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A simple expedient for obtaining large quantities of Neurospora

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Abstract

Large scale growth in carboys

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Procedures hove been developed to permit aseptic withdrawal and oddition of media in carboys to facilitate the preparation of large butches 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 ond closed with a Hoffman clomp.

Neumsporo was grown fmm a conidiol inoculum in these carboys at 30°C with vigorous oerotion from on aseptically filtered bubbler system according to the method of Mohler ond 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 asepticclly connected to the tubulature of a corboy 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 doily 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 Neumsporo 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 mycelia/l of medium per day, while growing batches from conidiol inocula once every three days yields 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 doily yield is approximately 2.4 times the quantity of Neurospora that con be obtained growing botches once every three days. The tryptophan synthetase activities in extracts of the powders (Mohler 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 con obtain a 2.4-fold increase in yield per doy 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 rote.

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