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Comments on Metzenberg's procedure
for isolating unordered Neurospora
asci.

Metzenberg (1988 Fungal Genet. Newsl. 35) has devised a Tris-EDTA agar which clearly has advantages as a holding substrate for asci, avoiding overgrowth by contaminants or spontaneous germinants during the extended period required for ripening. We expect to adopt his formula, using 4% agar to expedite isolation. However, hypochlorite-treated water-agar as described by Perkins et al. (1966 Neurospora Newsl. 9:11; 1986 Can. J. Genet. Cytol. 28:971-981) remains a viable alternative. Overgrowth on the water-agar, if it occurs, is so thin and transparent that this has not impeded visibility or deterred us from recovering asci. In our procedure, individual rescued ascospores are tubed and subjected to heatshock, which kills any hyphal fragments or conidia that may have been carried over. If contamination with Neurospora mycelia is known to have occurred, heatshock in a 60°C water bath may be extended to 50 minutes from the usual 30 minutes as double assurance that no vegetative cells survive.

While adopting the new substrate for ripening, we shall probably retain our established procedure for picking up octads and for separating the eight ascospores of each octad. It is quick and simple and requires no new tools. The octads are picked to the storage medium and later transferred to fresh agar, all with the same platinum-iridium blade that we use for isolating random ascospores. Preparation of the blade and its use in isolation have been described (1959 Genetics 44:1185-1208; Fungal Genet. Newsl. 33:35-41, item 13). To pick up an octet (or an individual ascospore) on its underlying 4% agar, the hand-held blade is inserted only once into the agar, at a shallow angle entering ~0.5 mm from the object. The blade is then lifted carefully, fracturing the agar along lines that encompass the octet (or spore). The loosened agar piece is lifted out by manipulating the blade so that surface tension adheres the block to the blade rather than to the underlying agar. 40 to 60x magnification is used. The blade is sterilized by flaming.

The most critical step is separation and transfer of the eight individual ascospores comprising each ascus. It is important at the beginning that octads for storage be picked up on a piece of agar large enough (0.5-1 mm diameter) to be placed right-side-up on the ripening medium. (We'll call the agar piece a "block", although, it is not carved out with regular sides, but is bounded by irregular fracture surfaces. Only the original top agar surface holding the spores is smooth and regular.)

After storage, each block is lifted again on the platinum-iridium blade and is placed face down on an unmarred 4% water-agar surface which has dried sufficiently that there is no water film. As a result, the eight spores lie sandwiched between the underlying agar and the inverted block. The block is then slid gently to one side by pushing with the blade. When this is done, the eight ascospores are usually left behind on the under lying surface, spread out more or less in a line. These are readily accessible for routine isolation, just as though they were random ascospores. Each spore is picked up on its underlying agar and transferred to a 12 x 75 mm slant for heat-shock.

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