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H. E. Brockman

F. J. de Serres

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Abstract

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Brockman, H.E. and F. J. de Serres.*

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Anhydrous silica gel has been used for the preservation of Neurospora cultures by various investigators for several years, and the ability to recover such cultures has been comparable to that following lyophilization (Perkins, unpublished observations). We have initiated experiments to determine whether the viability of conidia from such cultures is strain-specific and whether survival is enhanced by storing the silica gel cultures at 4°C. A description of the procedure, which is similar to that used by other investigators, is given below.

Culture tubes (13 x 100 mm) with teflon-lined screw caps are filled approximately three-fourths full of 6-12 mesh anhydrous silica gel (Eagle Chemical Co., Inc., Industrial Canal, Mobile, Ala.), dry-sterilized at 160°C for 1-1/2 hours, and stored at 60°C until needed. These tubes later are chilled and kept in an ice bath until after the conidial suspension has been added. Three cc of Carnation instant nonfat dry milk (7.5 g/100 cc; autoclaved 10 minutes) is added to a 5-7-day-old culture slant (20 x 150 mm). The conidia are then suspended by placing the tube on a Vortex mixer, and 1 cc of the suspension is added to a cold silica gel tube. The tubes are stored at room temperature (23°C) for 7 days with the screw caps on loosely. The caps then are tightened and the tubes are stored over silica gel in Nalgene pans (12 x 5 x 4-1/2 inches) at 4°C.

To assay conidial viability, a number of silica gel crystals are shaken into 1-2 cc of cold distilled water, the conidia are suspended with a Vortex mixer, the suspension is decanted, and total conidia are counted in a hemocytometer. Various dilutions of the conidial suspension are plated in Westergaard's medium supplemented with 1.5% sorbose, 0.1% sucrose, 1.5% agar, and the necessary mutant requirements. This medium is autoclaved for the period of time required for maximum viability (de Serres, Kølmark, and Brockman, Nature, 193: 556, 1962). Plates are incubated at 30°C for 3-4 days, and the percentage of viable conidia is determined from colony counts.

Table 1 shows the results of an experiment in which a wild-type strain, 74A-OR, and an ad-3 mutant strain, 1-155-0011, were assayed for conidial viability immediately before adding the suspension to silica gel (0 hours) and 2 hours and later after adding the suspension. Viability decreases rapidly during the 7 days at room temperature and less rapidly during storage at 4°C.

In another experiment, the conidial suspension was added to two silica gel tubes--one treated as described in the above procedures and the other stored continually at room temperature. The viability of these strains after 183-224 days is given in Table 2. All strains are ad-3 mutants induced in wild-type strain 74A except 74-OR21-6a, which is hist-3, nic-2, al-2, pan-2. Viability is better at 4°C than at room temperature in every strain, but the difference is much greater for some strains. There is also a considerable difference in viability at each temperature from strain to strain. In all platings the morphology and

growth characteristics of the colonies were strikingly uniform and indistinguishable from those grown from fresh vegetative cultures.

We conclude that conidial viability is greater from silica gel cultures stored at 4°C than at room temperature and that the viability is high enough for routine stock preservation and for experiments utilizing stored conidia.

Table 1

Age of Silica Gel Culture	Viability (%) 74A-OR	1-155-0011
0 hours	75.0	74.8
2 hours	82.3	61.2
8 days	18.0	34.8
14 days	12.5	23.2
35 days	16.0	31.9
97 days	13.2	7.1
255 days	9.6	8.0

Table 2

Strain	Age (days) of Silica Gel Culture	Viability (%) 4°C	Room temp. (23°C)
2-015-0095	223	5.1	0.09
2-015-0128	223	7.7	0.03
1-154-0022	224	6.9	2.9
2-016-0001	212	2.8	2.3
2-016-0006	212	31.7	0.1
1-234-0106	211	48.6	5.4
1-234-0084	211	29.3	0.4
2-017-0002	183	21.7	0.1
2-017-0006	183	10.1	2.1
74-OR21-6a	211	57.6	16.2

*From Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee. Operated by Union Carbide Corporation for the U. S. Atomic Energy Commission.