Hybrid strains useful in transferring genes between species

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Abstract
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This communication describes conditions under which phenethyl alcohol will reveal the cryptic compartment and will render all of the enzyme susceptible to acid inactivation. Phenethyl alcohol has been previously shown (Lester 1965 J. Bacteriol., 90: 29) to inhibit uptake of various amino acids and glucose in germinated conidia. In addition, 0.3% phenethyl alcohol prevented germination of Neurospora conidia for 8.5 hrs. at 30°C without loss of viability.

Conidia from the strain eth-1 (r), cya-5 (85518) A were grown under conditions of derepression for aryl sulfatase synthesis as previously described (Scott and Metzenberg 1967 Neurospora News1., 11: 8). Conidia were harvested, filtered twice through a glass wool, washed twice with 0.1 M Na-acetate/acetic acid buffer, pH 5.0, and treated with HCl at a pH of 1.3 at 4°C for 15 minutes; then the pH was readjusted to 4.8 with NaOH. Conidia so treated were centrifuged and resuspended in 0.1 M Na-acetate/acetic acid buffer, pH 5.0, containing 0.1 mM cycloheximid and were incubated with various concentrations of phenethyl alcohol at 37°C for 30 minutes. Conidia were kept in suspension by adding a glass bead and agitating on a shaker. During the incubation, the conidial concentration, measured by turbidity of a suitably diluted sample, was OD, 76045 µm = 1.0. This corresponds to 2.8 mg protein per ml by the method of Lowry et al. (1951 J. Biol. Chem. 193: 265), modified by incubating the sample of conidio in the Lowry alkali reagent at least 12 hours before adding the copper reagent. This modification gives reproducible values for protein but probably does not measure all of the protein present in the conidia.

At the end of the thirty minute incubation, an aliquot from the incubation mixture was treated with HCl at pH 1.3 and 4°C for 2 minutes; then the mixture was readjusted to pH 4.8 with NaOH. Another aliquot was diluted with NaCl as control. Samples from the acid-treated and control tubes were collected by vacuum filtration onto filter paper discs and were washed on the paper with cold 0.1 M Na-acetate/acetic acid buffer, pH 5.0. Additional samples were collected and washed as above and further washed with ice-cold chloroform. Eberhart and Tatum (1961 Am. J. Botany 48: 702) reported on analogous technique using acetone. All of these samples were assayed for aryl sulfatase by shaking the filter disc and conidia under the previously established assay conditions (Metzenberg and Parson 1966 Proc. Natl. Acad. Sci. U.S. 55: 629) with the addition of 0.1 mM cycloheximid. The results are shown in the accompanying figure.

It can be seen that, after incubation in the absence of phenethyl alcohol, 34% of the enzyme is not detected in the assay unless the conidia have been treated with chloroform (cryptic compartment). In addition, about 12% of the enzyme has become vulnerable to acid inactivation during the incubation. Low concentrations (0.25% and 0.50%) of phenethyl alcohol reveal the cryptic compartment almost completely, but a large part of the enzyme is still protected from acid inactivation. After incubation with 0.75% or 1.00% phenethyl alcohol, all of the cryptic compartment her become accessible to substrate and all of the enzyme has become susceptible to acid inactivation. The conidia are still able to retain enzyme molecules, however. Even after incubation of the conidia at the highest phenethyl alcohol concentration, washing the conidia removed no more than 10% of the enzyme.

Repetition of this experiment with the same conidial suspension on the same day gave results differing, at most, by 10%. If conidia collected on different days were used, qualitatively the same results were obtained, but there was some variation in the concentration of phenethyl alcohol required to "permeabilize" the conidia in a fixed period of time. Metzenberg, R.L. and S.K. Ahlgren. Hybrid strains useful in transferring genes from one species of Neurospora to another.

The investigation of natural variation in the genus Neurospora has been limited by the absolute or relative infertility of interspecific crosses, especially where it is desirable to move moderately deleterious genes, such as auxotrophic markers, from one species to another. A number of workers have done this successfully, but we have found that, by using the "transfer kit" - a
series of interspecific hybrids that allow one to move a given trait quite easily from one species to another in two or more small phylogenetic steps, rather than one large one. The kit is of purely utilitarian value, and we have made no attempt to determine which chromosomes, or how many, are derived from a particular species. For example, in the crassa-tetrasperma gradient, the strain designation C1, T3 means only that the strain had one N. crassa grandparent and three N. tetrasperma grandparents, and does not imply that it contains N. crassa and N. tetrasperma genes in precisely that ratio. Some miscellaneous exotic strains cross reasonably well with one or more members of this gradient even though they are infertile with both of the parent species.

The nomenclature we have adopted for these interspecific hybrids is as follows. Each hybrid is identified by letters and numbers that refer to the most recent cohort of ancestors that were not laboratory hybrids. For example, C17, S15-a had 17 N. crassa great-great-grandparents and 15 N. sitophila great-great-great-grandparents. Similarly, C4, T4-a had 4 N. crassa great-grandparents and 4 N. tetrasperma great-grandparents, but some of its grandparents were hybrids.

In establishing the N. tetrasperma = N. crassa hybrid line, we plated 343.6AE (FGSC1606, actually found to be mating type a) on Westergaard-Mitchell medium (1947 Am. J. Botany 34: 573). In our hands this isolate shows the highest fertility of any N. tetrasperma strain with N. crassa, and is also quite fertile with several N. intermedia strains. (Crosses of the "type" strains of N. tetrasperma, 85A and 85a (FGSC1270 and 1271, respectively) to N. crassa did not, in our hands, give any viable spores.) After 4 days at 25°C, the plate was treated with a suspension of N. crassa 74-OR23-1A (FGSC1987) conidia. After 3 weeks, a modest number of spores had collected on the lid of the Petri plate. These were suspended in water and heated at 55-60°C for 30 minutes to induce germination. Of 39 germinated spores, 37 grew into cultures of mating type A, and 2 were of mating type A. A similar assay has been described previously (Hae and Haysman 1966 Genetics 54: 292). The two a strains were suspected of having a very atypical chromosome complement, and were discarded; an a strain with roughly the desired ancestry (C4, T4-a) was derived as described in the pedigree below. All other crosses were made by simultaneous inoculation on Westergaard-Mitchell medium. All of the N. crassa = N. tetrasperma hybrids chosen for this kit were a and Srbaleless. The strains comprising the kit are being placed in the collection of the Fungal Genetics Stock Center.

Many of the strains used by Dodge were retrieved from his laboratory after his death, and there was some uncertainty about their identity. As noted above, 343.6AE is actually mating type a. Earlier, H. B. Howe found this strain to contain the allele (unpublished data). 394.5ae (FGSC1609) is a self-fertile heterocaryon. Last of all, the strain designated "N. intermedia, no #, secondarily homothallic" (FGSC1688) should be listed as Neurospora toruloides and is identical with the Centraalbureau voor Schimmelcultures (CBS) stock 25935 (Farratt, personal communication) (See Tai 1935 Mycologia 27: 328). FGSC1688 is extremely fertile with N. tetrasperma testers, and gives the appearance of being N. tetrasperma.

We gratefully acknowledge advice from D. Novak and A. Srbová and also thank them for furnishing their strain of N. intermedia, NIT-A, (FGSC1755) and other useful strains. (It should be noted that NIT-A does have some N. crassa ancestry; Srbová prepared this strain by "caring the mating type allele from N. crassa, conveyed by 10 generations' backcross, to N. intermedia." The original hybrid was between N. crassa and NIT-a (FGSC1754). Hence, in our nomenclature, NIT-A would be C1, 12047-A).

We are likewise indebted to R. H. Davis for N. sitophila 3A. He and M. Grindle have described its origin as follows. "N. sitophila 2a and 3A were kindly provided by J. Fincham. Strain 2a was pure N. sitophila, while 3A was a third-generation backcross of an N. crassa x N. sitophila hybrid to N. sitophila 2a." Fincham obtained the latter strain from H. L. K. Whitehouse (1942 New Phytologist 41: 23). Whitehouse obtained it from J. Rambottom and F. L. Stephens (1935 Trans. Brit. Mycol. Soc. 19: 215), who, in turn, got it from W. H. Wilkins, who found it growing on beech batters in a lumberyard kiln in Chichester, Great Britain, in 1933. In our nomenclature, N. sitophila 3A would be called C1, S15-A.

The origin of the new hybrid stocks is as follows:

- N. tetrasperma 343.6AE x N. crassa 74-OR24-1A = C1, T1-A.

- N. tetrasperma 343.6AE x C1, T1-A = C1, T3-A and C1, T3-a

- C1, T3A x C3, T1-a = C4, T4-a.

- N. crassa 74-OR8-1a x N. intermedia NIT-A = C2049, 12047-a and C2049, 12047-A.

- N. crassa 74-ORE-1a x N. sitophila 3A = C17, S15-a.

The new stocks which have been deposited in the Fungal Genetics Stock Center collection have been assigned the following numbers: N. sitophila 3A (FGSC1769); C1, T1-A (FGSC1770); C1, T3-A (FGSC1771); C1, T3-a (FGSC1772); C3, T1-A (FGSC1773); C5, T1-a (FGSC1774); C2049, 12047-A (FGSC1775); C2049, 12047-a (FGSC1776); C17, S15-a (FGSC1777); C4, T4-a (FGSC1778); N. sitophila 2a (FGSC1779).