A simple expedient for obtaining large quantities of Neurospora

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A simple expedient for obtaining large quantities of Neurospora

Abstract
Large scale growth in carboys
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 clump.

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 tubulation, leaving behind about 10% of the culture as an inoculum. The tubulation was then aseptically connected to the tubulation of carboy of fresh medium which was allowed to enter under gravity flow. To prevent contamination during harvesting, the flow of fresh medium, the aeration can be stopped. Collection and restoration was repeated daily as long as desired. Occasionally, when it was evident that the mycelium were in clumps large enough to clog the tubulation during harvesting (vigorously aeration 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 mycelium/l of medium per day, while growing batches from conidial inocula once every three days yields a total of 2.9 ± 0.2 g dry weight of mycelium/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.

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 multinucleate conidio and in other types such as yeast or Aspergillus which produce diploid heterozygous single cells or conidio.


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