
Neurospora crassa strain KJT 1960 is grown in shaker flasks (Kiritani 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 on 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 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 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 tubes 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.

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Previous studies of Neurosporacarotenoids have been hampered by low total carotenoid yields (0.08 = 1% of dry weigh+) 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 ylo-b, ALS-4, ALS-23. These 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, M-carotene, neurosporene, 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 intermediate. M-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.


by treatment with one of several agents. In addition, to acid inactivation of the enzyme which has been previously been observed after the conidia have been permeobilized by acid and acid (cryptic compartment) corresponds to truly intracellular aryl sul-
This communication describes conditions under which phenethyl alcohol will reveal the cryptic compartment and will render all of the enzyme susceptible to acid inactivation. Phenethyl alcohol has been previously shown (Lester 1965 J. Bacteriol. 90: 29) to inhibit uptake of various amino acids and glucose in germinated conidie. In addition, 0.3% phenethyl alcohol prevented germination of *Neurospora* conidio for 8.5 hrs. at 30°C without loss of viability.

Conidio from the strain eth-1 (r), cys-5 (85518) A were grown under conditions of derepression for aryl sulfatase synthesis as previously described (Scott and Metzenberg 1967 *Neurospora News* 11: 8). Conidia were harvested, filtered 12 hours before adding the copper reagent, and glucose in germinated conidie. In *Am. J. Bot.* through incubation with discs and were washed twice through gloss wool, washed twice with 0.1 M Na-acetate/acetic acid buffer, pH 5.0 and treated with HCl at a pH of 1.3 at 4°C for 15 minutes; then the pH was readjusted to 4.8 with NaOH. Conidia so treated were centrifuged and resuspended in 0.1 M Na-acetate/acetic acid buffer, pH 5.0, containing 0.1 mM cycloheximide and were incubated with various concentrations of phenethyl alcohol at 37°C for 30 minutes. Conidio were kept in suspension by adding a gossow bead and agitating on a shaker. During the incubation, the conidial concentration, measured by turbidity of a suitably diluted sample, was OD \( \frac{2}{2} \text{cm} = 29.2 \). This corresponds to 2.8 mg protein per ml by the method of Lowry et al. (1951 J. Biol. Chem. 193: 265), modified by incubating the sample of conidio in the Lowry alkali reagent at least 12 hours before adding the copper reagent. This modification gives reproducible values for protein but probably does not measure all of the protein present in the conidio.

At the end of the thirty minute incubation, an aliquot from the incubation mixture was treated with HCl at pH 1.3 and 4°C for 2 minutes; then the mixture was readjusted to pH 4.8 with NaOH. Another aliquot was diluted with NaCl as control. Samples from the acid-treated and control tubes were collected by vacuum filtration onto filter paper discs and were washed on the paper with cold 0.1 M Na-acetate/acetic acid buffer, pH 5.0. Additional samples were collected and washed as above and further washed with ice-cold chloroform. Eberhart and Tatum (1961 Am. J. Botany 48: 702) reported on analogous technique using acetone. All of these samples were assayed for aryl sulfatase by shaking the filter disc and conidio under the previously established assay conditions (Metzenberg and Parson 1966 Proc. Natl. Acad. Sci. U.S. 53: 629) with the addition of 0.1 mM cycloheximide. The results are shown in the accompanying figure.

It can be seen that, after incubation in the absence of phenethyl alcohol, 34% of the enzyme is not detected in the assay unless the conidio have been treated with chloroform (cryptic compartment). In addition, about 12% of the enzyme has become vulnerable to acid inactivation during the incubation. Low concentrations (0.25% and 0.50%) of phenethyl alcohol reveal the cryptic compartment almost completely, but a large port of the enzyme is still protected from acid inactivation. After incubation with 0.75% or 1.00% phenethyl alcohol, all of the cryptic compartment her become accessible to substrate and all of the enzyme has become susceptible to acid inactivation. The conidio are still able to retain enzyme molecules, however. Even after incubation of the conidio at the highest phenethyl alcohol concentration, washing the conidio removed no more than 10% of the enzyme.

Repitition of this experiment with the same conidial suspension on the same day gave results differing, at most, by 10%. If conidia collected on different days were used, qualitatively the same results were obtained, but there was some variation in the concentration of phenethyl alcohol required to permeabilize the conidia in a fixed period of time. Department of Physiological Chemistry, University of Wisconsin, School of Medicine, Madison, Wisconsin 53706.

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**Metzenberg, R.L. and S.K. Ahlgren.** Hybrid strains useful in transferring genes from one species of *Neurospora* to another.

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The investigation of natural variation in the genus *Neurospora* has been limited by the absolute or relative infertility of interspecific crosses, especially where it is desirable to move moderately deleterious genes, such as auxotrophic markers, from one species to another. A number of workers have done this successfully, but we have found to circumvent this difficulty, we have developed a "transfer kit" - a