SHORT COMMUNICATION

DNA Restriction Endonuclease Analysis of *Mycobacterium tuberculosis* and *Mycobacterium bovis* BCG

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DNA preparations from two reference (H37Ra and H37Rv) and two wild strains of *Mycobacterium tuberculosis* and one re-isolated strain of *Mycobacterium bovis* BCG were analysed using 17 restriction endonucleases. The enzyme *Bst*EII revealed the greatest differences between strains. Electrophoretic DNA patterns from the wild *M. tuberculosis* strains differed from each other and from the reference strains at relatively few positions. At the highest resolution attained, patterns from the two reference strains remained indistinguishable from each other. The pattern of the *M. bovis* BCG strain was substantially different from, but had many bands in common with, the *M. tuberculosis* patterns.

INTRODUCTION

The classification and identification of mycobacteria has presented many problems that have hindered genetic and epidemiological studies. In particular, typing of *Mycobacterium tuberculosis* has been difficult to achieve because the species appears to be a single homogeneous serogroup (Jones & Kubica, 1968) and strains exhibit a high degree of taxonomic similarity (Wayne, 1981). Phage typing is the only approach that has shown any promise in subdividing the species (Grange, 1982; Jones *et al.*, 1982).

Restriction endonuclease analysis has recently been applied successfully to the typing of bacteria (Marshall *et al.*, 1981; Kaper *et al.*, 1982). In this technique, high molecular weight DNA is digested with a restriction enzyme and the fragments produced are separated by agarose electrophoresis. Strains are characterized and compared on the basis of their DNA fragment patterns. We have now applied this technique to *M. tuberculosis* and *M. bovis* BCG.

METHODS

Mycobacteria. *Mycobacterium tuberculosis* strains H37Rv and H37Ra were obtained from the National Institutes of Health, Bethesda, Md., USA; *M. tuberculosis* strains T1 and T2 were isolated from patients with tuberculosis. *Mycobacterium bovis* BCG was isolated from a patient with an abscess at a BCG inoculation site. The classification of the strains was confirmed by standard biochemical tests (Vestal, 1975) and also, in the case of *M. bovis* BCG, by lack of virulence to guinea pigs.

Preparation of DNA. Strains were cultured in 200 ml Tween/albumin broth (Vestal, 1975) at 37 °C for 6–10 weeks. Cultures were killed by heating at 70 °C for 15 min, centrifuged at 5000 *g* for 30 min, and washed three times with 0.15 M-phosphate-buffered saline pH 7.3. The final pellet was suspended in 1 ml buffer consisting of 100 mM-EDTA and 100 mM-Tris/HCl pH 8.5. DNA was extracted by the method of Marshall *et al.* (1981), modified as follows: cells were treated with 1 mg lysozyme ml⁻¹ instead of 0.3 mg ml⁻¹, lysed in 1% sodium dodecyl sulphate instead of 0.75%, and after lysis the solution was made 0.7 M in perchlorate instead of 1 M. The DNA content of each preparation was measured by fluorimetry (Le Pecq & Paoletti, 1966).

Restriction endonuclease analysis. Samples of mycobacterial DNA (2 µg) were digested with 20 units of each of the following restriction enzymes, using the temperature and buffer specified by the supplier: *Apa*I, *Bam*HI, *Bcl*I, *Bgl*I, *Bst*EII, *Bst*XI, *Eco*RI, *Hind*III, *Kpn*I, *Nae*I, *Pst*I, *Sac*I, *Sal*I, *Sma*I, *Tth*III, *Xba*I, and *Xho*I. Restriction

Fig. 1. Fragment patterns after *Bst*EII digestion of mycobacterial DNA. 1, *M. tuberculosis* H37Rv; 2, *M. tuberculosis* H37Ra; 3, *M. tuberculosis* T1; 4, *M. tuberculosis* T2; 5, *M. bovis* BCG. Lane 6 contains a *Bst*EII digest of λ phage with fragment sizes (kb) given on the right.

enzymes were obtained from New England Biolabs except for *Bam*HI, *Sal*I and *Sma*I which were obtained from Sigma. Digests were analysed by gel electrophoresis on 20 cm long horizontal slabs of 0.7% agarose (Bio-Rad; ultrapure DNA grade) in a 30 cm long electrophoresis tank. Gels were run at 90 V for 4 h immersed in a buffer consisting of 40 mM-Tris/acetate, 5 mM-sodium acetate, and 1 mM-EDTA pH 7.8. In later high resolution electrophoresis, 26 cm long gels of 1% agarose were used, the gels were run for 18 h at 70 V, and the buffer was re-circulated from the anode to the cathode compartment at 8 ml min⁻¹ using a peristaltic pump. After electrophoresis the gels were stained for 30 min in ethidium bromide (0.5 µg ml⁻¹), placed on an ultraviolet trans-illuminator, and photographed on Kodak Tri-X film by means of a 120 format plate camera with a Wratten 23A gelatin filter.

RESULTS AND DISCUSSION

Harvested mycobacterial cells weighed 0.4–1.2 g wet weight and yielded 12–75 µg DNA in a final volume of 2–3 ml. Restriction enzymes giving the clearest fragment patterns were *Bcl*I, *Bgl*I, *Bst*EII, *Eco*RI, *Pst*I, *Sma*I and *Xho*I. Of these, *Bst*EII digests gave the best differentiation.

The patterns were highly reproducible, and DNA harvested from separate cultures of the same strain and cut with the same enzyme gave identical results.

On the 20 cm long 0.7% agarose gels, each *Bst*EII digest displayed a dense sequence of DNA fragments from a size of 8 kb to below 1 kb where the pattern became indistinct. On these gels, differences in patterns between strains were only apparent in the region above 6 kb. The resolution of the larger DNA fragments was markedly improved by increasing the electrophoresis running time. The use of a much longer running time necessitated other modifications to the electrophoresis system, including the introduction of buffer recirculation to maintain a relatively constant pH across the gel. In this high resolution system, RNA and all DNA fragments with sizes less than 1.6 kb travelled off the end of the gel. The patterns obtained using 3.5 µg DNA samples are shown in Fig. 1.

The most noticeable feature of the DNA fragment patterns with the five strains studied was their general similarity. All strains had many fragments of apparently identical size, particularly below 4 kb in length. The patterns for strains H37Rv and H37Ra were indistinguishable. The T1 pattern was very similar to the H37 patterns but differed at fragment sizes of 9.7–10.4, 7 and 4.2 kb. The T2 pattern was different in several regions from both the T1 and H37 patterns, but was more similar to them than to the *M. bovis* BCG pattern.

Restriction endonuclease analysis successfully characterized strains of *M. tuberculosis* and *M. bovis* BCG and gave an indication of how closely these strains are related. The technique should therefore be useful in studying the epidemiology of *M. tuberculosis* and may find application in the wider field of mycobacterial taxonomy.

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