, others look alike. Colonial morphology, however, is influenced by the media and other growth conditions. The colonial morphology of the same bacteria may vary on different media or under different conditions. Cultural characteristics or morphology are determined by culturing microorganisms on nutrient agarplates, in nutrient broth and slants. After incubation, the characteristics are observed. Observation of such factors also helps in recognizing types of bacteria. Differences in the macroscopic appearance of a microorganism's growth; are used as a basis for separating microbes into taxonomic groups; contained in Bergey's Manual of Systematic Bacteriology.

CHARACTERISTICS IN SOLID NUTRIENT AGAR

SIZE

It is a cultural characteristic in nutrient agar plates; pinpoint, small, moderate, or large. Different types of bacteria produce different sizes of colonies. From the punctiform or pinpoint colonies of Streptococcus pyogenes and Staphylococcus epidermidis to the large colonies of Klebsiella pneumonia, the size of a colony can give some important clues as to the identity of the organism under observation.

PIGMENTATION

A cultural characteristic in nutrient agar plates; color of colony. Some bacterial cells are pigmented, as in the case of Micrococcus luteus and Chromobacterium violaceum. Some bacteria, like Pseudomonas aeruginosa, produce a water-soluble pigment that diffuses into the agar.

FORM/SHAPE

A cultural characteristic in nutrient agar plates; the shape of the colony as described as follows: circular, irregular, or rhizoid

Punctiform

A type of "form" in nutrient agar plates; colonies which are very

small but not microscopic, having a diameter of less than 1 mm

Circular

A type of "form" in nutrient agar plates; unbroken, peripheral edge

Filamentous

A type of "form" in nutrient agar plates; colonies which are composed of long, interwoven, irregularly disposed threads

Irregular

A type of "form" in nutrient agar plates; indented peripheral edge

Rhizoid

A type of "form" in nutrient agar plates; root like, spreading growth

Spindle

A type of "form" in nutrient agar plates; spindle-like, bulged at middle while pointed at the ends

ELEVATION

A cultural characteristic in nutrient agar plates; the degree to which colony growth is raised on the agar surface is described as follows: flat, raised, convex, umbonate

Flat

A type of "elevation" in nutrient agar plates; elevation not discernible

Raised

A type of "elevation" in nutrient agar plates; slightly elevated

Convex

A type of "elevation" in nutrient agar plates; dome-shaped elevation

Pulvinate

A type of "elevation" in nutrient agar plates; cushion-shaped, swelled.

Umbonate

A type of "elevation" in nutrient agar plates; raised, with elevated convex central region

MARGIN

A cultural characteristic in nutrient agar plates; the appearance of the outer edge of the colony; described as: entire, lobate, undulate, serrate, and filamentous

Entire/Smooth

A type of "margin" in nutrient agar plates; sharply defined, even at margins.

Undulate

A type of "margin" in nutrient agar plates; wavy indentations

Lobate

A type of "margin" in nutrient agar plates; marked indentations

Erose/Irregular

A type of "margin" in nutrient agar plates; irregular at margins, tooth-like appearance

Filamentous

A type of "margin" in nutrient agar plates; threadlike, spreading edge

Curled

A type of "margin" in nutrient agar plates; layered curls.

## CHARACTERISTICS IN NUTRIENT BROTH CULTURES

Uniform Fine Turbidity

A cultural characteristic of nutrient broth cultures (distribution of growth); finely dispersed growth throughout

Sediment

A cultural characteristic of nutrient broth cultures (distribution of growth); concentration of growth at the bottom of broth culture may be granular, flaky, or flocculent.

Ring

A cultural characteristic of nutrient broth cultures in which a film of growth clings to the test tube in the form of a ring at the liquid air interface requiring oxygen.

Pellicle

A cultural characteristic of nutrient broth cultures (distribution of growth); thick, pad like growth on surface

Flocculent

A cultural characteristic of nutrient broth cultures (distribution of growth); flaky aggregates dispersed throughout.

Membranous

A cultural characteristic of nutrient broth cultures; growth is as a thin skin at the surface.

## CHARACTERISTICS IN NUTRIENT GELATIN CULTURES

Crateriform

A cultural characteristic of nutrient gelatin (liquefaction); liquefied surface area is saucer-shaped

Napiform

A cultural characteristic of nutrient gelatin (liquefaction); bulbous-

shaped liquefaction at surface

Infudibuliform

A cultural characteristic of nutrient gelatin (liquefaction); funnel-shaped

Saccate

A cultural characteristic of nutrient gelatin (liquefaction); elongated, tubular

Stratiform

A cultural characteristic of nutrient gelatin (liquefaction); complete liquefaction of the upper half of the medium

CHARACTERISTICS IN NUTRIENT AGAR SLANTS

ABUNDANCE OF GROWTH

A cultural characteristic in nutrient agar slants; the amount of growth is designated as none, slight, moderate, or large

PIGMENTATION

A cultural characteristic in nutrient agar slants; the coloration of organisms as seen in surface colonies. Most organisms will lack chromogenesis (pigment production), exhibiting a white, beige, or gray growth. Pigmentation within the organism may be red, yellow, violet, or other colors. Soluble pigments may be blue, green, yellow, brown, or other colors. Pigmentation occurring within the organism itself; or water soluble pigment that diffuses into the surrounding medium.

OPTICAL CHARACTERISTIC

A cultural characteristic in nutrient agar slants; evaluated on the basis of the amount of light transmitted through growth; described as opaque, translucent, or transparent

FORM/SHAPE

A cultural characteristic in nutrient agar slants; the appearance of the single-line streak of growth on the agar surface is designated

as:

filiform, echinulate, beaded, effuse, arborescent, rhizoid

Filiform

A type of "form" to describe nutrient agar slants; continuous, threadlike growth with smooth edges

Echinulate

A type of "form" to describe nutrient agar slants; continuous, threadlike growth with irregular edges

Beaded

A type of "form" to describe nutrient agar slants; nonconfluent to semiconfluent colonies

Effuse

A type of "form" to describe nutrient agar slants; thin, spreading growth

Arborescent

A type of "form" to describe nutrient agar slants; thin, treelike growth

Rhizoid

A type of "form" to describe nutrient agar slants; rootlike growth

CONSISTENCY

A cultural characteristic in nutrient agar slants; can be described as dry, buttery, or mucoid

Dry

A type of "consistency" in nutrient agar slants; free from moisture e.g., Bacillus subtilis

Buttery

A type of "consistency" in nutrient agar slants; moist and shiny

Mucoid

A type of "consistency" in nutrient agar slants; slimy and glistening e.g., Klebsiella pneumonia