

Prakash, V. Spore isolation in order  
(Tetrad Dissection).

The following sequence has been found effective during the dissection of asci:

- a) Treatment for softening of the perithecial wall
- b) Pre and post-treatment washing of perithecia
- c) Use of 12% agar blocks
- d) Use of sharply pointed tungsten needle for isolation of spores
- e) Transferring of ascospores directly to the tubes, instead of first carrying to 1 x 1 x 0.5 mm agar blocks.

Care is taken to exclude as many conidia as possible while removing plump, black perithecia of appropriate ripeness. The perithecia are first washed in five to six changes of sterile distilled water in small separate tubes, so as to remove conidial contamination by vigorous shaking and by draining off the water by means of a pipette. The large difference in density soon separates conidia. For softening the tough perithecial walls and for killing any of the remaining conidia still left over, the perithecia are then transferred to a few drops of fresh 40% clorox (or 2% sodium hypochlorite) and 1.5% ethyl alcohol solution for three to four minutes. Further careful washing in three to four changes of sterile distilled water is carried out before placing them on a block of 12% agar. The perithecia are squeezed open and asci are expressed into a drop of sterile water. Asci containing eight spores in linear order are attached to each other at their bases and extended radially from the point of attachment. The cluster of asci is pulled to one end of a block of 12% agar and each ascus is separated from the cluster with a pair of sharply pointed needles. Only complete asci are spread, one by one with a space inbetween each of the adjacent asci, on the other end of the block. A drop of water with a pipette facilitates this operation considerably, in carrying the asci from one end of the block to the other. The block is then allowed to reach a condition of optimum dryness before dissecting asci. The needles are made by dipping tungsten wire in molten sodium nitrite, heated in a crucible.

A 3 mm thick block (1.5 x 3.5 cm) of 12% agar, instead of the 4% one which is so frequently used, has been found to provide a very convenient, or rather an adequately tough, base for a clean dissection of asci and without any fear of picking up a stray ascospore. The surface of 12% agar attains the right stage of dryness very easily because of its quick absorption power. In addition, as agar with higher concentration has more water retaining capacity for a particular length of time, a block of 12% agar can last comparatively for a longer time before getting totally dried out, due to evaporation, than the one with 4% agar. Such an agar block affords an added advantage, particularly when a number of asci are to be dissected at a long stretch of three to five hours. The use of the 4% agar block can almost lead to complete disruption of its surface especially when comparatively immature asci are dissected, whereas, the latter can be dissected on agar blocks with a higher concentration than 4%, with great ease.

Since it is considered a waste of time in first transferring the ascospores onto 1 x 1 x 0.5 mm agar blocks and then carrying these blocks separately to fresh agar slants in 3 x 3/8 inch test-tubes, the ascospores instead are transferred directly into the tubes containing appropriate medium. There was no evidence that a stray ascospore was picked up at any time.

By employing the above method, it is quite possible, under favorable conditions, to dissect twenty to twenty-five asci in an hour. ---Department of Botany, University of Malaya, Kuala Lumpur, Malaya.