Preparation of Large Molecular Weight DNA from the Fungus *Aspergillus nidulans*

By N. RONALD MORRIS

Department of Pharmacology, CMDNJ-Rutgers Medical School, P.O. Box 101,
Piscataway, New Jersey 08854, U.S.A.

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Development of the molecular biology of the filamentous fungi has been limited by the unavailability of a good general method for preparation of large molecular weight DNA for restriction analysis. Large molecular weight DNA has been prepared from isolated nuclei of *Aspergillus nidulans* (Morris, 1976) and *Neurospora crassa* (Hautala *et al.*, 1977), but to my knowledge total cellular DNA of large molecular weight has not been prepared in large amounts either from the spores or from the vegetative mycelium of any filamentous fungus. There have been two major technical problems. The first is that fungi contain very small amounts of DNA and have very active DNAases. The second is that both the spores and the vegetative mycelia of filamentous fungi have extremely tough walls and there have been no good, gentle methods for opening either spores or mycelia without causing shearing of DNA. Both of these problems have recently been solved. Spores can be opened by vortexing with glass beads (Van Etten & Freer, 1978) and mycelia can be made into protoplasts (de Vries, 1974) which are then easily lysed with detergent. I have discovered, during the course of trying to develop a new method for isolation of nuclei from *A. nidulans*, that the intracellular DNAases of *A. nidulans* are inhibited by EDTA. The purpose of this short communication is to describe a method for the preparation of large molecular weight DNA from spores and from protoplasts prepared from vegetative mycelium of *A. nidulans*.

DNA can be prepared from conidial spores by the following procedure. Conidia (2 g) are harvested into a 30 ml siliconized glass centrifuge tube and washed twice with 20 ml of cold lysis buffer (5 mM-spermidine, 100 mM-KCl, 10 mM- Na_2EDTA , 10 mM-Tris/HCl pH 7.4, 0.25 M-sucrose). The conidia are ruptured and nuclei are released by vortexing with glass beads (Van Etten & Freer, 1978): 10 g acid washed 0.17 mm Braunwell (Braunwell Industries) glass beads and 2.5 ml of cold lysis buffer are added to the conidial pellet, mixed well with a glass rod and the suspension is vortexed on a Vortex-Genie Mixer at a setting of 10 until 90 % of the spores are broken, as determined by inspection under the phase microscope. This requires 60 to 90 s of vortexing (smaller quantities of conidia can be broken in shorter times). The ruptured spores are harvested by stirring with two additions of 3 to 4 ml of cold lysis buffer followed by sedimentation of the glass beads by gravity for a few minutes. The spores are harvested with the supernatant liquid. The purpose of the spermine buffer system is to maintain a compact nuclear morphology until lysis in detergent so as to minimize DNA shearing during the vortexing procedure. Sodium dodecyl sulphate (SDS) is added to the suspension of ruptured spores to a final concentration of 1 % (w/v). The lysate is heated to 60 °C for 20 min and then incubated with proteinase K (200 $\mu\text{g ml}^{-1}$, Boehringer) for 16 h (overnight) at 42 °C. The lysate is then deproteinized by gentle mixing with an equal volume of freshly distilled phenol equilibrated with 0.5 M-Tris/HCl pH 8.0. After centrifugation, the aqueous phase is removed and deproteinized again with phenol. The phenol is then removed by dialysis against 0.05 M-Tris/HCl pH 8.0, 10 mM- Na_2EDTA , 100 mM-KCl. RNAase A (preheated to 80 °C for 30 min) is added to a final concentration

Fig. 1. Comparison of the electrophoretic mobilities of *A. nidulans* spore and protoplast DNA with known molecular weight markers. From left to right: T7 DNA (2.6×10^7 daltons); λ DNA (3.2×10^7 daltons); 1 μ g protoplast DNA; 2 μ g protoplast DNA; 1 μ g spore DNA; 2 μ g spore DNA; λ DNA; T7 DNA. Electrophoresis was for 4 h at 40 V in 0.5 % (w/v) agarose (Seakem) with 40 mM-Tris/acetic acid pH 7.4, 2 mM-EDTA.

of 200 μ g ml⁻¹ and the lysate is incubated for 1 h at 42 °C. RNAase is removed by treatment with phenol and the phenol removed by dialysis. The volume of the DNA solution is then reduced by burying the dialysis bag in polyethylene glycol (Carbowax 4000, Union Carbide) for 30 to 60 min. This removes 50 to 75 % of the volume. A final dialysis is used to put the DNA into any desired storage buffer. We routinely store *Aspergillus* DNA at 4 °C in 20 mM-Tris/acetic acid pH 7.4, 2 mM-Na₂EDTA. Recovery of DNA is approximately 250 μ g DNA per g spores.

Large molecular weight DNA can also be made from protoplasts. Protoplasts are prepared by a modification of the method of de Vries (1974). *Aspergillus nidulans* is grown from an inoculum of 3×10^6 conidia ml⁻¹ for 12 to 14 h at 30 °C in Czapek-Dox broth (Difco), supplemented with growth factors as required, in a reciprocating incubator shaker (Controlled Environment Incubator Shaker, New Brunswick Scientific Co.) at 150 rev. min⁻¹. Drained mycelium (5 g) is resuspended at 25 mg ml⁻¹ in 0.5 M-MgSO₄, 0.05 M-sodium maleate buffer pH 5.8, and to this suspension is added *Trichoderma* lytic enzyme (de Vries, 1974) at 4 mg ml⁻¹ plus 1 % of a snail gut lytic enzyme preparation (Glusylase, Endo Labs, extensively dialysed against the above buffer). The suspension is shaken gently at 30 °C with a large surface/volume ratio (100 ml in a 500 ml flask). Under these conditions protoplast formation appears to be nearly complete in 1 to 2 h; however, incubation is continued for 10 h to allow the metabolically active protoplasts to develop large vacuoles. The protoplasts are then centrifuged at 10000 rev. min⁻¹ (16300g) for 10 min in the Sorvall HB-4 swinging bucket rotor to float vacuolated protoplasts, and the vacuolated protoplasts are harvested by skimming the surface with a spatula or with a Pasteur pipette. Typically 0.5 to 0.75 g vacuolated protoplasts can be obtained from 5 g mycelium. The skimmed protoplasts are diluted with 2 vol. of cold 0.5 M-KCl and sedimented by centrifugation at 10000 rev. min⁻¹ for 5 min in the HB-4 rotor. The protoplast pellet is washed twice with 30 ml of cold 0.5 M-KCl to remove residual MgSO₄. The protoplasts are suspended in 10 to 15 vol. of 50 mM-Tris/HCl pH 8.0, 50 mM-Na₂EDTA, SDS is immediately added to a final concentration of 1 % (w/v) and the lysis mixture is heated to 60 °C for 20 min. The remainder of the procedure is the same as for obtaining DNA from spores. The yield is about

450 μg DNA per g protoplasts. The molecular weight of the bulk of the DNA obtained from both spores and protoplasts by this method is about 3×10^7 (Fig. 1).

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REFERENCES

- HAUTALA, J. A., CONNER, B. H., JACOBSON, J. W.,
PATEL, G. L. & GILES, N. H. (1977). Isolation
and characterization of nuclei from *Neurospora*
crassa. *Journal of Bacteriology* **130**, 704-713.
- MORRIS, N. R. (1976). Nucleosome structure in
Aspergillus nidulans. *Cell* **8**, 357-363.
- VAN ETEN, J. & FREER, S. N. (1978). A simple
procedure for disruption of fungal spores. *Journal*
of Applied and Environmental Microbiology (in
the Press).
- DE VRIES, O. M. H. (1974). *Formation and cell wall*
regeneration of protoplasts from Schizophyllum
commune. Thesis, University of Groningen, The
Netherlands.