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### 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* mycelio for enzyme studies. Two-gallon polypropylene bottles were modified by inserting a polypropylene tubulature of 3/4 inch bore near the base (modified on special order by Laboratory Plasticware Fabricators, Kansas City, Mo. ). Rubber tubing of 9/16 inner diameter was attached to the tubulature 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 Mohler and Suskind (1960 *Biochim. Biophys. Acta* 43: 288) except that after three days of growth the mycelio were harvested via the tubulature, leaving behind about 10% of the culture as an inoculum. The tubulature was then aseptically connected to the tubulature of a carboy of fresh medium which was allowed to enter under gravity flow. To prevent contamination during harvesting, the aeration must be continued; but to increase the flow rate during addition of fresh medium, the aeration can be stopped. Collection and restoration was repeated daily for as long as desired. Occasionally, when it was evident that the mycelio were in clumps large enough to clog the tubulature during harvesting (vigorous 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  $2.6 \pm 0.2$  g dry weight of mycelia/l of medium per day, while growing batches from conidial inocula once every three days yields a total of  $2.9 \pm 0.2$  g dry weight of mycelia/l. Since only 90% of the culture is being harvested in order to leave an 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 (Mohler and Suskind, loc. cit.) were  $0.29 \pm 0.04$  units/mg and  $0.27 \pm 0.02$  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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