

Rapid sampling and collection of Neurospora mycelial suspensions.

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Abstract

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Many experiments with Neurospora involve the taking of multiple samples of mycelial suspensions as a function of time after various treatments. One of the difficulties in working with mycelia is that they are often too coarse to be pipetted easily. Furthermore, because parameters to be measured may change rapidly, it may be important to collect the samples very rapidly to avoid changes during collection.

This note describes procedures we have found useful both for rapid sampling of small aliquots of Neurospora mycelial suspensions and for rapid filtration of such samples.

Neurospora liquid shaking cultures (1-24 h, 25" in liquid minimal medium, density of about 1 mg dry wt/ml) can be sampled using a variety of dispensers which fit into 24/40 ground joint erlenmeyer flasks (variously named "Repeater Pipets", "Tipets", "Prepipettors", "Tilt-a-Pets", etc.). One can typically take 10 ml samples varying by 15% with such medium density culture as described above.

Such samples can be collected on Whatman GF/A glass fiber filters. We typically collect such samples on 2.4 cm diameter filters using Millipore or similar filter holders having sintered glass bases. The samples can then be placed into TCA, PCA, ethanol or liquid nitrogen for extraction or rapid freezing. If it is not necessary to wash the samples, it is not difficult to take a sample, filter it and move it into TCA-containing tube in 3 to 5 seconds. We have shown that such samples may have ATP/ADP ratios of 7, showing that they retain a high energy charge throughout the collection procedure.

The GF/A filters are very fast in filtering mycelia of Neurospora and other fungi. Conidia, however, tend to clog the filters fairly rapidly. Consequently, it is important that conidia used to grow the mycelia are relatively fresh with good germination frequencies to avoid slowed filtration due to ungerminated conidia. (Supported by NIH grant GM-24368) Programs in Genetics and Cell Biology and Biochemistry/Biophysics.) Molecular Genetics, Washington State University, Pullman, Washington 99164-4350.