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R. L. Metzenberg

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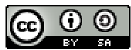

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An alternate way of collecting, storing, and dissecting Neurospora asci.

Abstract

Tetrad analysis may be accomplished either with ordered asci squeezed from perithecia, or with asci shot as an unordered group at a target slab of agar (Perkins 1966, Neurospora Newsl. 9:11).

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Metzenberg, R.L.

An alternate way of collecting, storing and dissecting *Neurospora* asci

Tetrad analysis may be accomplished either with ordered asci squeezed from perithecia, or with asci shot as an unordered group at a target slab of agar (Perkins 1966, Neurospora Newsl. 9:11). In either case, germination of the separated spores is usually poor unless the ascospores are allowed to ripen for at least a week, or preferably longer. Perkins (1966 NN 9:11; 1986 Can J. Genet. Cytol. 28:971-981) collected shot asci on a slab of 4% water-agar, and then cut agar blocks, each with a group of eight shot spores, and moved the blocks to petri plates, also containing water-agar, but previously spread with a drop of hypochlorite solution to kill vegetative forms of the mold. This "second generation" plate may then be stored until the ascospores are deemed ripe. At that time, the spores are isolated to tubes and heat-shocked to induce germination.

I have had generally satisfactory results with this method, but have had some failures, especially with the sterilization or ripening. In particular, the agar plate is often contaminated, either because the hypochlorite fails to kill all vegetative forms, or because, in some crosses, one or more of the ascospores germinates spontaneously and overgrows the plate. Increasing the rigor of the hypochlorite treatment may result in killing of ascospores as well as conidia, and it does nothing for the problem of spontaneously germinating ascospores.

To obviate these problems, the shot asci are collected onto 3% agar in 100 mM Tris-HCL buffer, pH 8.15, containing 2 mM disodium ethylene diamine tetraacetate (EDTA). (Ordered asci from squeezed perithecia can be collected on this medium as well). The EDTA at this pH completely prevents growth of *Neurospora* or casual contaminants. The collection plate of slab is stored at room temperature in a plastic box for at least a week, or better, several weeks. During this time, the ability of the ascospores to germinate rises to nearly 100%, while conidia which may have accompanied them to the Tris-EDTA plate tend to die off. The ascospores remain viable for at least several months on these plates. However, the ascospores die if they are subjected to heat-shock before transfer to a plate on which they can grow.

A suitable medium for the latter plate is 3% agar containing Vogel's salts, a carbon source, sorbose, and supplements as desired. I find square plates easier to use than round ones. To transfer the groups of shot asci from the Tris-EDTA plates to this plate for germination and growth, I pick them up on the ends of bristles, prepared as follows. Clear nylon bristles are cut from a toothbrush or fingernail brush and cemented individually to the tips of toothpicks. Just before use, the bristle is sterilized by dipping for a moment in 70% ethanol, and the tip is then touched to the surface of a drop of mucilage (e.g. Ross Mfg. Co.). The tiny adherent drop of mucilage is then touched to the ascus (if the eight spores are contiguous) or, if the eight spores are a bit scattered, the droplet is used to gather them up successively. The bristle on its toothpick is hung, bristle and droplet down, from a lump of plasticene modelling clay stuck to the underside of a shelf while successive asci are collected in the same manner. Finally, drops of sterile water are put on the recipient plate, about 2 cm from the wall, one for each ascus. The tip of each bristle is dipped onto a drop; a little modelling clay wrapped around the toothpick and jammed onto the rim of the petri dish holds the tip of the bristle in the water, just off the surface of the agar. In a minute or two, the mucilage dissolves and releases the eight ascospores into a clean, well-isolated field (the absence of a block of agar makes it easier to find all eight spores). The ascospores are teased into a straight line using an eyelash cemented to a toothpick and dipped momentarily into 70% ethanol. The petri dish is heat-shocked at 60-65° C for 25-30 minutes. This modified technique has proven itself well worth the small amount of extra set-up time. - - - Department of Physiological Chemistry, University of Wisconsin Madison, WI 53706