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Abstract

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The preparation of acetone powders from animal tissues for enzyme extraction and purification is now a classical procedure. The following method has proven practical for the preparation of acetone powder of Neurospora mycelia.

All operations are made at room temperature in a well-ventilated room. Mycelia are frozen in liquid nitrogen and disrupted by grinding for a few minutes in the metal cup of a homogenizer (Virtis homogenizer or Waring blendor) in the presence of excess liquid nitrogen. When the liquid nitrogen has evaporated and the temperature of the cell mass has risen to ca. -20°, three volumes of acetone (-20°) are added, and homogenization is continued for 1-2 minutes. The resulting slurry is immediately transferred to a Buchner funnel on Whatman No. 3 MM filter paper and vacuum is applied with a water aspirator. As soon as excess acetone is no longer visible above the filter cake, and before the cake has dried and cracked, a sheet of rubber dental dam is fixed over the funnel top and vacuum filtration is continued for 5-10 minutes. (The rubber sheet aids in expressing the last traces of acetone and prevents excessive oxidation of the filter cake.) The filter cake is re-suspended in acetone (3 vol., -20°) and the homogenization-filtration process is repeated two or three times until the final filtrate is free of orange pigmentation. When the last trace of acetone has been filtered off, the rubber sheet is removed, and the filter cake is washed with ether (0.5 vol., -20°, peroxide-free) on the filter. The rubber sheet is fixed on the funnel and vacuum is applied for 30 min. The dried filter cake is removed to a sheet of filter paper, crumbled with a spatula, and transfered to a vacuum desicator over $CaCl_2$. The last traces of solvent are removed from the powder with an oil vacuum pump. (A condenser in liquid nitrogen serves to trap the solvent vapor). The powder is stored in vacuo at -20°, if not extracted immediately.

With homogenizers and Buchner funnels of appropriate size, lots of mycelia ranging from 10 gm. to 3 kg. may be processed in one operation. About 10-20 gm. of acetone powder are obtained from 100 gm. of fresh mycelia. Buffer extracts of the acetone powder, following centrifugation, are translucent to pale-yellow, lipid-free, and yield 10-20 gm. of soluble protein per 100 gm. of powder.

The following enzymes exhibit activity in Neurospora acetone powder extracts: dihydrouracil hydrase, dihydroörotic dehydrogenase, dihydroörotase, amino imidazole carboxamide ribotide transformylase, inosinicase, adenylosuccinase, ribonuclease, lactic dehydrogenase, and malate dehydrogenase.