9 d- ISOLATION,ENRICHMENT ,PRESERVATION

Yeast and mould are the fungus and they occur in nature as mixed cultures. After isolation, it is essential to carry out the purification so as to study the single cultures . Dilution is the basic principle involved in purification that leads to the development of single colony ,which has been developed from the single cell. Though mould and yeast are coming under the fungus the technique of purification and preservation are different

A. Methods for the purification of fungus :

Fungi are ubiquitous in habitat. The thallus or body of the fungus consists of mycelium and spores. The mycelium is a complex of several filaments called hyphae. Hyphae are made up of a thin transparent, tubular wall called lumen, filled or lined with a layer of protoplasm varying in thickness. New hyphae generally arise from a spore, which on germination put out a germ tube or tubes. These tubes elongate and branch to form hyphae.

The fungi reproduce naturally by various methods. A sexual reproduction does not involve union of nuclei, sex cells or sex organs. It is also called as somatic or vegetative reproduction. The vegetative reproduction is accomplished by (1) fission of somatic cells yielding two similar daughter cells (2) budding of somatic cells or spores, each but a small outgrowth of the parent cell developing into a new individual (3) fragmentation or disjointing of the hyphal cells, each fragment becoming a new organism or (4) spore formation.

Fungi can be purified by the following two methods

1. Single spore isolation method
2. Single hyphal tip method

1. Single spore isolation method

This method can be employed, when the fungus produces spores, which are coloured and bold.

Procedure

- Melt 3 tubes of plain agar and cool them to 50°C.
- Transfer a loopful of the spore suspension of the mixed culture to the first plain agar tube with an inoculation needle.
- Shake well for uniform dispersion of spores.
- Transfer a loopful from the first dilution tube to the second tube shake thoroughly. Likewise prepare third dilution also.
- Pour the media with diluted spore into three separate petriplate and allow to solidify
- Observe Petri plates under low power objective of the microscope and locate isolated single spore.
- Transfer the single spore to PDA slant to obtain pure culture.

2. Single hyphal tip method

This method is employed for purifying fungi which either do not produce spores or produces small and hyaline spores.

Materials required

- Fungal culture plate
- Plain agar plates
- Inoculation needle
- Cork borer
- PDA slants
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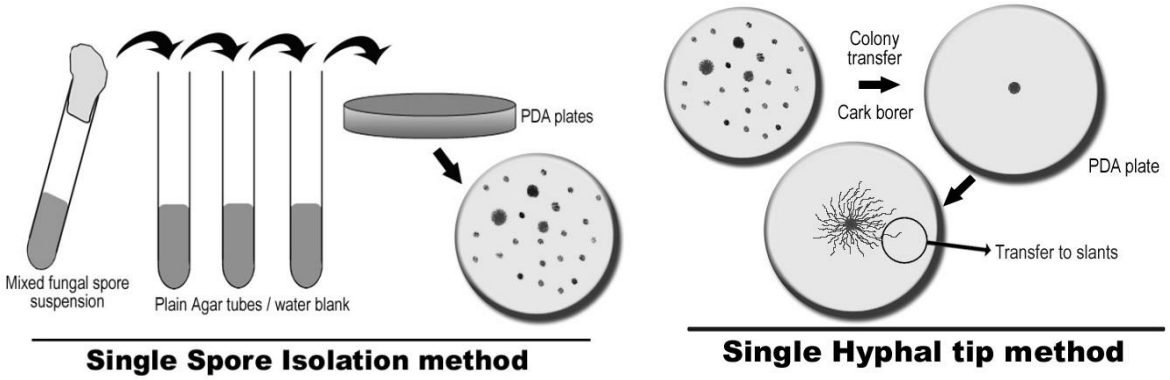
Procedure

- Using cork borer, take a disc of fungal colony and place it in the middle of a plain agar plate and incubate for 1-2 days.
- Place the Petriplate in the stage of a compound microscope and locate a hyphal tip using the low power objective.
- With the cork borer, remove it and place it in a PDA slant and maintain it as a pure culture.



B. Purification of yeast cultures :

Yeast are single celled eukaryotic microorganism and produces slimy white soft colonies resembling the bacteria . They will not produce mycelial filaments. Purification can be done like that of bacterial cultures using Streak plate method, pour plate method and spread plate methods

C. Preservation of yeast and mould

Mold cultures are usually maintained at 4°C in refrigerators in teaching and research laboratories. These cultures are regularly subcultured to fresh potato dextrose agar slants, every two weeks to six months, depending upon the type of the mold. Successive subculturing of molds often leads to the production of sterile mutant strains or death of the organisms. The easiest, most effective and efficient way to maintain mold cultures indefinitely, without change in isolate characteristics, is on

sterile distilled water. Although the isolates on distilled water remain viable for several years, ideally cultures are revived every two years and a fresh distilled water suspension is made from a typical sporulating colony.

Materials required

- Freshly isolated mold culture
- Potato dextrose agar (PDA) slant
- Sterile distilled water in screw-capped tube
- Sterile pipettes
- Sharp sterile blade
- Transfer needle
- Bunsen burner/spirit lamp

Procedure

- Subculture the mold on PDA slants from the original mold culture which has been freshly isolated from a substrate/material.
- Incubate the inoculated slant at 25°C for 3-5 days.
- Pipette 3-5 ml of sterile distilled water, from the screw-capped tube, and pour it over the surface of the slant.
- Scrap the surface of the mold colony to make a suspension of broken mycelium and spores in water.
- Transfer the suspension to the screw-capped tube containing sterile distilled water.
- Tightly close and seal the screw cap.
- Label the tube with the name of the isolate, with mold number and date.
- Revival of the culture : Cultures are revived by pipetting 1-2 ml of suspension on to an agar slant (PDA).