

On the isolation of DNA from *N. crassa* mycelium

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On the isolation of DNA from *N. crassa* mycelium

Abstract

Isolation of DNA

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On the isolation of DNA from N. crassa mycelium.

para Newsl. 7: 9). The second procedure used is that developed by Brandt and DeBusk (1964 Neurospora Newsl. 6: 17), which has yielded apparently pure preparations of native DNA.

We have applied these procedures to "young mycelium" obtained by incubating concentrated conidial suspensions in minimal medium (Wainwright and McFarlane 1965 Can. J. Biochem. 43: 1813). The product obtained by either procedure was grossly contaminated with material tentatively identified as carbohydrate, and was not adsorbed by nitrocellulose membranes used for hybridization studies. We have modified these two procedures to obtain material which is adsorbed by the nitrocellulose membranes, although not yet free of all material adsorbing in the ultraviolet.

In our experience, preparations obtained by the Marmur procedure are very grossly contaminated (Ratio of OD's 260/240 μ 1.3). On analysis by centrifuging in cesium chloride gradient as many as four distinct peaks, plus polydisperse background material, are observed (Figure 1 (a)). Much of the contaminating material is RNA which has survived the treatment with RNase presumably because of the presence of a potent inhibitor of the enzyme previously observed in other nucleic acid fractions (Wainwright and McFarlane, loc. cit.).

Considerable purification was achieved by removal of some of the carbohydrate before treatment with RNase. Crude DNA isolated by the Marmur procedure up to the stage of RNase treatment was dissolved in "standard saline citrate" (SSC) (100 ml per 100-500 gram wet weight of initial germinated conidia and incubated at 37°C for 30 mins. with 500 μ g/ml of a preparation of hemicellulase (Miles Chemical Co.). The mixture was deproteinized with chloroform-isoamyl alcohol and the DNA precipitated with two volumes of ethanol in the cold. For some preparations a second treatment with hemicellulase was required. The resulting precipitate was then treated with RNase according to Marmur and crude DNA precipitated with ethanol after deproteinizing. The crude DNA was re-dissolved in SSC and re-precipitated with 2-ethoxyethanol according to Kirby (1957 Biochem. J. 66: 495). A solution of the DNA in SSC adjusted to pH 6.0 was treated with 100 μ g/ml of chitinase for 45 min. at 37°C. deproteinized and re-precipitated with ethanol. The resulting precipitate was taken up in SSC, retreated with RNase (100 μ g/ml), chitinase (100 μ g/ml) plus RNase (250 μ g/ml), deproteinized and the DNA re-precipitated. Our best preparations obtained in this manner gave a single peak on centrifugation in cesium chloride gradients (Figure 1 (b)). The material was clearly partially degraded (as judged by band width) and still contaminated with material yielding a polydisperse background in the OD profile.

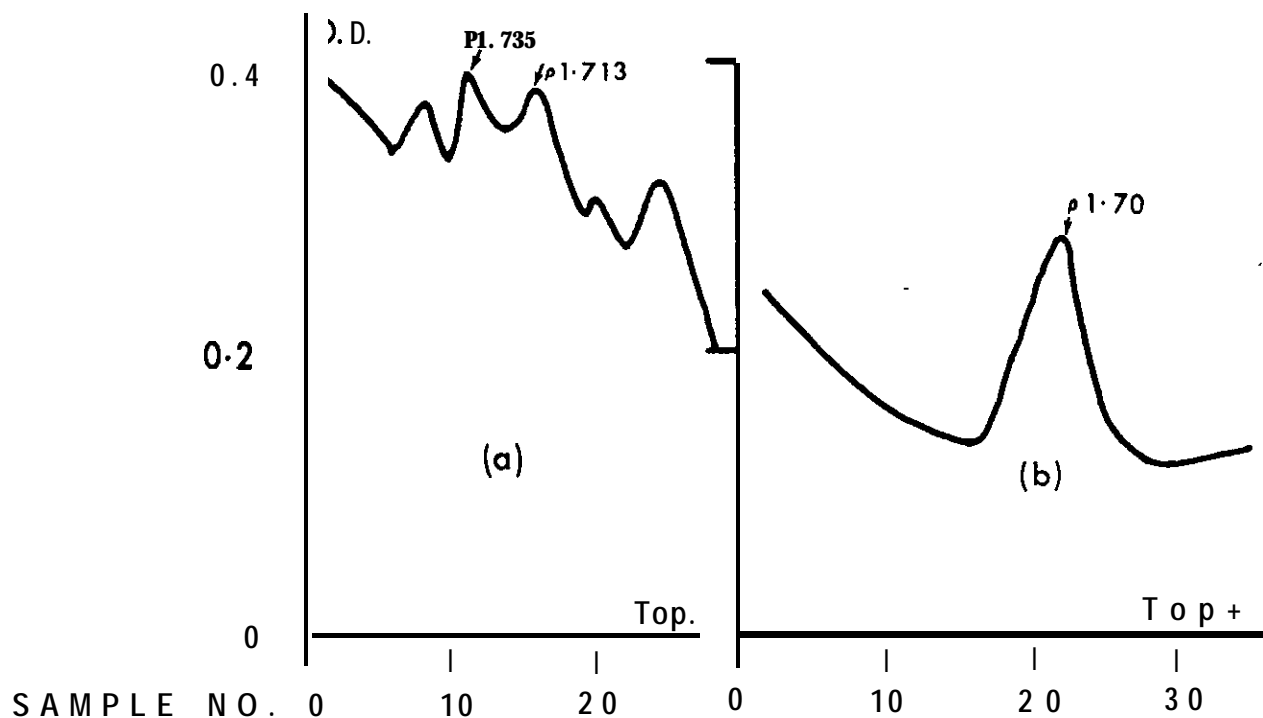


Figure 1. Optical density profiles at 260 μ for samples of DNA centrifuged in linear cesium chloride gradients at 18°C for 20 hrs. at 33,000 rpm in the Spinco SW 39 rotor and sampled by conventional procedures. (a) DNA isolated by the Marmur procedure; apparent quantity by OD at 260 and 280 μ 245 μ g. (b) Purified DNA 100 μ g.

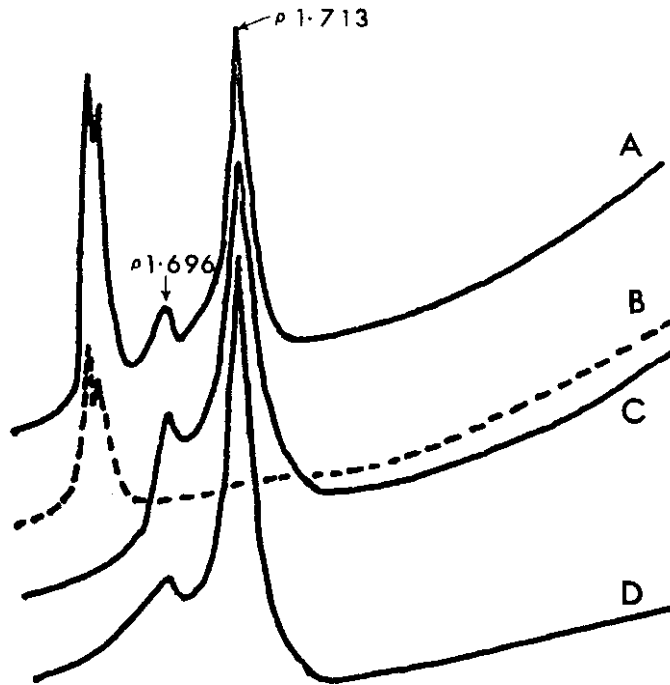


Figure 2. Optical density profiles at 260 $m\mu$ of DNA preparations centrifuged in cesium chloride gradients for ca. 20 hrs. at 20°C at 44,770 rpm in the Model E Spinco analytical centrifuge. A. 14 μ g "DNA" prepared according to Brandt and DeBusk. B. 5 μ g "DNA" preparation A after digestion with DNase. C. 12 μ g "DNA" preparation A after treatment with methoxyethanol and ethoxyethanol. D. 18 μ g DNA supplied by Brandt and DeBusk, to whom we express our thanks.

The product obtained by the procedure of Brandt and DeBusk contained two types of contaminant (Figure 2). After being dissolved in SSC, treated with methoxyethanol and precipitated with ethoxyethanol according to Kirby, the product showed only two major DNA peaks in cesium chloride gradients at buoyant densities agreeing with values given by Luck and Reich (1964 Proc. Natl. Acad. Sci. U. S. 52: 931) for *N. crassa* nuclear and mitochondrial DNA, respectively. We have, however, been unable to free our material of all contaminants contributing to the background of the optical density profile. - - - Department of Biochemistry, Dalhousie University, Halifax, Nova Scotia.