LaBrie, D.A. and R. P. Wagner. Isolation and purification of mitochlodria from N. crassa.

The fallowing describer two methods, used in this laboratory, for the preparation of mycelial homogenates from which Neurospora mitochondria may be isolated and purified.

Neurospora crassa strain KJT 1960 a is grown in shaker flasks (Kiritani et al. 1965 Biochim. Biophyr. Acta 100: 432 ). The mycelium is harvested after 16 hours of growth by fitration through a double layer of cheesecloth, resuspended in 0. 1 M sucrose in 0. 1 M Tris, pH 7.8 and filtered again. When the wet weight of mycelium exceeds 100 g, it is disrupted with on Eppenbach Micro Mill by the method of Greenawalt et al. (Methods in Enzymol. 10: 142). Smaller quantities of mycelium ore homogenized by grinding in a prechilled porcelain mortar and pestle with twice the mycelial wet weight of acid-washed sand. The mycelium is first ground to a coarse paste with sand alone, after which 0.24 M sucrose containing 0.15% BSA is added with continual grinding until a smooth paste is obtained. The final volume in ml of sucrose-BSA added need not exceed twice the wet weight of mycelium.

The crude mitochondrial pellet is obtained by differential centrifugation of the mycelial homogenate obtained by either of the above methods. The homogenate is centrifuged at 1500 x g for 10 minutes, and the supernatant, thus obtained, centrifuged again at 1500 x g for 15 minutes. This process removes sand, unbroken mycelium, nuclei and other large cell fragments. The supernatant is then centrifuged at 37,000 x g for 30 minutes, and the supernatant decanted. The residue consists in large part of crude mitochondrial pellet which is transferred to a glass homogenizer and resuspended with three stroker of a teflon pestle in a minimum of 0.25 M sucrose, 0. 15% BSA. An aliquot of the mitochondrial suspension, containing no more than 40 mg of mitochondrial protein, is layered on an 8.0 ml linear sucrose gradient (0.58 - 1.9 M; 20-65%, w/v ). The gradients ore then centrifuged at 50,000 rpm for 90-120 minutes in a Spinco 50 rotor, after which the bottoms of the gradient tuber are punctured and the mitochondrial band collected as a single fraction. Such mitochondria are relatively free of microsomes, arc capable of synthesizing certain amino acids and can be used in polarographic studies to determine oxygen uptake. - - - Department of Zoology, University of Texas, Austin, Texas 78712.