A simple device for rapid preparation of conidial suspensions of Neurospora

F. J. de Serres
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Abstract
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platinum gauze sieve, 150 mesh) to determine the volume to be added to a 250 ml Erlenmeyer flask of minimal medium to give a final concentration of $1 \times 10^5$ conidia/ml. For inoculation we use 2 ml Cornwall continuous pipetting outfits (No. 1251) fitted with 21-gauge hypodermic needles. To make all possible pairwise combinations of a given sample of mutants, the baskets are arranged in a grid and 1 ml aliquots of each strain are injected into the appropriate horizontal and vertical rows of tubes. Inoculation in a vertical direction is made over one side of each tube and in a position diametrically opposed in a horizontal direction to avoid cross contamination of suspensions. When inoculation of all strains has been completed, each tube contains two 1 ml aliquots, each containing $1 \times 10^5$ conidia. In this type of experiment, therefore, "selfings" of all mutants appear on the diagonal, and the two triangles thus defined contain mirror images of all possible pairwise combinations of mutants.

Syringes are cleaned and sterilized between suspensions by rinsing with sterile boiling water. With one person to adjust the concentrations of the suspensions and to sterilize the syringe and another to inoculate the tubes, we have been able to make all possible pairwise combinations of 100 mutants in a single experiment in 10-12 hours.

Observations are made daily and the day of appearance of each positive response recorded. Information on variation in the growth rate of individual heterokaryons can be obtained by comparing them with nonallelic controls. Each tube is, in effect, a miniature growth tube and since growth starts at the bottom with $2 \times 10^5$ conidia per tube, any delay in the timing of surface growth and/or conidiation should be an indication of a lower rate of growth.

Under the conditions defined, excellent agreement in duplicate tubes has been obtained with regard to time of formation and rate of growth. In addition, positive responses have been observed 26-35 days after inoculation with certain pairwise combinations of ad-3 mutants (de Serres and Brockman, unpublished) or hist-3 mutants (Webber and de Serres, unpublished).

*From Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.

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Heterokaryon and other simple screening tests often require the preparation of a large number of conidial suspensions from different mutant strains of Neurospora. Our approach to this problem is to use the suspensor described below (a modification of a device designed originally by K. C. Atwood and T. H. Pittenger) which makes it possible to make large numbers of suspensions of conidia (1) uncontaminated by constituents of the culture medium, (2) rapidly, and (3) with the possibility of cross contamination reduced to a minimum.

The suspensor shown in the diagram is made out of 8-mm glass tubing, with an egg-shaped bulb blown about 6 inches from the top end. A hole 6-mm in diameter is placed 2-1/2 inches from the end for suction control with the forefinger. The open end of the inverted U-shaped tube (see diagram) inside the bulb should be placed near the base of the bulb so that a suspension of conidia can be completely removed. The tip is drawn out into a tube 2 mm in diameter (O.D.) about 6 inches in length and bent at an angle of 45° 1/2 inch from the end. The bent tip and inverted-U in the bulb should be in the same plane and perpendicular to the suction control opening at the top. The dimensions given are for a suspensor to harvest conidia from slants in 20 x 150 mm test tubes.

Suspensions are prepared in a hood. Sterile materials include two stainless steel containers filled with sterile distilled water, one ice-cold and the other boiling on a hot plate, test tube racks filled with (1) 13 x 100 mm tubes (with aluminum caps) each containing 2 ml water and (2) 20 x 150 mm tubes (with aluminum caps) containing 9 ml water; and a vacuum "safety" flask of at least 1-2 liter capacity.
To prepare a suspension, 2 ml of water is drawn up into the bulb, and the length of glass tubing from the tip into the bulb is dried somewhat by drawing air in slowly. The tip is then inserted into the culture tube and held over (but not in) the conidia, the suction being controlled and varied with the forefinger covering the hole near the top. With care, contamination by culture tube medium constituents can be avoided completely. The conidia should be drawn up the tubing and through the water in the base of the bulb. If the conidia come out above the water level, most will wind up in the waste water in the vacuum flask.

When all the conidia have been removed from the slant, the suspensor is disconnected from the vacuum tubing and the process of suspending these conidia is completed by drawing up additional water from a 20 x 150 mm tube. The suspension is blown out into this test tube and all of the conidia are removed from the device with repeated flushing. The suspensor is re-connected to the vacuum tubing and the entire device cleaned and sterilized between cultures by filling it up with boiling water and turning it up on end to drain the water out through the top. This process is repeated once or twice to rinse out all mycelial fragments and conidia and to inactivate any remaining. The device is then filled with the sterile cold water and emptied to cool it thoroughly before preparing the next suspension.

With this device a conidial suspension containing a total of 1-3 x 10^8 conidia can be prepared from a single 5-7-day-old culture grown on an agar slant in a 20 x 150 mm tube.

Figure 1. Device for rapidly preparing conidial suspensions.

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Frost, L. C. Conidiating media for Neurospora. The following media, promoting conidiation, have been found effective for over 100 different wild, morphological or biochemical mutant strains of Neurospora spp. when large supplies of conidia were required experimentally or for the maintenance of frequently used stock cultures:

1. **Bacto-peptone Medium.** 0.4% bacto-peptone (British Drug Houses) is added to Fries salt solution (Beadle and Tatum, 1945), biotin (4 per litre) 2% glucose and 2% agar. The pH of the medium is about 5 and no adjustment is made (but see McNelly this Newsletter for histidine mutants). Wild, morphological and amino-acid mutant strains conidiate abundantly, especially arginine and lysine strains and arginine, lysine double mutants. However, some purine, pyrimidine and vitamin mutants give poor results. This medium was devised by Frost, 1955 (Ph.D. Thesis, Cambridge University).

2. **Malt, Peptone and Yeast Medium.** To the Fries salt solution and biotin as above are added: glycerol 15 ccs., glucose 2.5 grms., malt extract (British Pharmacopoeia) 2.5 ccs., bacto-peptone (BDH) 0.5 grms., yeast extract (Difco) 2.5 grms., agar 20 grms. and distilled water to 1 litre. No adjustment of pH is made. This medium has given good results with wild and all classes of mutant strains tested, with very few exceptions.

The salt solution, Medium 'N' of Vogel, gives poor results with either of the above media.