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Advantages of using the inactive-mating-type a^{m1} strain as a helper component in heterokaryons.

Griffiths and DeLange (1978 Genetics 88: 239-254) and Griffiths (1982 Can. J. Genet. Cytol. 24: 167-176) have obtained numerous null mutations of the mating-type alleles in Neurospora crassa Typically the mutant strains have lost simultaneously both mating ability and the vegetative incompatibility (heterokaryon incompatibility) that is also a normal property of the mating-type alleles. Loss of the mating reactions cannot be restored by complementation. Unlike normal A and a strains, the mating-type null mutants are capable of forming heterokaryons both with A and a partners (provided the strains are alike at other heterokaryon-incompatibility loci). When such a heterokaryon is used as parent in a cross, only genes from the component having an active mating-type allele are transmitted. The component with the inactive mating-type allele does not contribute appreciably to the progeny.

We have found an inactive-matying-type strain to be ideal as a component of heterokaryons used to rescue or to normalize disadvantaged strains having recessive genotypes that render the strains lethal, weak, infertile, or unstable. Heterokaryons containing such a disadvantaged component together with the inactive-mating-type helper are phenotypically wild-type in morphology, nutrition, and growth rate. They are also fertile in crosses when used as either female or male parent.

I have employed as helper in heterokaryons a strain kindly provided by Dr. A.J.F. Griffiths -- a^{m1} ad-3B cyh-1 (alleles Nos. 1,2-17-114,KH52(r); now deposited as FGSC #4564). (We have found the notation a^{m1} more convenient than a^{m1} used earlier.) Allele a^{m1} is one of three nonreverting a mutants reported in 1978. (Seemingly contradictory statements in the 1978 Genetics paper are due to four typographical errors: "mutant 1" should read instead "mutant 11" at line 15 on page 249, lines 7 and 19 on page 250,. and in Table 2. -- Griffiths, personal communication.) ad-3B serves as forcing marker. cyh-1 can be ignored in the present context. The strain will be referred to as "a^{m1} helper" or simply "helper".

The a^{m1} helper is heterokaryon compatible with the Oak Ridge Laboratory wild types, which are het-C het-d het-e.

Two constraints that limit ability to form heterokaryons with the am1 helper will be apparent. These entail heterokaryon incompatibility genes (het-genes) and forcing markers. (1) The second component must be identical to the a^{m1} helper strain at all het-loci other than mating type. Mutants already in Oak Ridge (74A) genetic background should be fully heterokaryon-compatible (het-compatible) with the helper. But genes in Emerson, Rockefeller or other wild-type backgrounds must be transferred by crossing into OR.

(2) A forcing marker must be present in the second component. Often the detrimental trait of the second component itself acts as a forcing marker (E.G. colonial morphology, slow growth due to cytochrome deficiency). But this is not true of all traits for which shelter in the heterokaryon may be desired (e.g. sterility mutants, unstable alleles). The forced heterokaryon with a^{m1} ad-3B cyh-1 must be maintained under appropriate selective conditions, on minimal medium.

Examples of uses of the a^{m1} helper

1. Rescuing lethals or poor growers. Extremely slow-growing cytochrome mutants are found among progeny of crosses involving the mutant eas Many of these do not grow appreciably after germination. They can be rescued for analysis, however, by adding the a^{m1} helper, wfth which they form vigorous heterokaryons.

Barry (1984 Genetics abstract) has analyzed a chromosome rearrangement which produces a class of progeny containing a small deficiency. Ascospores containing this deficiency are capable of germination, but the germlings die. The a^{m1} helper strain was used to rescue the inviable deficiency progeny, which can be maintained in heterokaryotic condition and transmitted through crosses.

2. Sheltering unstable genes. Known ufa mutants have apparently been revertable (S. Brody, personal communication), and those from the original study have all been lost. Even with optimal supplement, revertants may be at an advantage, and may outgrow the mutant in stock cultures. A new ufa mutant P73B118 has been put into heterokaryons with the a^{m1} helper in hope that selective pressures will be minimized in the sheltered environment of the heterokaryon and ufa nuclei will not be lost. (ufa heterokaryons: FGSC Nos. 4442, 4443).

3. Restoring or promoting fertility in crosses. Mutants at many loci result in loss or impairment of fertility. Examples are fs, ff, pp, cya, cyt, ty, ssu. (See Perkins et at., 1982 Microbiol. Rev. 46: 426-570.) A sizeable proportion of morphological mutants, especially slow growing ones, are difficult or impossible to use as female parents, so that intercrosses cannot be used to test for allelism or to construct double mutants. Infertility is often due to recessive genes and has been circumvented by using heterokaryons. (See, e.g. Mylyk and Threlkeld, 1974 Genet. Res. 24 : 91-102.) We have used am1 helper heterokaryons to expedite mapping of the slow growing female-sterile strain cya-8, to carry out crosses among colonial mutants bn, col-2 and col-3, and to enable multiply marked linkage-tester strains to be used as female parents on unsupplemented crossing medium. The functional mating type of each heterokaryon is that of the nonhelper component.

4. Testing for heterokaryon compatibility. When it is desired to transfer markers from other het-compatibility backgrounds into OR background, progeny from crosses to an OR parent must be tested for heterokaryon compatibility. Testing has normally been somewhat laborious, requiring that the mating types of each progeny be determined prior to heterokaryon testing, or that het-compatibility testers of both mating types be used. Because a^{m1} ad-3B cyh-1 is mating-type neutral, it forms vigorous heterokaryons with het-compatible strains of either mating type, so that mating types need not be determined prior to testing. The effort required for het-compatibility testing is thus reduced by at least one-half. The need for time-consuming additional backcrosses can be avoided if more progeny from an early cross can be tested readily.

5. Stock preservation. Nonconidiating morphological mutants cannot ordinarily be lyophilized successfully. Preservation on silica gel is generally successful but laborious. Cryopreservation is expensive and vulnerable. We have found that phenotypically wild-type heterokaryons with the a^{m1} helper circumvent all these problems, and conidia of the heterokaryons are readily preserved by the most convenient and least expensive method, on anyhdrous silica gel. The same easy solution applies to preservation difficulties with fragile strains. (The cell-wall-less slime strain has long been kept in heterokaryotic condition, though not with the a^{ml} helper.)

Individual components of the heterokaryons can be extracted by plating or streaking conidia on appropriately supplemented sorbose medium, or the heterokaryon can be used directly for crossing and the desired markers recovered in the progeny. - - - Department of Biological Sciences, Stanford University, Stanford, CA 94305.