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Abstract
Hyphal anastomoses are common in filamentous fungi, and they have been described in detail. Little information beyond descriptions is available. These descriptions indicate that the process is complex, and probably involves the activity of several genes. We describe a mutant of *Neurospora crassa* in which hyphal fusions cannot be found. It has no other morphological abnormalities. To our knowledge it is the first of its class.

A hyphal fusion mutant in *Neurospora crassa*

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Hyphal anastomoses are common in filamentous fungi, and they have been described in detail. Little information beyond descriptions is available. These descriptions indicate that the process is complex, and probably involves the activity of several genes. We describe a mutant of *Neurospora crassa* in which hyphal fusions cannot be found. It has no other morphological abnormalities. To our knowledge it is the first of its class.

Although hyphal fusions constitute a major avenue for the formation of heterokaryons in *Neurospora crassa*, the genetics involved are unknown. Observations of hyphal growth and fusions within a strain, between strains, and between species of *Neurospora* suggest the process is controlled by a number of genes. There is a complex stimulus-response pattern in the anastomoses between small branches of intra-strain hyphae, and between small inter-strain hyphae of a given wild type (Garnjobst and Wilson 1956 Proc. Natl. Acad. Sci. U.S. 42:613-618). This pattern is missing between hyphae of Oak Ridge-St. Lawrence (OR-SL) and Rockefeller-Lindegren (RL) wild types. However, a relatively few fusions do occur where the hyphae are in prolonged contact. There are also a number of incomplete fusions. The hyphae of some isolates of the two wild types show an "avoiding reaction", in which the lead hyphae curve away from each other instead of interlacing as they approach. The hyphae of *N. crassa* and *N. sitophila* show no response to each other; they grow as if the each were alone (Wilson, unpublished observations). Some of these responses were described by Buller (1931 Researches on Fungi, 5: Part I Toronto, Longmans, Green & Co.). We wish to report the discovery of a hyphal anastomosis mutant (*ham*) in *N. crassa* which may serve as a starting point in unraveling the complexities involved.

The mutant was found during the analysis of a cross made to determine the *Hl/hi* genotype of Oak Ridge-St. Lawrence (Wilson, et al. Fungal Genet. Newsl. 46:25-30. See also for methods). One slow-growing inositolless isolate would not form a heterokaryon with any *het C,D,E* tester in the standard time period of 24 hr at 30°C. After subculturing, it did form a stable heterokaryon at 34°C with *het CDe i a* after 72-96 hours. The isolate grew on agar as a puff of aerial hyphae at the inoculum site before a hyphal frontier was established. The hyphal frontier was unusually irregular, with single hyphae growing millimeters ahead of the rest. Conidiation was relatively sparse.

A cross with *pan-1;het CDe i* established that the slow growth phenotype segregated as a single gene mutation. Microscopic examination of several of these slow growers paired with normal strains showed a complete absence of intra- and inter-strain fusions, and no cytoplasmic flow between contiguous hyphae. There was only a barely perceptible cytoplasmic movement toward the hyphal tips. Normally, there is rapid movement of cytoplasm through hyphal fusions as hyphae nearest the edge of the coverslip start to grow (Wilson and Garnjobst Genetics 1966 53 :621-631) . We tested further by pairing with strains of opposite mating type and incompatible heterokaryon genotype. Successful inter-strain fusions would be accompanied by incompatibility reactions which are easily seen. None were found. We also punctured with a microneedle one of the segments involved in several possible inter- and intra-strain fusions. If there had been an actual opening between the fused segments, cytoplasm would have run out of both connected segments. There was no such flow, proving that there was no opening. The correlation between slow growth and the absence of hyphal fusions indicated that slow growth could be used as a simple scoring character.

Slow growth isolates from the above cross regained the ability to form heterokaryons with normal strains at 25 and 30°C. The delay in heterokaryon formation remained, and fusions still were not detectable. Growth curves of heterokaryons (either *ham* + *ham* or *ham+ham+*) showed slow but steady growth, ranging from 2.0-2.5 mm/hr in the former, and from 3.0-3.5 mm/hr in the latter.

The paradox of heterokaryons forming without detectable fusions has not been resolved. Although we have not analyzed conidia from the heterokaryons, the components (*inl* (37401) and *pan* (15300) were originally chosen for hetero-karyon work because of their stability (Garnjobst 1953 Am. J. Bot. 40:607-614). We also have never seen a reversion in either strain in years of research on heterokaryon incompatibility. Conidial fusion is not likely, because we have formed *ham* heterokaryons with vegetative hyphae. The *ham* character differs from the *scot* phenotype in that it is a slow grower at temperatures at which *scot* grows normally. The most probable explanation is that the fusions are too rare to be seen in the limited area and limited period of hyphal growth we can examine microscopically.

With the discovery of this mutant, we now have examples of three distinct types of *het* mutants: fusion mutants, mutations that result in heterokaryotic cell death, and mutations that produce unstable heterokaryons. Perhaps it is time to develop a nomenclature based on the sequence of events involved in producing successful heterokaryons, instead of lumping them all under the term, "incompatibility".

In the meantime, *ham* will be sent to FGSC without classifying it.