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Abstract

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Survival of *Neurospora* conidia on silica gel.

We have each established extensive culture collections maintained on silica gel. Recently it has become apparent that while one of the collections has maintained full viability, the other has not. Comparison of the methods for preparation and conditions of storage suggest that two factors may be involved in determining this differential viability: the number of conidia per gram of gel and the control of moisture regain.

Stocks in the DGC collection are prefixed F, those in the DEAC collection are prefixed T.

The materials and methods used are similar to those described by D.D. Perkins (1977 *Neurospora News* 1, 24: 16-17). For the T stocks, cultures were grown on 1.5 ml slopes for 5-7 days and conidia were suspended in 1.5 ml water and then mixed with 1.5 ml of reconstituted nonfat milk (10g powder/100ml, autoclaved 5 min at 10psi and steamed for 30 mins on two successive days). Conidia were allowed to settle and half or more of the supernatant was discarded. The conidia were resuspended in the remaining supernatant and up to 0.8 ml was distributed evenly onto about 3.5g of silica gel (12-20 mesh dry sterilized at 180° for 1.5 hrs in 3x 100mm tubes closed with plugs rolled from cheese cloth; the tubes were stored over anhydrous CaCl₂ prior to use). The tubes were tapped or vibrated mechanically to distribute the inoculum evenly but were not cooled during gel hydration. The gel became dry and free running within a few minutes. Tubes were kept in vacuo over anhydrous CaCl₂ for 3-5 days prior to checking viability and closing with Parafilm. The sealed tubes have been kept at 4° in heavy duty plastic bags containing open tubes of indicator gel which are replenished as required. Parafilm closures have needed replacement at about 5 year intervals. For the F stocks, the only identifiable variations from the methods used for the T stocks were: i) the conidial concentrations step was omitted and ii) following closure, the tubes were stored in plastic bags which did not contain desiccant.

TABLE 1

Viability of rilico-gel preservation stocks

| | DATE OF PRESERVATION (month.year) | NUMBER OF TUBES | | GENOTYPES REPRESENTED |
|----------|-----------------------------------|-----------------|------------|--|
| | | VIABLE | NOT VIABLE | |
| T stocks | 12.63 | 14 | 0 | <i>wild-type: N. sitophila, N. crassa. ad-1-3A-8, d-2, arg-1-2-5, cho-1, cot-1, col-4, ftr, his-s, lys-1-5, met-1-3-5-7, mtr, mts, nic-1, pab-1, pyr-1-3, sfo, sp, trp-1-2-3.</i> |
| | 1.64 | 12 | 0 | |
| | 9.64 | 11 | 0 | |
| | 5.66 | 13 | 0 | |
| | | 50 | | |
| F stocks | 11.63 | 0 | 1 | VIABLE: <i>wild-type N. sitophila. al-1, arg-1-4-7-10, aro-1, aur, col-4, his-1, inl, leu-1, met-6, pab-1, pyr-3, sfo, suc, thi.</i> NOT VIABLE: <i>wild-type N. crassa. ad-3B-4, al-2, arg-2-3-5-6-8-9-11, cho-1-2, col-4, cot-*, cys-1-10, his-1-3-4, hom, ilv, Lys-1-2-3-4-5, met-1-2-3-5-6, nic-1-2, nit-1, nt, pdx-1, pyr-2-3, rib-1, suc, trp-1-2-3-4, vat.</i> |
| | 3.65 | 8 | 25 | |
| | 4.65 | 2 | 12 | |
| | 5.65 | 3 | 6 | |
| | 6.65 | 3 | 2 | |
| | 8.65 | 1 | 0 | |
| | | | 17 | |

TABLE 2

Conidial density in viable and inviable rilico-gel tubes.
Five tubers were selected at random from each group.

| CONIDIA PER GRAM OF SILICA GEL x 10 ⁶ | | |
|--|-------------|-------------|
| | VIABLE | NOT VIABLE |
| | T STOCKS | F STOCKS |
| | 5.24 | 0.39 |
| | 2.24 | 0.45 |
| | 3.98 | 0.64 |
| | 5.36 | 0.21 |
| | 1.96 | 0.44 |
| mean | 3.76 ± 1.61 | 0.43 ± 0.15 |

The viability of silica gel stocks held for 12 to 15 years was checked in October 1978 by shaking a few granules (50-100 mg) of gel onto slopes of the appropriately supplemented medium. The stocks screened were selected to represent a wide range of genotypes and a number of different dates of preservation. The T series stocks were all viable (Table 1). In contrast 73% of the F series stocks were inviable. Inviability is not correlated with the date of preservation ($\chi^2_5 = 7.3$, $p \approx 0.2$) and is not apparently associated with particular genotypes. The effect of the size of conidial inoculum was investigated by mixing a weighed sample (~100 mg) of gel with 1 ml of water and counting the conidia released into suspension. Five tubes were sampled from each group (Table 2). Inviability is clearly correlated with the number of conidia recoverable in this way. If it is assumed that the processes which lead to inviability do not also cause changes in the conidia making them unrecognisable or interfering with their release from the gel, then the results indicate that a high conidial density in the gel is important for their survival. However, it is not clear whether there is any specific threshold density of conidia above which survival is good or if survival time is proportional to conidial density.

The data suggest that for good survival, a conidial density in excess of 10⁶/g of gel is desirable. The effect of water regain by the gel is unclear. However, storing the tuber in the presence of activated silica gel as a desiccant clearly does no harm.

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