TECHNIQUE OF ISOLATION OF MICROORGANISMS

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FOR BOTANY
B.SC. PART-3 (H)
PAPER - VI
GROUP - A

- Microorganisms occur in natural environment like air, water, soil, and on food items. They are mixed with several other forms. The separation of desired microbe or one type of microbe from a mixture is called isolation and a culture containing just one species of microbe is known as a pure culture.

- Various techniques are developed for the isolation of pure culture from mixed culture. Some of these are as follows —

1. Use of Micromanipulator :

The small size of most microbes makes mechanical separation of single cell impossible. With the recent development of micromanipulater, it is now possible to obtain a single viable cell from a developing colony of mixed microbial population. The cell is transferred on the culture medium to develop axenic pure culture. This technique uses microscope for picking up the cell.

2. By Streaking :

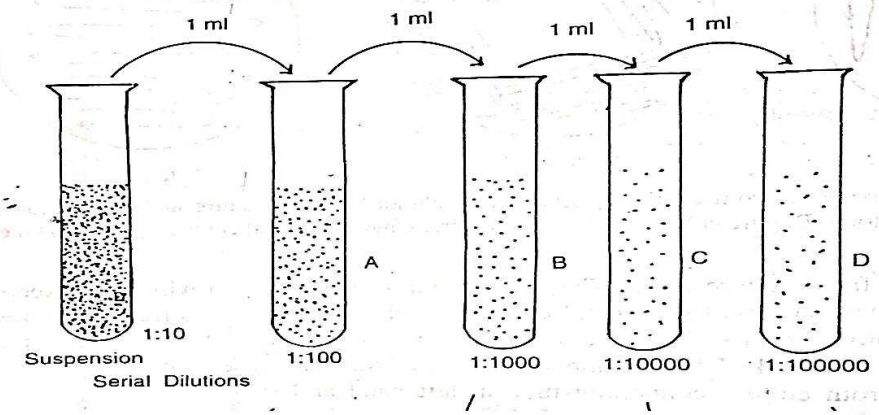
This is most widely used method of isolation. The technique consists of pouring a suitable sterile medium into sterile petriplate and allowing the medium to solidify. By means of a sterile loop of inoculation needle, a small amount of growth preferably from a broth culture or suspension is streaked back and forth across the surface of agar medium until about one third of the diameter of the plate is covered. The needle is then flamed and streaking is done at right angles to and across the first streak. This serves to drag the microbe out in a long line from the initial streak. When this streaking is completed the needle is again flamed and streaking is done at right angles to the second streak and parallel to the first.

In this way by multiple streaking, pure colony may develop which can be isolated and maintained in culture tube.

3. Dilution plate method:

It involves the serial dilution and the inoculation to sterile medium contained in petriplate.

. Serial dilutions of the sample: Serial dilution is done in order to reduce the microbial concentration so that pure culture could be obtained. For this a known amount of original sample, for example soil, is mixed with a known volume of sterile water to have a soil suspension which contains microbial mixture. To one ml. of this original sample 9 ml. of water is added, it will give 1:10 or 10^{-1} dilution i.e, the original sample has be diluted to $1/10^{th}$. Similary 1:1000 (10^{-2}), 1:1000 (10^{-3}), 1:10,000 (10^{-4}) and so on dilutions are prepared. Finally one ml. aliquot of any dilution is added to culture medium by placing either pour plate method, streak plate method or Spread plate method.



I- Pour plate method: The agar is maintained in molten state at 45°C. One ml of the dilution is added to the petri plate to which 9 ml of sterile, cool agar medium is poured. The contents are thoroughly mixed and allowed to solidify. The plate are incubated at a suitable temperature. After few days, different kinds of microbes grow as separate colonies. Cells from individual colony may be picked up for the subculture.

II. Streak plate method: A small amount of sample is transferred on to the surface of a suitable, solid agar medium either by loop or transfer needle. This is then streaked. The successive dilution gives different types of colonies. The colonies may give pure culture at the maximum dilution.

III- Spread plate method: An aliquot of the diluted sample is placed onto the agar surface and is spread uniformly with a sterile, bent glass rod. After incubation, the microbes may be isolated from the developing colonies.

4. Special methods: There are other methods which are considered special because these are used for the isolation of a particular type of microorganism. The methods may be —

I- Enrichment medium: The principle of enrichment medium is to provide specific nutrients and culture conditions in such a way that suit only to a specific microbial species. For example, if a medium containing salt solution with $NaNO_2$ at PH 8.5 and incubated in air at 25-30°C in dark condition can give pure colonies of Nitrobacter from garden soil. Similarly other microbes can be selectively isolated.

II- Selective medium: Such medium contains specific chemicals which do not affect the growth of the bacterium to be isolated but discourage the growth of other bacteria in the mixture. For example, addition of sodium azide at

specific concentration into medium selectively isolates lactic acid bacterium. There are many other selective agents used as dyes (for brucellas), cetrimide (for Pseudomonas aeruginosa), tetrazolium (for halophytic bacteria etc.

III - Differential medium : It contains, reagents or chemicals which allow the observer to distinguish between types of microbial colonies developed after incubation. For example, if raw sewage is streaked or spread on eosin methylene blue (EMB) agar some bacteria produce brilliant green colonies, others gummy, pink colonies with dark centres. Escherichia coli imparts metallic sheen due to precipitation of eosin-methylene blue complex. Similarly, on Mac Conkey agar medium, E. coli colonies are brick red in colour due to fermentation of lactose.