A method for obtaining double mutants within single genes or gene clusters

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Abstract
Method for double mutants within genes or gene clusters

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Gorrick, M. D. A simple expedient for obtaining large quantities of Neurospora.

Procedures have been developed to permit aseptic withdrawal and addition of media in carboys to facilitate the preparation of large batches of Neurospora mycelium for enzyme studies. Two-gallon polypropylene bottles were modified by inserting a polypropylene tubing order by Laboratory Plasticware Fabricators, Kansas City, Mo.). Rubber special order and closed with a Hoffman clamp.

Neurospora was grown from a conidial inoculum in these carboys at 30°C with vigorous aeration from an aseptically filtered bubbler system according to the method of Mahler and Suskind (1960 Biochim. Biophys. Acta 43: 286) except that after three days of growth the mycelium were harvested via the aeration, leaving behind about 10% of the culture as an inoculum. The mycelium was then aseptically connected to the carboy of fresh medium which was placed in or under the aeration flow. To prevent contamination during harvesting, the aeration must be continued; but addition of fresh medium, the aeration can be stopped. Collection and restoration of the culture was repeated daily for as long as desired. Occasionally, when it was evident that the mycelium were in clumps large enough to clog the tubing during harvesting (vigorously aeraion usually made this a rare situation), the carboy of fresh medium was inoculated by gravity flow from the carboy containing Neurospora and a fresh bubbler system was inserted to continue growth. This modification made it possible to harvest the clumped Neurospora, although not aseptically.

Typically, using strain C-B4 (hist-1) grown on medium N (Vogel 1956 Microbial Genet. Bull. 13: 42) supplemented with 53 mg of L-histidine/liter, this method yields 2.6 ± 0.2 g dry weight of mycelial/l of medium per day, while growing batches from conidial inoculum once every three days yields a total of 2.9 ± 0.2 g dry weight of mycelial/l. Since only 90% of the culture is being harvested in order to leave on inoculum, the daily yield is approximately 2.4 times the quantity of Neurospora that can be obtained growing batches once every three days. The tryptophan synthetase activities in extracts of the powders (Mahler and Suskind, loc. cit.) were 0.29 ± 0.04 units/mg and 0.27 ±0.02 units/mg, respectively. Thus, for a little added investment of effort, one can obtain a 2.4-fold increase in yield per day of growth with no change in the quality of the material. Similar results may be obtained with other strains, with the amount or timing of the harvesting modified according to the growth rate.

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for genetic mapping studies at many loci in Neurospora, as well as in other organisms which form heterocaryons producing multienzyme conidia and in other types such as yeast or Aspergillus which produce diploid heterozygous single cells or conidia.

Double mutants within the hist-3 region have been obtained by a technique utilizing heterocaryons similar to that described by de Serres and Osterbind (1962 Genetics 47: 793). This procedure makes possible the recovery of double mutants within single genes (cistrons) or within operon-type systems. This technique should be of general applicability for genetic mapping studies at many loci in Neurospora, as well as in other organisms which form heterocaryons producing multi-enzyme conidia and in other types such as yeast or Aspergillus which produce diploid heterozygous single cells or conidia.

Basically, the procedure in Neurospora involves forming a heterocaryon between two complementing mutants within the same cistron or operon with each of the strains carrying a different, unrelated mutation. Conidia from such a heterocaryon are then treated with an appropriate mutagen, subjected to the filtration concentration procedure on minimal medium and then plated on minimal medium containing only the growth supplement normally required by the single original complementing mutants. Under these conditions, selection will occur for heterocaryotic conidia containing induced double mutants (in either of the two parental nuclei) which now cannot complement with the original single parental type nucleus.

In the studies at the hist-3 region, two different heterocaryons were used (both mating type A). The first heterocaryon combined a hist-3A mutant (M127) carrying an adenine requiring mutant ad-6 and a hist-3D mutant (M234) carrying a niacin requiring mutant nic-2 (43002). The second heterocaryon involved the same hist-3A parent with the ad-6 mutant and a hist-3B mutant (M1352) with the same nit-2 strain. The double mutants were detected by their inability to grow on minimal medium and were extracted from the heterocaryons either by conidial plating or by outcrossing. The second site mutants in the resulting homocaryotic double mutants were then characterized by their complementation pattern with the tester strains hist-3A (M127), hist-3B (M1352) and hist-3D (M234), by their mapping pattern with the other hist-3 mutants, and by enzymatic assays for the three reactions in histidine biosynthesis controlled by the hist-3 region. By using this procedure, a large number of presumptive double hist-3 mutants were obtained. Many of the double mutants involved lethal mutations which could not be extracted from the heterocaryons either by plating or by outcrossing. Fifteen double mutants were completely characterized. Eight double mutants were recovered in the hist-3B strain. Five of these second site mutants were noncomplementing, two were hist-3A mutants, and one was a hist-3D mutant. Seven double mutants were recovered in the hist-3D strain. Six of the second site mutants were noncomplementing, and one was a hist-3A mutant. (Supported by AEC contract AT (30-1)) 3098.)

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