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Abstract

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Growth and harvesting of microconidia. The following procedures have been employed to obtain large numbers of microconidia. 125 ml Erlenmeyer flasks containing 40 ml of a complete medium (1 liter of 1x Vogel's medium containing 20 g. sucrose, 1 g. yeast extract, 1 g. malt extract, 0.1 g. liver extract, and 2% w/v agar) are each inoculated with a drop of a microconidial suspension from a pe, fl strain (Y8743m,L) (FGSC#867). These flasks are incubated for 15 days at 20° C (Reissig 1963 J. gen. Microbiol. 30: 317).

Microconidia are harvested by adding 25 ml of water to each flask and placing the flasks on a rotary shaker for 15 minutes. The shaking can be done by hand. The microconidial suspension is poured from the flask and filtered through cheesecloth and glass wool to remove mycelia and clumps of conidia. The net harvest of filtered microconidia averages about 10^9 cells/flask with an average viability of about 10% when plated on minimal medium (1 liter 1x Vogel's medium, 0.5 g. fructose, 0.5 g. glucose, 20 g. sorbose, and 2% w/v agar). When the pe, fl strain was grown on minimal medium, the net harvest was only about 20% as great as that obtained on complete medium.

The filtration concentration technique. This technique was described in detail by Mary Case (1964 NN#3: 7). We will not present another complete description here, but will only relate some information obtained in this laboratory in the course of filtration concentration experiments.

Four liter Erlenmeyer flasks containing three liters of medium were used in most experiments. The concentration of viable microconidia was about 20×10^6 per liter of medium. Lowering the conidial concentration seems to increase the efficiency of filtration concentration so that a conidial concentration should be selected which fits the needs of the experiment.

The filters were made of one layer of nylon monofilament screen cloth (Nitex #53). It is assumed that this fine mesh with 35 micron openings is more efficient than cheesecloth because of its ability to remove conidia at an earlier stage of germination.

Microconidia germinate more slowly than macroconidia and consequently the intervals between filtrations and the duration of the experiments are longer. When incubation was at room temperature on a rotary shaker, the first filtration was made at 18 hours. For 48 hours following the first filtration 8 hour intervals were used. During the second 48 hours, filtrations were at 12 hour intervals and thereafter the intervals were 24 hours. Mary Case has stressed the importance of regulating the filtration intervals so that mycelium will not develop to the point of fragmentation. Still another reason exists for avoiding the development of mycelium. We have found that a mycelial mat is itself an effective filter. In fact, a relatively small mycelial mat trapped in a filter was found to remove one half of the conidia from a conidial suspension passed through it.

The efficiency of the filtration concentration technique is high when the experiments have a duration greater than 10 days. It was found that usually over 75% of the survivors of an experiments lasting 10 days or more were unable to grow or grew very slowly on minimal medium. Of course, as Mary Case has indicated, the duration of an experiment will be determined by the ability of the desired mutant to remain viable under conditions of starvation. The volume of filtrate remaining at the end of an experiment was often too large for plating without concentrating the survivors. Centrifugation was found to be the most satisfactory method of concentration. A small amount of potato starch added to the centrifuge bottle will aid the formation of a pellet. In this way fewer conidia will be lost as the supernatant is decanted.

Control of bacterial contamination. Certain types of experiments, e.g., filtration concentration, require repeated opening of the culture vessels during the course of the experiment. In such experiments losses may result from bacterial contamination. We have found chloramphenicol to be effective in controlling airborne bacterial contamination. Chloramphenicol concentrations of 500µg/ml autoclaved with the medium were used in most experiments. This concentration of chloramphenicol failed to affect three-day pad weight. Also, we were unable to demonstrate that chloramphenicol had any effect on mutation frequency. Less extensive experiments indicate that lower concentrations of chloramphenicol (e.g., 250 µg/ml) may be equally effective in eliminating bacterial contamination.

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