An efficient grinder for ascospores and mycelium

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Abstract
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C. Tyrosine estimation: (Udenfriend and Cooper 1952 J. Biol. Chem. 196: 227):
Reagents: (1) 1-nitroso-2-naphthol solution; 0.1% nitrosonaphthol in 95% ethanol. (2) Nitric acid - sodium nitrite; 2.6 M nitric acid containing 0.05% sodium nitrite. This may be conveniently prepared by adding 0.5 ml of 2.5% sodium nitrite to 24.5 ml of 2.6 M nitric acid immediately before use.

Assay: The reaction mixture contains 1 ml of supernatant + 0.5 ml nitrosonaphthol solution + 0.5 ml nitric acid-sodium nitrite. Mix and heat at 55°C for 30 minutes. Cool, add 5 ml 1,2-dichloroethane to each tube, mix thoroughly and centrifuge to break the emulsion. The dichloroethane layer contains the unreacted nitrosonaphthol. Measure the optical density of the upper layer at 450 μm. Reagent blanks and tyrosine standards should be run with each set of assays. --- Division of Biology, California Institute of Technology, Pasadena, California.


mounted a glass syringe barrel containing a sample of Chroococcus in a lathe, and by forcing the plunger into the slowly turning barrel got effective breakage. Using this idea, we have developed an efficient instrument from common and inexpensive materials.

In this machine the syringe plunger is mounted in the chuck of a high torque stirrer motor through a bolt cemented to a rubber stopper with epoxy resin. The end of the plunger is cemented to the other end of the stopper. This is a secure, yet flexible, connection. The tip of the barrel is sealed with epoxy resin and the barrel is mounted in a chuck mode of heavy-walled rubber tubing (13/16 x 3/8”) which, in turn, is fastened by a hose clamp to a wooden block on the end of a threaded rod. This arrangement for holding the barrel also is designed to impart flexibility in order to prevent excessive breakage of the plunger. The 3/8” threaded rod is aligned with the plunger by a U-shaped brace of 1/4” steel. A handle at the end of the rod is “red to turn it through the brace, thus advancing the barrel over the plunger. Because of the force necessary to advance the barrel over the plunger, it is necessary to mount the individual parts to a sturdy one-inch plywood board.

In practice the grinder is loaded by tipping it up and pipetting the spore suspension into the barrel. We generally “see 100-200 mg of ascospores in 5 ml of suspending medium in a 10 ml syringe. The barrel is fitted over the plunger, and all the air is forced out. The grinder is then laid flat, the motor turned on, and the barrel advanced over the slowly turning plunger. The homogenate, which drips slowly from the end of the barrel, can easily be caught in a beaker. A 5 ml sample in a 10 ml syringe generally is ground in 15 minutes if the syringe is close-fitting. In the case of syringes with unusually close tolerances it is necessary to reduce the closeness of fit by a few seconds grinding with fine corundum. If the grinder is “red in a cold room, and if the plunger is turned slowly, no serious heating occurs.

By “sing a 50 ml syringe with a conical tip on the plunger, the grinder works well on mycelium that has been pre-ground in a blender. --- Department of Botany, University of Michigan, Ann Arbor, Michigan.


In recent years we have extracted Neurospora by hand grinding with alumina, which releases more protein than any other method we have tried, and we still “see it on a small scale. For large scale preparations we now “see a “laboratory homogenizer” manufactured by the Manton-Gaulin Company of Everett, Mass. This press, which occupies little floor space although it weighs 450 pounds, extracts 3 times more protein than an alumina does and is a great deal more agreeable to see. A kilogram of freshly harvested mycelium is dispersed in acetone containing 0.1% sulfur (Flavin 1965 Biochem. Biophys. Res. Commun. 20: 652); the cell mass is 35% and the extractable protein 15% of normal. The extracts are run for 4 hours in the Spinco 21 rotor. Besides the pellet and the fat pellicle, it is essential to discard about the top 15% of solution, even though no refractive difference can be seen. Otherwise, sooner or later the protein will float when a salt fraction is centrifuged, or a viscous solution will be obtained which can’t be passed through sephadex. --- Enzyme Section, National Heart Institute, Bethesda, Maryland.