NOTES

Simple Method for Preparation of Homogeneous Spore Suspensions Useful in Industrial Strain Selection

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Homogeneous suspensions of streptomycete spores were desirable for plating after mutagenesis. Filtration through 8-μm membrane filters (Millipore Corp.) more effectively separated hyphae and spore clumps from single spores than did filtration through cotton wefts or paper.

Colonies to be picked and tested in screens for high-yielding mutants of antibiotic-producing streptomycetes should be genetically homogeneous to allow full expression of the rare, desirable mutation. Vegetative growth and sporulation after mutagenesis can be insufficient as a segregation procedure if hydrophobic spores clump randomly after sporulation and clumps are not separated before plating. A genetically superior cell that has become physically associated with the parental type cell or with cells harboring undesired mutations can easily produce a heterogeneous colony which will test equivalent to or inferior to control in the fermentation test. The method commonly used to physically separate vegetative cells and spore clumps from single streptomycete spores is filtration through paper or cotton wefts (1, 2). Using four industrially significant streptomycete cultures, filtration of suspensions containing hyphae and spores through 8-μm membrane filters (Millipore Corp.) proved more effective in separating hyphae and spore clumps from single spores than did filtration through Whatman no. 1 paper or cotton wefts.

Ten milliliters of 0.1% sterile Tween 80 was added to a tube (20 by 150 mm) containing a confluent lawn of the sporulated organism on a 12-ml agar slant. The medium was optimized for the sporulation of that particular streptomycete. The slant was sonicated for 1 min in a model 8845-1 Cole-Parmer sonic cleaner. The resulting spore suspension was vacuum filtered through a sterilized 8-μm membrane filter disk (Millipore Corp., SWP02500, 8 μm, white plane, 25 mm). The filtered spore suspension was counted in a hemocytometer chamber, and the percentage of single spores was calculated as follows: each bioparticle (single spore, multi-spore clump, or hyphal fragment) was counted in five fields of dimension 50 by 50 by 100 μm under a Zeiss photomicroscope II. Photographs were taken at the time of counting. The number of single spores divided by the total number of bioparticles times 100 was referred to as percentage of single spores. The appropriate dilution of filtered spores was plated for determination of spore viability. Operationally, percentage of viability was defined as the viable plate count on nutrient agar divided by the microscopic bioparticle count times 100. For paper or cotton filtration, essentially the same procedure was followed except that the sonicated suspension of spores was filtered by gravity either through Whatman no. 1 paper or through a 4-cm diameter cotton wool.

Average results are reported from three independent and separate experiments for each streptomycete. Ranges are reported when significant standard deviations occurred. Observation of filtrates at 500 times magnification showed that those obtained from cotton or paper filtration were qualitatively identical; hence, only paper filtrates were quantitatively compared to the membrane filtrates.

Fewer bioparticles were passed by membrane filters than by paper except for Streptomyces cinnamonensis. However, the concentration of bioparticles in membrane filtrates was greater than 107/ml for each streptomycete (Table 1); thus, concentration was suitable for most mutation experiments and for plating on selective media. Some S. cinnamonensis multisporo clumps present before filtration appeared to be
Fig. 1. Photomicrographs of streptomycete spore suspensions comparing 8-μm membrane filters (Millipore Corp.) and Whatman no. 1 filtrations. (A) S. fradiae, paper filtered. Magnification 640×. (B) S. fradiae, 8-μm membrane filtered. Magnification 640×. (C) S. tenebrarius, paper filtered. Magnification 500×. (D) S. tenebrarius, 8-μm membrane filtered. Magnification 500×.
broken down to single spores by passage through the membrane filters. This disassociation did not occur in the paper or cotton filtrations and did not appear to occur with the other streptomycetes.

The correct interpretations for the percent viability data were not always clear, as discussed below; what was clear was the fact that membrane filtration more effectively removed multisporic clumps than did paper filtration. This was evident by direct visual observations as noted by the greater percentages of single spores in Table 1 and by Fig. 1.

The contrasting viable count results for *S. tenebrarius* and *S. cinnamonensis* clearly illustrated that both visual homogeneity and high plate count viability were necessary to assure that most colonies had grown from single spores. In the case of *S. tenebrarius*, 97.5% of the bioparticles in the membrane suspension were observed to be single spores, and the corresponding plate count was 82.5%. If all spore clumps gave rise to single colonies and all nonviable bioparticles were single spores (18% would have to be nonviable), then a minimum of 97% of the colonies would have been produced from single spores. However, in the case of *S. cinnamonensis*, an average of 90% of the bioparticles in the membrane filtrate were observed to be single spores, and the corresponding plate count was only 6%. With *S. cinnamonensis*, 9.5% of the bioparticles in the membrane filtrate were doublets of two clumped or unseparated spores. Hence, mathematically it appeared possible that single spores could be nonviable and that only doublets gave rise to colonies.

A factor complicating the interpretation of percent viabilities was the evidence for disassociation of spore clumps upon plating, i.e., the 179 and 111% viability counts obtained for paper filtrates of *S. fradiae* and *S. tenebrarius*, respectively. Such disassociations would be expected to inflate viability counts of all preparations containing large concentrations of spore clumps. Consistent with this expectation was the observation that all paper filtrates had higher viabilities from the corresponding membrane filtrates.

In summary, membrane filtration provides a convenient means of obtaining a relatively homogeneous suspension of single, unclumped spores for use in industrial screens or in the study of the plating efficiency of isolated spores.

**LITERATURE CITED**

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