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# Isolation of Deoxyribonucleic Acid from Mycobacteria

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Mycobacteria are refractory organisms, and it has usually been necessary to resort to rigorous grinding to permit extraction of deoxyribonucleic acid (DNA) from these bacilli (H. Venner, Acta Biol. Med. Ger. **11**:806, 1963; M. Tsukamura et al., Am. Rev. Respirat. Diseases **81**:403, 1960). In addition to the obvious health hazards attending grinding of masses of pathogenic organisms, the grinding with glass powder or sonic treatment for the prolonged periods which are necessary to disrupt mycobacteria has deleterious effects on DNA (J. Marmur, J. Mol. Biol. **3**:208, 1961).

Our recent observations on autolysis of tubercle bacilli grown in glycerol-rich medium under strongly aerobic conditions and then abruptly subjected to oxygen limitation (L. G. Wayne and G. A. Diaz, J. Bacteriol. **93**:1374, 1967) have provided the basis for a relatively gentle, safe, and efficient procedure for obtaining mycobacterial DNA.

Sauton medium, with 1.5% agar, is dispensed in 40-ml amounts to 160-ml prescription bottles, autoclaved, and slanted. Inoculum from stock mycobacterial cultures is suspended in Sauton liquid medium which is distributed in 7-ml amounts over the agar slants, yielding a biphasic culture system. These cultures are incubated in a slanted position at 35 to 37 C for 3 days to 6 weeks, depending on growth rate. In this manner, it is possible to cultivate an average of about 1 g of wet cells per bottle. As soon as growth has become luxuriant, the slurry from several bottles of a given organism is pooled in a sterile 300-ml screw-capped centrifuge bottle containing a magnetic stirring bar. The slurry is adjusted to *p*H 7.5 and aerated vigorously on a magnetic stirrer at 37 C for 72 hr. The agitation is then stopped, and 10 $\mu$moles of sodium ethylenediaminetetraacetate and 1 mg of Pronase (Calbiochem, Los Angeles, Calif.) are added per ml of slurry. The bottle is transferred to a desiccator or anaerobic jar, the air is evacuated, and the bacilli are permitted to incubate anaerobically for 24 hr at 37 C. To the suspension is then added 0.1 volume of 5% sodium deoxycholate; the *p*H is again adjusted to 7.5, and the mixture is heated, with occasional gentle agitation, at 56 C for 90 min. At this point, the DNA has been liberated from the bacilli, and the further processing of the product is carried out by conventional means. In our laboratory, the crude mixture is cooled and gently rocked with an equal volume of phenol (K. S. Kirby, p. 5, *in* J. N. Davidson and W. E. Cohn [ed.], *Progress in Nucleic Acid Research and Molecular Biology*, Academic Press, Inc., New York, 1964) for 10 min and then centrifuged. The aqueous phase is carefully removed with a wide-bore pipette, and 2 volumes of 95% ethyl alcohol are layered over it. A glass rod is used to mix the two phases and wind the precipitated nucleic acid into a spool. This material is washed with several changes of 95% ethyl alcohol and then suspended in standard saline citrate (SSC), treated with ribonuclease (Sigma Chemical Co., St. Louis, Mo.), and subjected to further deproteinization and reprecipitation of DNA threads on a rod.

To date, this technique has been applied successfully to cultures of *Mycobacterium tuberculosis*, *M. kansasii*, *M. avium*, *M. gastri*, *M. flavescens*, *M. smegmatis*, *M. phlei*, and group II scotochromogenic mycobacteria (i.e., slow-growing mycobacteria that produce pigment in the dark) with an average yield of DNA (estimated by ultraviolet adsorption at 255 m$\mu$, its peak adsorption frequency) of 0.67 mg per bottle of culture, and a range of 0.16 to 2.10 mg. In five instances, diphenylamine assay was performed, and in those cases the results were within 90 to 100% agreement with those obtained by adsorption at 255 m$\mu$. Formic acid hydrolysis and paper chromatography of the DNA has demonstrated the presence of guanine, cytosine, adenine, and thymine, but not uracil.

In one experiment, three bottles of biphasic culture of *M. tuberculosis* were harvested separately. The packed cell volumes harvested were 0.5, 0.75, and 1.25 ml, and these yielded 0.45, 0.72, and 1.26 mg of DNA, respectively, for an average of 0.96 mg of DNA per ml of wet packed cells. The ratio of optical density in SSC at 255 m$\mu$ to that at 280 m$\mu$ averaged 1.96. Sedimentation velocities were determined on two of these samples at a concentration of 20 $\mu$g/ml in SSC and were found to have values of 21*S* and 25*S*, corresponding to molecular weights of $6 \times 10^6$ and $10 \times 10^6$, respectively. The molecular weights

were determined from uncorrected values according to the formula of Burgi and Hershey (J. Mol. Biol. **3**:458, 1961).

We have also studied thermal denaturation of this DNA in the low ionic strength buffer of Bohacek et al. (J. Gen. Microbiol. **46**:369, 1967) and noted a hyperchromic rise in optical density of 26.6% with a $T_m$ of 80.2 C, corresponding, according to the formula of J. Bohacek et al., to a guanine plus cytosine ratio of 64.8%. Hydrolysis of triplicate samples in formic acid at 180 C, chromatography in *n*-butyl alcohol-ammonia, and elution with 0.1 N HCl yielded a guanine plus cytosine ratio of 65.0 ± 0.4%. Buoyant density in CsCl was found to be 1.724 g/cc, corresponding to a guanine plus cytosine ratio of 64.9%, with *Escherichia coli* DNA at 1.710 g/cc as reference.

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