USE OF SNAIL DIGESTIVE JUICE IN ISOLATION OF YEAST SPORE TETRADS[1]

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Tetrad analysis of yeast is somewhat hampered by the difficulties associated with ascus dissection. This note describes a method found to simplify this procedure.

The contents of the crop of the garden snail, *Helix aspersa*, have been shown to digest the cell wall of yeast (Eddy and Williamson, Nature, **179,** 1252, 1957). This suggested a possible method for the release of ascospores similar to that reported by Wright and Lederberg (Proc. Natl. Acad. Sci. U. S., **43,** 919, 1956).

The crop contents of a number of snails were suspended in distilled water to a concentration of 29.3 mg dry weight per ml (Bawden and Pirie, Brit. J. Exptl. Pathol., **27,** 81, 1946). This preparation, sterilized by filtration, was added at 5 per cent concentration by volume to a suspension containing approximately 10^7 cells per ml of the sporulated culture of *Saccharomyces cerevisiae*, and a sample was observed microscopically. It was noted that within 10 to 15 min most of the ascus walls had disappeared. The spores from a single ascus, however, remained together, providing the basis for the use of the procedure to obtain spore tetrads.

A loopful of the treated suspension was streaked along one edge of a thin slab of nutrient agar (yeast extract, 1 per cent; peptone, 1 per cent; glucose, 2 per cent; agar, 2 per cent) that had been spread on a 40 by 22 mm cover slip. With a mechanical micromanipulator, tetrads were pulled into the uninoculated region of the slab and sister spores separated and spaced at 2-mm intervals. Up to 17 tetrads could be spaced on each agar slab, which was then removed from the cover slip with a sterile spatula and placed, spores upward, on the surface of nutrient agar contained in a petri dish. After incubation at 30 C for approximately 48 hr, spore colonies of approximately 1 mm diameter (figure 1) were available for transfer to nutrient agar plates (40 cultures per plate). These plates were later used for replica plating on various omission media. Viability for a number of crosses analyzed by this technique averaged about 95 per cent, similar to that obtained using regular dissection techniques (Fowell, J. Appl. Bacteriol., **18,** 149, 1955).

The crosses used in this study were segregating for a number of genetic markers (7 to 16) facilitating detection of "false tetrads," i. e., tetrads resulting from chance association of nonsister spores. These cases would be expected to exhibit irregular segregations for a number of markers. Because of their low frequency of occurrence (approximately 1 per cent) and the ease of their detection, such tetrads are not considered to detract from this method. With relatively little practice, it was possible to isolate 20 to 30 tetrads per hr.

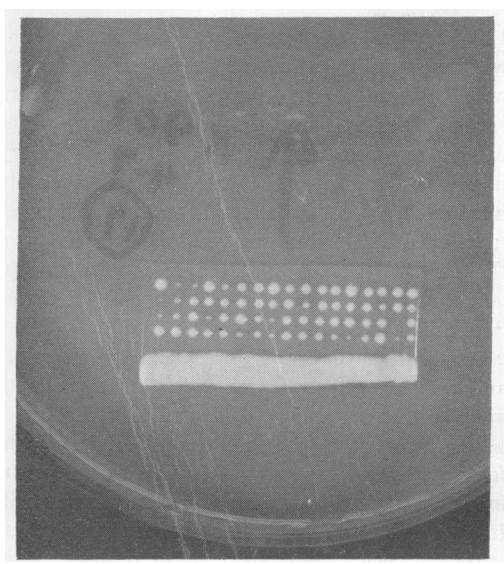

Figure 1. Colonies produced by spores isolated from 17 yeast tetrads. The four spores from a single ascus are aligned vertically. The growth from the original inoculum is across the bottom of the slab.

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