Preservation of Neurospora stock cultures with anhydrous silica gel

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Abstract
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This technical note is available in Fungal Genetics Reports: http://newprairiepress.org/fgr/vol1/iss1/17
Ogata, W. N. Media used for culture of and distribution of stocks. The media used by the Fungal Genetics Stock Center for culturing (1) and distributing (2) stock cultures are as follows:

(1) Vogel's Neurospora (Medium N) concentrated 50X which is published in the Microbial Genetics Bulletin #13 p. 43, January, 1956 - Vogel, H. J. This medium N supplemented with 2% sucrose is used to grow all stocks to be lyophilized or stored on silica gel. For the culture of nutritional mutants the specific chemical required for growth is added to the medium.

(2) The medium used for regrowing silica gel cultures for shipment from the FGSC is Difco Neurospora Agar #0321-15, Difco Laboratories, Detroit, Michigan. The FGSC has found this medium to be ideal for growing the majority of the cultures in its collection. There are only a very few stock cultures which do not grow well on this medium. These few are grown on Vogel medium N plus the biochemical constituent which these cultures require.

Ogata, W. N. Preservation of Neurospora The use of silica gel for preserving Neurospora Stock Cultures by the Fungal Genetics Stock Center was suggested by D. D. Perkins as described in his recent publication (Canadian Jour. Microbiology, in press). A few variations have been made by FGSC from the method described.

(1) Powdered non-fat milk at 7 gms/100 ml of distilled water is used exclusively to suspend conidia of conidiating strains. Non-conidiating strains are processed as described by D. D. Perkins before mixing with reconstituted powdered non-fat milk.

(2) 9 in. dispo-pipettes used at the FGSC are obtainable quite inexpensively from most any biological supply house. These pipettes have a constriction at the mouth end which is advantageous for plugging with cotton to maintain aseptic conditions during pipetting of the resuspended sample into silica gel tubes or lyophil tubes.

(3) Screw cap tubes of size 16 mm x 125 mm are used by the FGSC for holding the silica gel (grade and firm as described by D. D. Perkins). The tubes are filled about one half full with silica gel and dry heat sterilized at 180°C for 1 1/2 hours. After the tubes have been inoculated with their respective samples and the caps screwed down tightly, the cap end of the tube may also be dipped into a container of melted paraffin to further seal the tube from moisture. The tubes are stored in racks in a plastic container at 4-5°C. FGSC also places a container of Tel-Tale indicating silica gel (Davison Chemical Corporation, Baltimore 3, Maryland, 6-16 mesh, grade 42 dehydrating agent-dessicant, activated) into the plastic containers to observe the amount of moisture being taken up during storage. These containers are checked weekly.

(4) Prior to pipetting the conidial suspension onto the sterilized silica gel both the silica gel tubes and the milk conidial suspension are cooled in an ice bath. Considerable heat is liberated when an aqueous suspension makes contact with anhydrous silica gel, as also observed by D. D. Perkins. Brockman and de Serres have found a direct correlation between the per cent of viability and the temperature of the silica gel when inoculated with a liquid suspension of the culture material.

(5) Plastic containers in which the silica gel tubes are stored at the FGSC can be purchased from Althor Products, Brooklyn 14, New York, size L-615 (15" x 11" x 6") and also size AFC-1 (9 1/2" x 4 3/4" x 3/4").

The silica gel preservation method has been used to preserve over 750 conidiating stocks over the past 20 months. To date no tubes have failed to produce viable cultures. Whereas, less reliable results have been obtained with aconidial strains.