Isolation of Fungi

MYCOLOGY LAB
(3rd Grade)

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2. Selective methods

Selective isolation techniques are quite specific in their effects to isolate a new set of fungi from a familiar habitat.

A. Stress techniques
B. Surface sterilization
C. Selective nutrients
D. Selective temperatures
E. Osmophily
F. Spore printing
A- Stress technique

All fungi are capable of withstanding environmental stresses, but eventually, when the stress is great enough, they will be killed.

Not all fungi have the same tolerance to stress, however, and we can take advantage of this property by subjecting a material to just enough stress to kill some fungi but not others.

In other terms: some fungi will not germinate until they have been subjected to conditions that kill most others.

Other fungi produce spores that germinate only after they are exposed to freezing.
Stress technique Procedure

1. Take a sample of soil, dung, wood, etc.
2. Subject it to some kind of treatment that we kill most fungi.
   - We can steam the substance in an autoclave or steamer (without pressure).
   - soak it in alcohol, acids, bases, or other chemicals.
   - Alternately freeze and thaw it for several weeks.
   - Almost any drastic treatment will yield a few fungal "holdouts" that might not otherwise appear.
3. After treatment, the substance can be handled in any of the normal ways, such as plating, or moist chambers.
B- Surface sterilization

Sterilize the surface of the sample by soaking it in:

- 10% commercial bleach
- 3% hydrogen peroxide (H2O2) for about 1-10 minutes to kill some fast growing and surface adhering spores.
- Ethanol 70 %
This technique is essentially the same as the baiting methods discussed Lab 3.

We might, for example, wish to isolate those fungi that can utilize cellulose.

To select for these fungi, make a medium such as Czapek's, but using cellulose in place of sucrose. If a fungus is to grow well here it must make use of the cellulose; those that cannot will be excluded or grow only very poorly.
Fungi that grow easily at room temperature are said to be **Mesophilic**.

Most fungi are Mesophilic and are inhibited or killed at unusually high or low temperatures.

There are some, however, that actually require unusual temperatures.

Fungi requiring high temperatures (45°C or more) are said to be **thermophilic**.

Those requiring low temperatures (15°C or less) are **psychrophilic**.

Bird nests, for example, yield abundant **thermophiles** when incubated in a moist chamber at 45-50°C, while **rabbit dung** or compost materials produce several **psychrophiles** in moist chambers incubated at 0-5°C.
Many stored products, such as grain, museum specimens, and hides, undergo degradation by fungi. Most of us have also experienced fungal growth on materials stored in damp places at home. Both phenomena are caused by fungi that can withstand unusually dry conditions. Such fungi are said to be osmophilic, a term that refers to their prevalence in environments of high osmotic potential.
Osmophiles occur on relatively dry or sugary substances in the home. Most of the fungi encountered on old cloth or leather materials in the basement are osmophiles as are the fungi growing on the surface of jams and jellies.

Osmophilic fungi grow poorly on normal culture media and often sporulate abnormally or not at all.

To isolate them the water activities of media must be drastically decreased and inoculate at high-sugar agar media. For example, increasing the sucrose in Czapek's Agar from 20 g per liter to 100-500 g per liter.
F- Spore printing

❖ For mushrooms and bracket fungi a piece of the gill or tube tissue can be attached, perpendicular to the agar, on the lid of a Petri dish.
❖ The spores will then float down on to the agar surface and later germinate.
❖ With large mushrooms it is sufficient to attach a single gill, flat side down, to the lid; with small ones the whole cap can be stuck on.
Spore print on the paper or sterile petridish

Add sterile DW to make suspension

Inoculate spore suspension on PDA
THANKS!