How to obtain unbroken linear asci that are extruded individually from the perithecium.

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Background

When perithecia are opened, a ball of asci folds out into a 'rosette', with individual asci attached at the base. Detaching asci and separating them intact from others in the rosette is painstaking and sometimes difficult. However, two procedures have been described for obtaining intact, ripe asci that detach spontaneously and are extruded from the perithecium. This occurs in crosses homozygous for the mutant *crisp-1* (Maling 1960). It also occurs in crosses where asci are formed but the perithecia dry down before ascospores have been ejected (Patricia St. Lawrence, 1958 personal communication). In both situations, individual asci detach spontaneously from the rosette. They remain intact and are extruded when the perithecia are immersed in water.

Procedure

Crosses homozygous for crisp-1: Maling (1960), using *cr-1* allele B320, found that intact linear asci could be collected in large numbers 3–4 weeks after crosses were initiated. In *cr-1* × *cr-1* crosses, asci remain inside the perithecia and very few spores are shot. To obtain asci, a surface layer of agar bearing perithecia is immersed in 10–15 ml of sterile water in a Petri dish. Extruded asci settle to the bottom of the dish, where they can be picked up and placed on the surface of a block of 4% agar. A fine capillary pipette is used, and suction is applied by mouth through a rubber tube attached to the pipette. For efficiency, many asci can be sucked up together and blown out in a drop of water in the center of the agar block. Asci are then picked up individually with the pipette and arranged so that the ascospores can be separated easily. Ascospores from well-aged perithecia are already mature and can be transferred and heatshocked immediately, without need for further ageing. Ripe asci from crosses stored at 5°C remain intact for at least 3 months

Other crosses: In the absence of *crisp*, intact mature asci can be obtained if crosses are made under conditions such that perithecia dry down before ascospores are shot. Asci then remain inside the dehydrated perithecia while they mature. St. Lawrence found that dehydration of cross tubes occurred at an appropriate time to block the shooting of asci if crosses were made on ~0.5 ml agar crossing medium in small (10 × 75 mm) slants and placed at 25°C in a nonhumid incubator. Intact asci are extruded when well-aged, dehydrated perithecia are immersed in water. Cross tubes with dried perithecia can be stored indefinitely with little or no loss of ascospore viability.

Reference

Maling, B. 1960. Replica plating and rapid ascus collection of Neurospora. J. Gen. Microbiol. 23: 257-260.

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