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Abstract

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Various difficulties encountered in attempts to increase (1) the numbers of pairwise combinations or (2) the length of the observation period in heterokaryon tests on the ad-3 mutants with the agar plate method (de Serres, Genetics 41: 668, 1956) made it desirable to develop an alternate technique. A method was needed that would permit (1) making a large number of pairwise combinations, in individual containers, so that observations could be made easily over a number of weeks and (2) precise control over the inoculum density per unit area, or per unit volume, of medium so that replicate combinations would agree both in the time of formation of a heterokaryon and the rate of subsequent growth.

The method that was developed makes use of the facts that baskets of test tubes can be "plugged" by wrapping the unit completely in a double layer of Saran Wrap, and that tubes so plugged can be inoculated with suspensions of conidia in liquid minimal medium by means of a pipetting syringe fitted with a hypodermic needle. The two inoculation holes over each test tube effect such a sharp humidity gradient that aerial growth of the heterokaryon is confined in each tube to a region 1-2 cm above the meniscus. The rate of evaporation under average conditions ($\sim$ 23°C, 30-50% relative humidity) is low enough so that with 2 ml of medium per tube observations can be made for long periods of time (30-45 days). Aerial contamination can be controlled by covering the top of each basket of tubes with a square of blotting paper.

To prepare for this type of experiment we first autoclave wire baskets of 100- 75 x 100 mm test tubes (in a 10 x 10 grid) and then wrap the unit in a taut double layer of Saran Wrap. These are covered with a square of sterile blotting paper and packaged in groups of four in heavy-weight paper bags. The bags are placed in a drying over at 60°C for 60-72 hours to insure sterilization of all components and to make the Saran Wrap more taut.

Hemocytometer counts are made on each conidial suspension (after filtration through a

platinum gauze sieve, 150 mesh) to determine the volume to be added to a 250 ml Erylenmeyer flask of minimal medium to give a final concentration of 1 x 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 x 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 x 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).

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