Deoxyribonucleic Acid of *Mycobacterium lepraemurium*: Its Genome Size, Base Ratio, and Homology with Those of Other *Mycobacteria*

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Deoxyribonucleic acid of *Mycobacterium lepraemurium* grown in mice was isolated and analyzed spectrophotometrically. The genome molecular weight and guanine-plus-cytosine content of this *M. lepraemurium* deoxyribonucleic acid were 1.8×10^9 and 65.5 mol%, respectively. Among selected strains of mycobacterial species, four strains of *Mycobacterium avium* showed the highest degree of deoxyribonucleic acid homology (74.2 to 92.9%) with *M. lepraemurium*, suggesting a close genetic relatedness.

Although *Mycobacterium lepraemurium* is difficult to cultivate in vitro, analyses of masses of this bacterium produced in mice have indicated that the ultrastructure of its cell wall and the chemical structure of a mycolic acid-arabinogalactan-peptidoglycan complex are characteristic of *Mycobacterium* (1-3, 7, 15). Additional evidence for interrelatedness between *M. lepraemurium* and other mycobacteria has come from serological cross-reactions (4, 8, 11, 14) and patterns of biochemical tests (13) established for organisms grown in vivo or in vitro or both. However, until now there has been no information concerning the deoxyribonucleic acid (DNA) of *M. lepraemurium*: the size of its genome, its guanine-plus-cytosine (G+C) content, or the extent of homology between its DNA and DNAs of other mycobacteria. In this paper we provide such information derived from spectrophotometric studies of DNA from *M. lepraemurium*, both free and in association with DNAs from other species of *Mycobacterium*.

The bacterial strains used in this study and their sources and culture media are listed in Table 1. The preparation of DNA samples from bacterial cells grown in vivo or in vitro, the determination of the melting temperature for calculation of the G+C content, and the conditions for DNA-DNA reassociation for estimation of the genome size and DNA homology were as described recently (6). Briefly, DNA samples obtained by phenol-chloroform extraction were further treated with cetyltrimethylammonium bromide to remove the polysaccharides which interfere with DNA-DNA reassociation (5). The cetyltrimethylammonium bromide treatment was repeated until the ratio of absorbance

at 260 nm to absorbance at 230 nm of a DNA solution in $0.1 \times \text{SSC}$ (0.015 M NaCl plus 0.0015 M sodium citrate) was greater than 2.0. The purified DNA samples were dissolved in $0.1 \times \text{SSC}$, sheared by passage either through a 26-gauge needle or through a Ribi cell fractionator at $15,000 \text{ lb/in}^2$, and stored at -20°C . The melting temperatures of various DNA samples in $0.1 \times \text{SSC}$ and the $C_{0t_{0.5}}$ values in $6 \times \text{SSC}$ containing 25% (vol/vol) formamide were determined spectrophotometrically with a Gilford model 2600 system equipped with thermal cuvettes and a thermoprogrammer. The DNA of *Escherichia coli* K-12 ATCC 23724 was used as a standard (genome molecular weight, 2.5×10^9).

Table 1 shows the results of a comparison of a DNA sample from *M. lepraemurium* with the DNAs of various species representing *M. tuberculosis* and nontuberculous mycobacteria. With the exception of *M. leprae*, the G+C contents of the strains examined ranged from 61.7 to 74.1 mol%. The genome molecular weights ranged from 1.7×10^9 to 3.0×10^9 . Strains characterized by slowness of growth in vitro contained smaller genomes (e.g., *M. bovis* BCG and *M. lepraemurium*). In this connection, reference should be made to our previous observation that the genome molecular weight of *M. leprae* grown in armadillos is 1.3×10^9 (Table 1). Therefore, a relationship may exist between genome size and biosynthetic ability as reflected by rate of replication in vitro.

Of the mycobacterial strains tested, *M. leprae* showed the lowest degree of DNA homology with *M. lepraemurium*, as reported previously (6). In contrast, four strains of *M. avium* showed

TABLE 1. Genome size, base ratio, and DNA homology between *M. lepraemurium* and selected mycobacteria

Organism ^a	Genome mol wt ($\times 10^9$) ^b	G+C ratio (mol %) ^c	Homology with <i>M. lepraemurium</i> (%) ^b
<i>M. lepraemurium</i> Hawaiian	1.8 $\pm$ 0.1 ^d	65.5 $\pm$ 0.0 ^d	100
<i>M. avium</i> TMC 701 ^e	2.6 $\pm$ 0.1	74.1 $\pm$ 0.1	92.9 $\pm$ 0.8
<i>M. avium</i> TMC 706	2.3 $\pm$ 0.1 ^d	71.5 $\pm$ 0.1 ^d	87.7 $\pm$ 3.1
<i>M. avium</i> TMC 721	2.6 $\pm$ 0.1	73.9 $\pm$ 0.1	91.7 $\pm$ 2.8
<i>M. avium</i> ATCC 19421 ^f	2.1 $\pm$ 0.0	69.6 $\pm$ 0.1	74.2 $\pm$ 4.1
<i>M. intracellulare</i> TMC 1403	2.5 $\pm$ 0.0	65.3 $\pm$ 0.1	53.7 $\pm$ 1.7
<i>M. intracellulare</i> TMC 1406	2.0 $\pm$ 0.0 ^d	68.6 $\pm$ 0.0 ^c	44.1 $\pm$ 4.3
<i>M. scrofulaceum</i> TMC 1302	2.8 $\pm$ 0.0	66.1 $\pm$ 0.1	50.5 $\pm$ 2.2
<i>M. scrofulaceum</i> TMC 1323	2.4 $\pm$ 0.1 ^d	66.1 $\pm$ 0.0 ^d	48.8 $\pm$ 0.8
<i>M. tuberculosis</i> TMC 201	2.0 $\pm$ 0.0	63.3 $\pm$ 0.1	51.1 $\pm$ 2.2
<i>M. tuberculosis</i> TMC 327	2.1 $\pm$ 0.1 ^d	63.3 $\pm$ 0.0 ^d	55.9 $\pm$ 0.5
<i>M. tuberculosis</i> ^g	2.1 $\pm$ 0.0	63.5 $\pm$ 0.1	48.5 $\pm$ 3.6
<i>M. kansasii</i> TMC 1204	3.0 $\pm$ 0.0 ^d	68.8 $\pm$ 0.0 ^d	46.3 $\pm$ 2.5
<i>M. marinum</i> TMC 1218	2.5 $\pm$ 0.1 ^d	67.9 $\pm$ 0.1 ^d	49.8 $\pm$ 3.0
<i>M. bovis</i> TMC 410	2.9 $\pm$ 0.0	68.7 $\pm$ 0.0	37.6 $\pm$ 1.5
<i>M. bovis</i> TMC 1011 (BCG)	1.9 $\pm$ 0.0	65.9 $\pm$ 0.1	37.3 $\pm$ 3.7
<i>M. vaccae</i> TMC 1526	2.5 $\pm$ 0.1	68.5 $\pm$ 0.1	34.7 $\pm$ 3.8
<i>M. gordonae</i> TMC 1324	2.8 $\pm$ 0.1 ^d	63.3 $\pm$ 0.0 ^d	31.0 $\pm$ 2.3
<i>M. chelonae</i> TMC 1544	2.5 $\pm$ 0.0 ^d	61.7 $\pm$ 0.1 ^d	29.5 $\pm$ 1.4
<i>M. fortuitum</i> TMC 1529	2.7 $\pm$ 0.1	68.1 $\pm$ 0.1	39.2 $\pm$ 3.6
<i>M. smegmatis</i> ATCC 607	3.0 $\pm$ 0.1	68.5 $\pm$ 0.0	48.9 $\pm$ 2.5
<i>M. leprae</i>	1.3 $\pm$ 0.0 ^d	55.8 $\pm$ 0.1 ^d	20.2 $\pm$ 2.3 ^d

^a *M. lepraemurium* was isolated from spleens and mesenteric nodules of infected Swiss white mice as previously described (6, 7). *M. leprae* was isolated from spleen tissues of two armadillos infected with *M. leprae* of human origin. The infected tissues were prepared at the National Hansen's Disease Center and were supplied through the National Institute of Allergy and Infectious Diseases. Other mycobacterial strains were grown in Middlebrook 7H9 medium (Difco Laboratories) supplemented with 0.5% bovine serum albumin fraction V (Sigma Chemical Co.).

^b Average of six assays.

^c Average of three to six assays.

^d Data from reference 6.

^e TMC, Trudeau Collection of Certified Mycobacteria Mycobacterial Culture Collection (9). These strains were prepared at the National Jewish Hospital and were supplied through the National Institute of Allergy and Infectious Diseases.

^f ATCC, American Type Culture Collection, Rockville, Md.

^g Isolated from a patient with pulmonary tuberculosis in the University of Medicine and Dentistry of New Jersey Hospital.

the highest degrees of DNA homology with *M. lepraemurium*, ranging from 74.2 to 92.9%. Such a close genetic relatedness between *M. avium* and *M. lepraemurium* has already been suggested on the basis of common antigens (4, 14). However, other members of the *M. avium*-*M. intracellulare*-*M. scrofulaceum* complex showed significantly lower degrees of DNA homology with *M. lepraemurium*. Although the G+C content of *M. lepraemurium* is lower than the G+C contents of *M. avium* strains, the present study suggests that these organisms belong to the same subgroup within the *M. avium*-*M. intracellulare*-*M. scrofulaceum* complex.

Three strains of *M. tuberculosis*, which reportedly show the highest similarity in various biochemical properties to *M. lepraemurium* grown in vitro (13), are not closely related to the

latter as far as DNA homology is concerned. This disagreement may be due to the phenotypic expression of *M. lepraemurium* grown in vitro or to the difference in strains used. Since our attempts to grow *M. lepraemurium* in both egg yolk medium (12) and NC-5 liquid medium (10) have been unsuccessful, a comparative analysis of the DNAs contained in this organism grown in vitro and in vivo was not possible in the present study.

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REPRINT REQUESTS

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