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Details for preparing silica gel stocks

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Abstract

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Anhydrous silica gel has been used for preserving Neurospora stocks since the 1950s. The method was first described in 1962 (Can. J. Microbiol. 8: 591). Some minor variations were suggested by other laboratories (Neurospora Newsl. 1: 8; 1:13). Since then the technique has gradually evolved, with incorporation of several changes to increase convenience or effectiveness. We now use a greater mass of inoculum, grown in larger culture tubes on 4% agar medium. Gel of finer mesh size is employed. Inoculated silica gel tubes are shaken thoroughly with a mixer, and chilled. The importance of using young cultures for high viability should be stressed.

Details of our present procedure are given below. Stocks prepared in this way consist typically of a 6 ml volume of dry, orange silica gel particles that can be sampled many times to obtain vegetative cultures, and that remain viable over many years if kept dry and cold.

1. Use 13 x 100 mm culture tubes, filled 65 mm deep with anhydrous silica gel, plugged with cotton, hot-air sterilized 2 hr at 180° C, stored at room temperature in a moisture-proof box. We find these much easier to handle than screw-cap tubes, whose constricted openings reduce access. Davison Refrigeration Grade Silica Gel, PA400, is very satisfactory. This is approximately 12-20 mesh size, and contains no indicator dye.

2. Grow up a fresh culture of the strain to be preserved on appropriate medium with 4% agar. One 13 x 100 mm slant usually gives enough material for one silica gel tube. For conidiating stocks, 5 or 6 days at 25° C is about right. Slow growing and nonconidiating strains should not be allowed to age beyond 6 or 8 days -- a larger amount of material should be obtained by using larger tubes or several tubes.

3. Suspend the material in sterile water to give a densely turbid suspension. The procedure differs for conidiating and for nonconidiating strains. With conidiating strains, suspensions can be made in the original culture slant if 4% agar medium is used. About 0.5 ml sterile water is gently introduced, using a thin-snouted disposable pasteur pipet. The cotton plug is replaced and conida are suspended by shaking, using a Vortex-type mixer. About 0.5 ml nonfat milk is then pipetted in and stirred gently to avoid breaking the agar surface, and the entire suspension is pipetted onto silica gel.

   (Both water and nonfat milk are conveniently dispensed in 10 x 75 mm tubes, and autoclaved 10 minutes. Grocery store powdered nonfat milk is dissolved in water to give a concentration at least full strength.)

   Nonconidiating strains, such as fluffy or multicent, or scanty growers, are not suspended in the growth slant. Instead, a sterile blade (platinum-iridium, nichrome, or stainless steel) is used to slice or peel the mycelial mat off the agar surface and transfer it to a 10 x 75 mm tube containing 0.5 ml water. The more mycelium the better, and the unavoidable small amounts of agar do not affect preservation. Do not use just aerial hyphae, but include mycelium from the agar surface.

   In the 75 mm tube, use a pipet or glass rod to grind the mycelium against the tube wall (stirring motion) until a smooth homogenate results. (Be patient! It may take several minutes.) Many revolutions are more effective than heavy pressure. After the homogenate is creamy and has been vibrated, add 0.5 ml milk and proceed as with conidiators.

4. The 1 ml suspension is pipetted dropwise over the silica gel in a prelabelled tube. (Before removing the plug and introducing the pipet, hold the silica gel tube horizontally and shake it so that the particles lie evenly along length of tube, providing clearance for the pipet tip to reach the butt. The pipet is then gradually moved up the tube as the suspension flows out onto the layer of particles.

5. Replace plug and recheck correspondence of labels.

6. Vibrate briefly with the mechanical mixer, so as to distribute inoculum over as many grains as possible throughout the silica gel tube, and to prevent lumping.

7. Place tube in an ice-water bath for 15 minutes.

8. After a few hours at room temperature the particles should appear dry. If they remain soggy because of over-saturation, add more sterile silica gel and mix. (Sigma Silica Gel, Chromatographic Grade, type 1, 60-200 mesh is convenient for adding, being a fine powder.) In a humid climate, storage of the unsealed tubes in a dessicator may be desirable.

9. One day after the tube appears dry, it is sealed against moisture by covering the plug and mouth of tube with a 20 mm square of Parafilm. At this time we etch a stock number on the glass with an engraving tool.

10. Sealed tubes are stored at 5° or -20° C, in moisture-proof boxes.

11. Sampling is accomplished by shaking out a few particles to appropriate medium, then resealing the silica gel tube and returning it to storage.

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