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Method for Isolation of Deoxyribonucleic Acid from Mycobacteria

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A relatively gentle and efficient procedure for obtaining deoxyribonucleic acid from mycobacteria by the use of glycine and lysozyme is described.

For extracting deoxyribonucleic acid (DNA) from mycobacteria, the only gentle method known which did not disrupt cells mechanically was that reported by Wayne and Gross (5). However, they reported that more than 4 days are required to induce cell autolysis. For certain experimental purposes, such as labeling DNA with radioisotopes, it is desirable that DNA extraction be completed within a shorter period of time.

We previously reported that lysis of mycobacteria by lysozyme was induced when the cells were pretreated with 0.2 M glycine, and that spheroplasts were produced by the treatment of glycine and lysozyme in the presence of 15% sucrose (2). These observations provided the idea for a relatively gentle, quick, and efficient procedure for obtaining mycobacterial DNA.

Mycobacterium smegmatis ATCC 607, grown on Ogawa's egg slant, was inoculated in Heart Infusion Broth (HIB; Difco) supplemented with 0.06% Tween 80 (Atlas Chemical Industries, Inc., Wilmington, Del.), and incubated overnight at 37 C on a shaker. In this manner, it is possible to cultivate an average of about 1 g of wet cells per 500 ml of HIB. Glycine was added to the growing culture at a final concentration of 0.2 M, and the mixture was shaken for an additional 2 hr at 37 C. The cells were then collected by centrifugation and resuspended in one-tenth volume of 0.15 M NaCl plus 0.1 M ethylenediaminetetraacetate (EDTA), *p*H 8. DNA extraction was carried out by a modification of the method of Saito and Miura (3). Lysozyme (grade I, Sigma Chemical Co., St. Louis, Mo.) was added to the suspension at a final concentration of 200 μg/ml and incubated for about 60 min at 37 C with gentle shaking.

Cell lysis was followed by noting the increase of viscosity and the decrease of optical density. The lysed suspension was shaken with an equal volume of phenol saturated with 0.1 M tris(hydroxymethyl)aminomethane buffer plus 1% sodium dodecyl sulfate plus 0.1 M NaCl (*p*H 9) and was then centrifuged. The aqueous phase was collected, and two volumes of ethyleneglycolmonoethylether (Wako Junyaku, Tokyo, Japan) were added to precipitate the crude DNA. Phenol extraction and DNA precipitation were then repeated in the intermediate layer in the centrifuge tube. The DNA spool on a glass rod was washed with ethanol and dissolved in a dilute saline citrate (0.015 M NaCl plus 0.0015 M sodium citrate). One-tenth volume of concentrated saline citrate (1.5 M NaCl plus 0.15 M sodium citrate) was added to the solution. Ribonuclease (Sigma Chemical Co.) was added to the solution at a final concentration of 50 μg/ml, and the resulting suspension was subjected to further deproteinization and reprecipitation.

In one experiment, 0.67 mg of the DNA estimated by ultraviolet absorption (λ = 260 nm) was obtained from 1 g of wet cells. The ratio of optical density in standard saline citrate at 260 and 280 nm was 1.92. Sedimentation coefficient of the DNA at a concentration of 20 μg/ml in standard saline citrate had a value of 21.7*S*, corresponding to molecular weight of 8.4×10^6 (1). Bouyant density in CsCl and thermal denaturation of the DNA of this bacteria were reported previously (4).

This technique has been applied successfully to *M. phlei* and the other rapidly growing mycobacteria. Instead of glycine, 150 to 200 μg of cycloserine per ml was also found to be useful.

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