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On the isolation of DNA from N. crassa mycelium.

Two methods hove been reported to hove been used for the isolation of preparations of N. crassa DNA. The first of these is basically the general method developed by Marmur (1961 J. Mol. Biol. 3: 208). used by Marmur and by Dutta, McWhorter and Woodward (1965 Neurospora 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 hove applied these procedures to "young mycelium" obtained by incubating concentrated conidial suspensions in minimal medium (Woinwright 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 hove modified these two procedures to obtain material which is adsorbed by the nitrocellulose membranes, although not yet free of all moteriol adsorbing in the ultraviolet.

In our experience, preporations obtained by the Marmur procedure ore very grossly contaminated (Ratio of OD's 260/240 mμ 1.3). On analysis by centrifuging in cesium chloride gradient as many as four distinct peaks, plus polydisperse bockground moteriol, ore observed (Figure 1 (o)). Much of the contaminating moteriol is RNA which has survived the treatment with RNose presumably because of the presence of a potent inhibitor of the enzyme previously observed in other nucleic acid fractions (Wainwright ond 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 RNose treatment was dissolved in "standard saline citrate" (SSC) (100 ml per 100-500 gram wet weight of initial germinated conidio and incubated at 3PC for 30 mins. with 500 μg/ml of a preparation of hemicellulase (Miles Chemical Co.). The mixture was deproteinized with chloroform-isoamyl alcohol and the DNA precipitoted 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 RNose 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 μg/ml of chitinase for 45 min. at 37°C. deproteinized and re-precipitated with ethanol. The resulting precipitate was token up in SSC, retreated with RNase (100 μg/ml), chitinase (100 μg/ml) plus RNose (250 μg/ml), deproteinized ond the DNA re-precipitated. Our best preparations obtained in this manner gave a single peok on centrifugation in cesium chloride gradients (Figure 1 (b)). The material was clearly partially degmded (as judged by band width) and still contaminated with material yielding a polydisperse background in the OD profile.

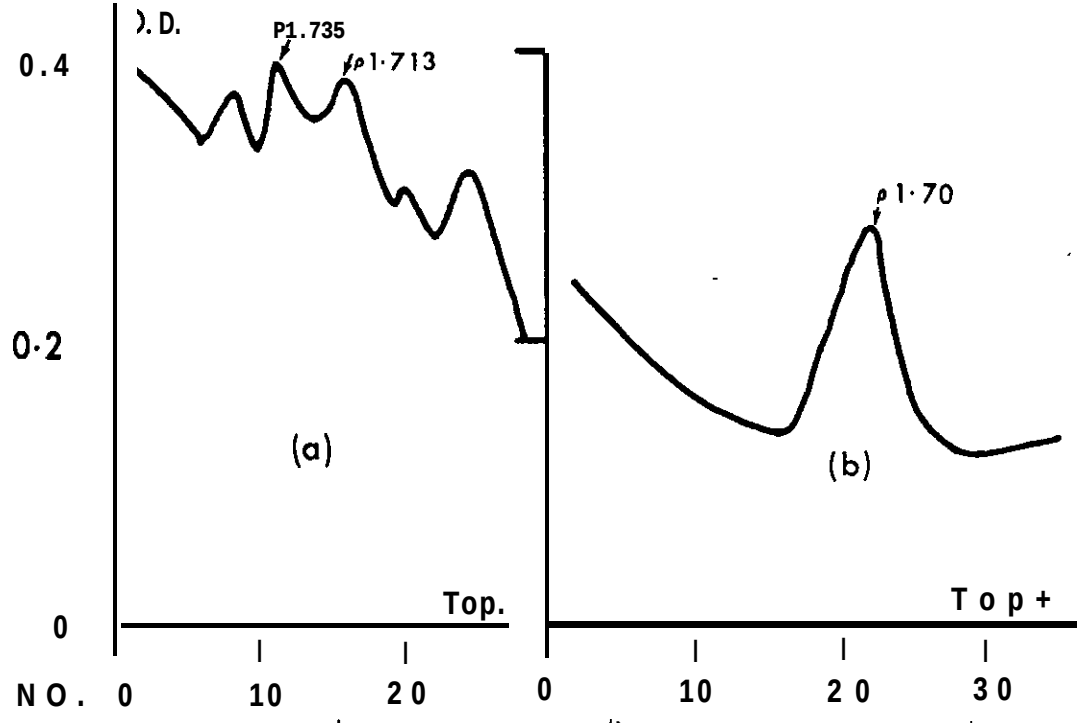

Figure 1. Optical density profiles at 260 mμ for samples of DNA centrifuged in linear cesium chloride gradients at 18°C for 20 hrs . at 33,000 rpm in the Spinco SW 39 rotor ond sampled by conventional procedures. (a) DNA isolated by the Marmur procedure; apparent quantity by OD at 260 and 280 mμ 245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

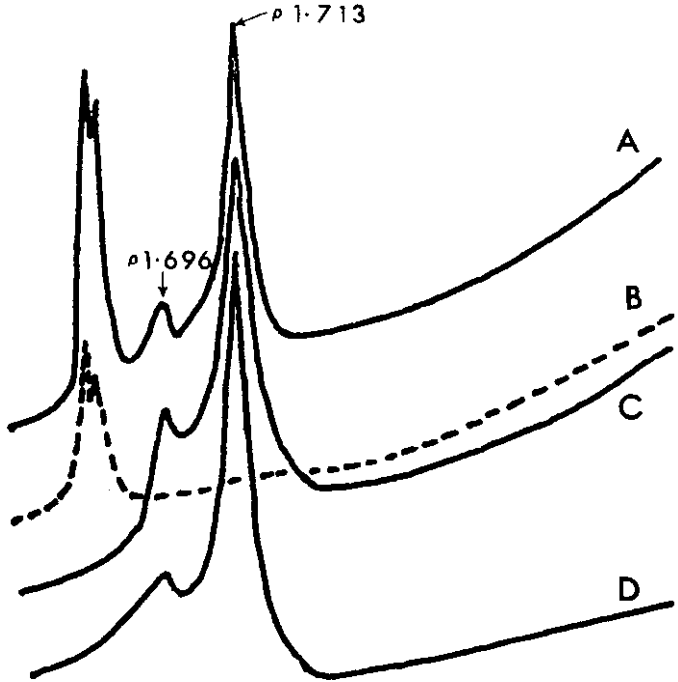

Figure 2. Optical density profiles at 260 mμ of DNA preparations centrifuged in cesium chloride gradients for ca. 20 hrs. at 20°C at 44,770 rpm in the Model E Spinco analytical centrifuge. A. 14 μg "DNA" prepared according to Brandt and DeBusk. B. 5 μg "DNA" preparation A after digestion with DNase. C. 12 μg "DNA" preparation A after treatment with methoxyethanol and ethoxyethanol. D. 18 μg 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 ond Reich (1964 Proc. Natl. Acad. Sci. U. S. 52: 931) for N. crassa nuclear ond mitochondrial DNA, respectively. We hove, however, been unable to free our material of all contaminants contributing to the background of the optical density profile. - - - Deportment of Biochemistry, Dalhousie University, Halifax, Nova Scotia.