Isolation of high molecular weight DNA from Neurospora

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Abstract
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The following procedure has been developed to isolate DNA with a minimal molecular weight of $5 \times 10^6$ (from ultracentrifugal sedimentation analysis) from Neurospora crassa.

1. Mycelia from cultures of N. crassa in early log phase are harvested, washed with distilled water, pressed dry and frozen in liquid nitrogen. The material is ground to a powder under liquid nitrogen in a mortar and pestle. Subsequent steps are carried out at 0-4°C.

2. The mycelial powder is suspended in 0.25 volumes of 0.1 M NaCl buffered at pH 7.75 with 0.1 M Tris and stirred for 10 minutes. Five volumes of ethanol-ether (1:1) are added and stirred for 20 minutes.

3. The suspension is centrifuged at 1000 X g and the supernatant discarded. The pellet is suspended in 0.1 M Tris at pH 7.75 and an equal volume of 5% aerosol OT (Fisher Scientific Co.) is added and stirred either overnight at 0-4°C or 2 hours at room temperature. This suspension is centrifuged at 11,000 X g for 20 minutes and the pellet discarded.

4. NaCl is added to the supernatant to a final concentration of 1 M and isopropanol (at -20°C) is added slowly while the DNA is wound onto a glass rod.

DNA thus isolated may be deproteinized by repeated treatment with 0.05 volumes of chloroform-octanol (8:1) and successive centrifugation to separate the two phases. The procedure is repeated at least 5 times or until there is no interphase (denatured protein). The aqueous phase is made to 1 M NaCl and the DNA precipitated with 2 volumes of cold ethanol. The precipitate is dissolved in 0.1 M NaCl and dialyzed.


Case, Mary. Procedure for filtration-concentration experiments.

The concentration of mutants by filtration from treated conidia was first utilized by Fries (Nature 159:199, 1947) for Ophistoma multianellatum and later adapted for Neurospora (Catcheside, J. Gen. Microbiol. 11: 34, 1954; Woodward, DeZeeuw, and Srb, Proc. Nat. Acad. Sciences, 40: 192, 1954). The principle involves the placing of treated conidia into a minimal medium and the filtering off of the growing mycelia as the wild type conidia germinate. Details have been described for these experiments by the above authors for Neurospora. Through the years, however, certain refinements of the experimental procedure have been made at Yale. (Various ideas for these experiments were contributed by Dr. F. J. de Serres while at Yale University and by Dr. Val Woodward).

Wild type strain: The wild type strain 74A is grown for 8 days on Fries minimal agar (1.5% sucrose). Conidia are harvested in distilled water, filtered through cotton, centrifuged, and washed before treatment with mutagens such as X-rays, ultraviolet, or chemicals. This filtering through cotton reduces the number of large conidia (presumed multinucleate) as well as the mycelial fragments. The conidial suspension is counted with a hemacytometer.

Filtration flasks: 500 ml. narrow mouth Erlenmeyer flasks are used for these experiments with not more than 300 ml. of Fries minimal medium/flask (1.5% sucrose). The concentration of conidia added to 300 ml. of medium should be no more than $30 \times 10^6$ viable conidia. Higher concentrations of conidia appear to reduce the efficiency of the recovery of mutants and to delay the germination of wild type conidia. In general, regardless of the mutagen used, a better recovery of auxotrophs appears to be obtained in the range of 40% to 60% survival.

Filtration process: The filtration flasks are incubated at 25°C on a low-speed reciprocating shaker. Since the control conidia begin to germinate within 8 to 10 hours after treatment, it is desirable to begin the incubation of the flasks at night, so that the first filtration occurs the next morning. For the next 20 hours the filtration flasks are filtered every 3 to 4 hours. After this period the flasks can be filtered every 8