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Abstract

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FGSC has collected and maintained stocks of Neurospora sp., Aspergillus nidulans and other filamentous fungi for nearly 25 years. Accounts of methods employed by the Stock Center appeared in early volumes of Neurospora Newsletter, but it has been suggested that many current workers are unaware of how strains are processed and preserved. Since the fungal genetics conrmunity is asked to trust the Stock Center to maintain materials which may be irreplaceable, it seems proper to explain what is done with these materials. Some methods may be new to some readers, and possibly useful to them in day to day operations. While I report the methods, I wish to make clear that they have been developed over the years by the Stock Center, and most were passed along to me by Ruth Rimbey and Ray Barratt. I have made certain adjustments to satisfy my own whims.

Most strains of Neurospora and A. nidulans produce large numbers of conidia, and can be routinely stored in silica gel and in lyophil. Both methods are employed for such strains. Upon receipt of a new culture, it is transferred to a fresh slant and grown at 25°. An adequate amount of conidiating material is generally produced within 4 days. The strain is then processed. In the case of sparsely conidiating cultures, the amount of fresh material can be increased by growing more than one slant. The aim is to maximize the number of viable conidia in the suspension fluid used for preservation. Some colonial strains require a longer period before finally producing abundant conidia. Recognition of this character comes through trial and error - with such strains the culture tube can be sealed with a permeable membrane cap to retard drying. fl mutants have long been difficult to preserve, but I have found a medium described by Turian (Nature 202:1240, 1964) to be very helpful. This is Westergaard-Mitchell (synthetic cross) medium supplemented with 10 mM Na-citrate. fl makes a spreading growth with few aerial hyphae on this medium, but I found that such preparations were qualitatively easier to lyophilize. I later measured the number of conidia (presumably microconidia) produced and found greatly increased numbers on this "conidiogenic" medium (Neurospora Newsl. 32:18, 1985).

When an adequate amount of material has grown, preservation on silica gel and in lyophil can be accomplished at the same time. The suspension fluid used is nonfat dry milk (7% w/v), autoclaved in 13 X 100 mm tubes for 10 minutes. 2.0 ml is an adequate volume. The mycelial-conidial mass is loaded into the milk with a sterile loop or needle. Normal concentrations of conidia can usually be suspended by agitation with the tip of a Pasteur pipette, but cultures that are largely aconidial will require grinding with a stouter implement. I use a small aluminum rod (4 mm diam) which can be flamed

with alcohol. When the vegetative matter is dispersed, the suspension is drawn into a pasteur pipette and dispensed onto the silica gel and into ampules for lyophilization. Silica gel (12-28 mesh) is loaded into 13 X 100 mm screw cap vials, half filling them. These are plugged with cotton, and dry sterilized in a 80° C oven. Immediately prior to use, the required number is removed and placed in an ice bath. A cooled tube is held horizontally to distribute the silica gel along its length, and approx. 0.25 ml of the suspension described above is dispensed drop-wise from one end to the other. The tube is then capped, shaken briefly but vigorously on a vortex mixer and returned to the ice bath. This dissipates the heat generated as the silica gel absorbs moisture. Once cooled, the silica gel tubes are refrigerated in sealed plastic boxes with a dessicant which is renewed as it absorbs moisture from the air. In humid areas, individual tubes may be sealed with parafilm to keep moisture from the silica gel.

Using the same pipette, seven ampules are prepared for lyophilization. Each receives approx. 0.15 ml of the milk-conidial suspension. The ampules are made of 6 mm OD glass tubing, 13-15 cm long, with one end flame sealed, the other plugged with cotton. Groups of ampules are quickly frozen by being suspended in LN vapor for approx. 2 minutes. This slow cooling reduces the chances of cracking the glass. After two minutes, they are lowered into the liquid nitrogen, which freezes the ampules and contents. Ampules are then quickly transferred to a dessicator kept in a -25° C freezer. The dessicator, which contains an open dish of P2O5, is evacuated. The moisture in the frozen samples sublimes and within a few days is absorbed by the dessicant, leaving only a dried pellet of milk and spores in each ampule. These are individually flame sealed while attached to a vacuum sleeve.

Strains that are absolutely aconidial may be impossible to preserve by lyophilization: though most can be preserved on silica gel. For these stocks, preservation over liquid nitrogen (LN) is the backup storage form. Of some 4300 different Neurospora strains, 23 are preserved in silica gel + liquid nitrogen. Only 4 strains [abn-1], [abn-2] and two slime-like variants) cannot be stored in silica gel nor lyophilized. All four are stored exclusively over LN.

Strains to be stored over liquid nitrogen are suspended in 10 % DMSO. Approximately 0.2 ml of the suspension is pipetted into glass ampules as used for lyophilization. These are flame sealed and secured to aluminum canes, two to a cane. Each ampule and each cane is marked with the FGSC number of the strain. These are frozen just as the samples were for lyophilization. Five ampules are usually prepared, with one to be used as a control. Immediately after freezing, the control ampule is dropped into a beaker of 35° C water. The contents are withdrawn with a pasteur pipette and dispensed onto an appropriate medium to check viability after freezing and thawing. If the control is viable, it is assumed the other four samples frozen at the same time are too.

Some additional steps are taken when freezing slime (fz;sg;os-1, FGSC 1118 and 4761). The freezing rate is slowed. Following a suggestion made by G.A. Scarborough, ampules are loaded into a balsa wood tube plugged with cotton. The whole tube is suspended in LN vapor for 15 minutes, by which time the samples have frozen. On thawing, the ampule contents are withdrawn and dispensed into 3 ml of liquid minimal medium in the belief that washing (by dilution) the DMSO is beneficial. A further note: C.P. Selitrennikoff has said he stores slants of slime at -70° C. Prior to our recent move to Kansas City, FGSC has not had access to a -70° freezer, so I have not tried this.

After being processed by any method, each strain is regrown to check for viability and for genotype. Silica gels we tested after a week, lyophils when the samples have dried, and LN stored samples immediately after freezing. Nutritional markers are scored, mating type is determined, unusual morphology is noted. Wild collected strains are crossed to testers for the respective species to verify both species and mating type. Aberration stocks are crossed to standard wild types to observe expected ascospore patterns. Such tests are also conducted when anomalous behavior is reported in any stock mailed by FGSC. After testing, one lyophilized ampule for each Neurospora strain is sent to Stanford, where a collection of such ampules is maintained to guard against loss of the all strains in the event of some major damage to the FGSC facility.

The need to make such tests points out the need for persons depositing strains to be complete in describing traits of each strain. - - - FGSC, Dept. of Microbiology, Univ of Kansas Medical Center, Kansas City, KS 66103