Isolation and purification of mitochondria

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

Neurospora crassa strain KJT 1960 a is grown in shaker flasks (Kirita et al., 1965 Biochim. Biophys. Acta 100:432). The mycelium is harvested after 16 hours of growth by filtration 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 an Eppenbach Micro Mill by the method of Greenwald et al. (Methods in Enzymol., 10: 142). Smaller quantities of mycelium are homogenized by grinding in a precooled 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 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 are then centrifuged at 50,000 rpm for 90-120 minutes in a Spinco 50 rotor, after which the bottoms of the gradient tube are punctured and the mitochondrial band collected as a single fraction. Such mitochondria are relatively free of microsomes, are 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.


Previous studies of Neurosporacarotenoids have been hampered by low total carotenoid yields (0.08 ± 1% of dry weight) or by a distribution of intermediate pool sizes which favored the end product; e.g., neurosporoxanthin accounts for up to 90% of the total carotenoid fraction.

Enhanced intermediate pool sizes have been obtained by using neurosporoxanthin-less or yellow "albino" strains; e.g., ylo-1 or yl-o-b, ALS-4, ALS-23. There strains have 55-75% of the total carotenoid fraction yields of the wild type strains, mostly in the form of the early precursor pools (phytofluene, b-carotene, neurosporone, etc.).

Huang (1964 Genetics 49:453) and Harding (1968 Neurospora News 13: 8) reported yield improvements by culturing in the dark in liquid medium for 5 days and then draining off the medium and exposing the spread-out mycelial mat to intense fluorescent light for 1 to 24 hours. Cold treatments (6 hrs. at 7°C) do seem to improve yield, but as yet no quantitative data are available.

Using the above techniques, it has been possible to obtain a yield of 1.8% (total carotenoid fraction/dry weight of mycelium) and isolate short-lived intermediates. b-zeacarotene has already been identified as a component of the Neurospora carotenoid fraction using this technique, which was developed in conjunction with a genetic study attempting to define the specific biosynthetic lesions caused by the "albino" gene cluster alleles. - - - Laboratory of General Microbiology, University of Geneva, Geneva, Switzerland.


by treatment with one of several agents. In addition, to acid inactivation of the enzyme, which has been previously observed after the conidia have been permeabilized with the enzyme assay and acid represents the periplasmic enzyme space. The acid-inaccessible, substrate-accessible compartment represents enzyme imbedded in the plasma membrane. The acid-inaccessible to both substrate and acid (cryptic compartment) corresponds to truly intracellular aryl sulfatase.

It was previously reported (Scott and Metzenberg 1967 Neurospora News 11: 8) that Neurospora conidia contain a cryptic compartment of aryl sulfatase which can not be detected in intact conidia but can be observed after the conidia have been permeabilized by treatment with one of several agents. Although direct evidence is lacking, it is useful to think of these compartments as follows: The enzyme accessible to both substrate and acid represents the periplasmic enzyme space. The acid-inaccessible, substrate-accessible compartment represents enzyme imbedded in the plasma membrane. The acid-inaccessible to both substrate and acid (cryptic compartment) corresponds to truly intracellular aryl sulfatase.