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Experiences with Metzenberg's method for preparing Neurospora DNA.

Metzenberg and Baisch (1981 Neurospora Newsl. 28: 20) reported an easy method for preparing high molecular weight DNA from Neurospora crassa RL3-8 A. We confirmed their results: however, in our hands the nucleic acid prepared by Metzenberg's method contained large amounts of RNA and palysaccharides. To reduce contamination in our DNA preparation the original procedure was modifie ed as follows: (a) digestion with RNAse was extended to 3 hours; (b) final precipitation of DNA was performed with 0.54 volume of iso-propanol.

Since the product obtained in this way still contained equal amounts of DNA and RNA, as determined by the diphenylamine (Richards 1974 Anal. Biochem. 57:369)and phloroglucine (Dische and Barenfreund 1957 Biochim. Biophys. Acta 23: 639) methods, respectively, an additional treatment with RNAse was performed. The RNA content of the final product was less than 10%.

The DNA prepared either by the method of Metzenberg or according to the above modification contained about equal amounts of DNA and polysaccharides (w/w): the latter was determined with the anthron reagent (Roe 1955 J. Biol. Chem. 212: 335).

The molecular weight of DNA was determined by agarose gel electrophoresis (Aaij and Borst I972 Biochim. 'Biophys. Acta 269: 192) with λ-phage DNA used as standard, and was found to be about 49 kilobase pairs (kb). About 5% of the product were fragments of lower molecular weight (between 23 and 49 kb).

In conclusion, although Metzenberg's mthod provides an easy way for obtaining high molecular weight DNA, the DNA prepared in that way is contaminated to a significant degree with RNA and polysaccharides.

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