

Alternate ways to preserve strains with silica gel

R. L. Metzenberg, Department of Biomolecular Chemistry, University of Wisconsin, Madison WI 53706-1532

The method for storing *Neurospora* strains described by D. D. Perkins (Can. J. Microbiol 8:591-594, 1962; *Neurospora Newsl.* 24:16-17, 1977) and elaborated in a collection of articles in *Neurospora Newsl.* 26 has made it possible to keep large collections with much less effort than would be required with the older lyophil method. In Perkins' method, conidia are suspended in sterilized non-fat milk, and the suspension is pipetted onto chilled sterile silica gel. For non-conidiating strains, mycelia are mulled or otherwise fragmented in milk to make the suspension. However, even this greatly improved method requires a non-trivial amount of manipulation when large numbers of strains are to be preserved, especially for non-conidiating strains. Preparing each stock consumes a pipet and at least two test tubes: one for growth of the strain, and one for preservation. Both tubes need to be labelled, which adds to the effort and to the chance of error. The following method requires no pipetting. The stock is preserved in the tube in which it was grown.

Perlite, a fluffy white volcanic ash available at any garden center, is put into culture tubes up to no more than 1/5 of their total length. A liquid medium of choice (see below) is added so as just to cover or nearly cover the Perlite and the tubes are autoclaved. The tubes are inoculated and then tapped in a near-horizontal position to form slants which holds their form when the tubes are gently returned to vertical. When the strains have conidiated well, or if non-conidiating, have grown to their limit, baked silica gel, 6-12 mesh, is added to about 4/5 the capacity of the tubes. The tubes are capped, sealed with pre-cut strips of Parafilm, and put at 4°C. At least two weeks later, they are shaken vigorously and stored at 4°C or in a deepfreeze. (The addition of silica gel should be done in a hood for obvious reasons. I have found it convenient to sterilize and dry the gel in an oven in a casserole or open beaker for two hours at 450°F (about 230°C) and store it in pre-sterilized bottles. Sterile 3 ounce paper dixie cups, upside down and separated by squares of toilet paper, are convenient single-use vessels, and the cups are squeezed to form a pouring lip for adding the gel to the tubes.) A fragment of the dry Perlite or a crystal or two of silica gel will start a new culture of a conidiating strain. For non-conidiating strains, the Perlite is necessary; silica gel crystals usually remain sterile. If the Perlite is difficult to disperse, breaking it up the mass with a sterile bamboo stick can be helpful.

Recently I have found that "seed beads" are a very convenient alternative to Perlite, especially for non-conidiating cultures. I use very small black or colorless beads from the Czech Republic, available at craft shops or at Discount Beads, POB 186, The Plains OH 45780; tel. 1-800-793-7592; \$25/kilo. I have used 5 g of beads per 18x150 mm tube with 1.5 ml of medium, and form slants by tapping, as with the Perlite. (Presumably, 13x100 mm tubes with about 0.75 ml of medium would work as well.) The holes in the beads trap medium and the mycelium grows into these protected holes. The beads disperse more easily after they are dried with silica gel than does Perlite. The convenience of seed beads often seems worth the greater cost, still only 12.5 cents per tube.

Finally a caveat. I have not had trouble with cultures dying, but my experience has been very short: about a year with most of the Perlite cultures, and negligible with the seed-bead cultures. Only time will tell how the longevity of these stocks compares with that of stocks made in the traditional ways. Others have found that non-fat milk as a suspending medium enhances survival of lyophil cultures. I have found that *Neurospora* grows well in otherwise permissive liquid medium to which non-fat powdered milk has been added to 10% w/v before autoclaving, and I have used this with beads to prepare stocks. However, I do not know whether this improves survival of the stocks. I would appreciate hearing the experience of anyone who decides to try this method.