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Abstract

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One of two general methods is usually employed in estimating the viable and total spore populations plated from interallelic crosses. In the first, the total spore population is estimated by counting the number of spores in an aliquot of the suspension to be plated and the viable spore population is estimated by counting the number of colonies arising when a suitable dilution of the spore suspension is plated on supplemented medium. In the second method, the number of spores counted in an aliquot of spore suspension is used as a rough estimate for plating purposes and the total and viable spore populations are determined by direct microscopic observation of germinated and ungerminated spores on the plates after several days' incubation.

Experiments with progeny from interallelic td (tryp-3) crosses indicated that different results are obtained when the same cross is examined by both methods. In general, much lower and less reproducible estimates of viability were obtained by the first method. The first method provides an estimate of only those spores able to give rise to colonies, while the second gives an estimate of all those able to germinate. The first class of spores may really be much smaller than the second class and more subject to the effect of external factors, but one must consider the possibility of selection for the wild type. Possibly any wild type spore which can germinate will form a colony while not all mutant spores capable of germination are able to form colonies on the supplemented plates. In fact, if one postulates that any spore that is wild type will germinate and form a colony, then it may be more realistic to base wild type frequencies on the total spore population regardless of the percentage of viable spores.

This reasoning is supported by results from interallelic td crosses (Lacy 1959 Ph. D. Thesis, Yale University). Crosses made at different times between the same two mutants sometimes give rise to widely differing viable spore percentages. When the wild type frequencies are based on the viable spore population, they also vary widely. However, when the wild type frequencies are based on the total spore population, they show reassuring similarity.

The following crossing and plating method used currently in our laboratory is more a product of

evolution than of original creation.

The basic crossing method is similar to that originally described by Ryan (1950 Methods in Med. Res. 3:51). One mutant strain is grown for 5-7 days at 25° C on slants of suitably supplemented (see following note on fertility) synthetic crossing medium (Westergaard and Mitchell 1947 Am. J. Bot. 34:573) in tubes which each contain a thin strip of Whatman #1 filter paper running about 80 mm up the tube under the agar slant. Then conidia from a mutant strain of the opposite mating type are either dusted onto the slant or pipetted onto it as a distilled water suspension. The crosses are placed at 25° C and incubated for about 4-6 weeks, at which time they are either plated or stored in the refrigerator until used.

Ascospore suspensions are made by adding 10 ml of sterile distilled water to each crossing tube and agitating vigorously to remove the spores from the wall of the tube. After perithecia and large hunks of mycelium are removed, the entire 10 ml suspension is added to 5 ml of minimal sorbose agar (maintained at 60° C). The medium used is Vogel's minimal (Vogel 1956 Microbial Genet. Bull. 13:42) modified to contain 0.1% sucrose and 1.0% sorbose. After thorough mixing, 0.01 ml and 0.1 ml samples are pipetted onto glass slides which have grids drawn on the undersides and the agar suspension is distributed in a thin layer over the grid. The total number of spores in each sample is counted under a dissecting microscope and the average result used as a rough estimate of the spore concentration of the suspension.

The suspension is then distributed evenly among several 50 ml quantities of minimal sorbose agar (maintained at 60° C) so that when each 50 ml is distributed over 10 already-layered minimal sorbose agar plates, each plate will contain an average of from 2-6,000 spores. Before plating, the 50 ml spore suspensions are heat-shocked for 65 min. at 60° C.

(To provide a comparison of methods for viable spore estimation, samples of the original suspension are added to several tubes containing 5 ml. of liquified minimal sorbose agar supplemented with 25 /ml. of L-tryptophan. After heat shocking, the contents of the tubes are poured onto plates already layered with the same medium, incubated, and examined for colonies for 2-4 days.)

The plates are incubated for 1 week (or longer, if being examined for slow-growing pseudo-wild type colonies) at 25° C or 30° C. (depending on the temperature sensitivity of the mutant strains involved) and examined nearly every day for the occurrence of prototrophic colonies. Pseudo-wild types and heterocaryotic colonies are usually eliminated by clear-cut differences in morphology or later time of appearance on the plates (most true wild type colonies appear during the first 2-4 days). In questionable cases, the prototrophic colonies are isolated, grown on minimal slants, and tested for the presence of mutant nuclei either by conidial plating or by crossing to wild type and testing for mutant progeny.

At the end of 4-6 days, the viable and total spore populations are determined. Ten fields/plate on 20 plates are examined under a dissecting microscope and the total number of germinated and non-germinated spores/field are counted. The average of these 100 counts is multiplied by a factor (the number of fields/average petri dish at 60X magnification on a specific microscope) to give the total number of spores and viable spores/plate. The difference in counts between sets of 10 plates in any one experiment is so small as to make it unnecessary to count each set. In cases in which many prototrophs are recovered, their even distribution among the sets of plates confirms the validity of this procedure. The estimation of germination percentage is sometimes determined by tabulating the first 100 spores observed on each of 5 plates/experiment. The results from the two methods are in very close agreement.

Map distances between allelic td mutants are expressed as the percentage of true wild types among the total progeny from a cross between two mutants. - - - Department of Biological Sciences, Goucher College, Towson, Maryland.