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 tubulation and closed with a Hoffman clamp.

Neurospora was grown from a conidial inoculum in these carboys at 30°C with vigorous aerostatic flow from an aseptically filtered bubbling system according to the method of Mahler and Suskind (1960 Biochim. Biophys. Acta 43: 288) except that after three days of growth the mycelium was harvested by tubing of 9/16 inner diameter attached to the tubing of 3/4 inch bore near the base (modified on order by Laboratory Plasticware Fabricators, Kansas City, Mo.). Rubber tubing was then connected in series to the tubing of corby of fresh medium which was allowed to enter under gravity. To prevent contamination during harvesting, the flow must be continued; but the aerostatic flow can be stopped. Collection and restoration was repeated daily for as long as desired. Occasionally, when it was evident that the mycelium were in clumps large enough to clog the tubulation during harvesting (vigorously aerated usually made this a rare situation), the corby of fresh medium was inoculated by gravity flow from the corby containing Neurospora and a fresh bubbling 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 yielded 2.6 ± 0.2 g dry weight of mycelia/l of medium per day, while growing batches from conidial inocula once every three days yielded a total of 2.9 ± 0.2 g dry weight of mycelia/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.


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

Basically, the procedure in Neurospora involves forming a heterocaryon between two complementing mutants within the same cistron or operon with each of the two strains carrying a different, unrelated mutation. Conidia from such a heterocaryon were then treated with an appropriate mutagen, subjected to the filtration procedure on minimal medium and then plated on minimal medium containing only the growth supplement normally required by the single original complementing mutant. Under these conditions, selection will occur for heterocaryotic conidio 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 a adenine mutant ad-6 and a hist-3D mutant (M234) carrying a nicotin forcing 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 hist-3B parent. 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 homocaryon double mutants were then characterized by a complementation pattern with the tester strain hist-3A (M127), hist-3B (M1352) and hist-3D (M234), by their mapping pattern with the other hist-3B mutants, and by enzymatic assays for the three enzymes 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 mutants 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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