Fungal Culture Prep for Teaching Labs

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**General Information**

Fungal cultures are used in Micro 210 and Biol 229. They are viewed under stereoscopes and in conjunction with prepared slides to view morphological structures.

Three organisms are viewed during the lab period: *Saccharomyces cerevisiae*, *Penicillium notatum*, and *Rhizopus stolonifera*.

**Storage Locations**

* *Saccharomyces cerevisiae* is stored in a screw-cap tube as a slant on the top shelf of the glass-front fridge in room 415.
* *Penicillium notatum* is stored in a 15-ml conical tube on the counter in room 415 as a spore water culture. Spore water cultures are regenerated once per year.
* *Rhizopus stolonifera* does not survive long-term storage well. Order *Rhizopus stolonifera* from Carolina Biological on a slant no more than 2 months before the date it will be used. Store the slant in the fridge in 415 until use.

**Inoculation and Incubation**

*From slants*

1. In a biosafety cabinet, dip a sterile cotton swab in sterile water.
2. Rub the moist swab over the culture on slant.
3. Rub the inoculated swab over a TSA plate. No need to streak for isolation.
4. It is only necessary to change swabs if possible contamination has occurred. Otherwise, it is acceptable to swab multiple TSA plates with the same swab, rotating the swab between plates.
5. Tape lids on plates so students are not tempted to open them, exposing fungal spores to the air.
6. Incubate at 30°C for 5 days (*Penicillium*) or 2-3 days (*Rhizopus*).

*From spore water culture*

1. In a biosafety cabinet, dip a sterile cotton swab into the spore water culture.
2. Rub the moist swab over the culture on slant.
3. Rub the inoculated swab over a TSA plate. No need to streak for isolation.
4. It is only necessary to change swabs if possible contamination has occurred. Otherwise, it is acceptable to swab multiple TSA plates with the same swab, rotating the swab between plates.
5. Tape lids on plates so students are not tempted to open them, exposing fungal spores to the air.
6. Incubate at 30°C for 5 days (*Saccharomyces*).

**Generating water spore cultures**

1. Incubate fungal culture on a slant for a specified period of time.
2. Add 5 ml sterile water to the slant.
3. Vortex gently.
4. Pipette 5 ml into a 15-ml conical tube.
5. Spore water cultures remain on the counter at room temperature in room 415 for up to one year.