Genome Size of \textit{Mycoplasma genitalium} \\
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The genome size of \textit{Mycoplasma genitalium} was determined by using restriction enzymes that infrequently cut its DNA. The calculated value of 577 to 590 kilobases is one-fourth smaller than the genome of \textit{Mycoplasma pneumoniae}, which is considered among the smallest genomes of self-replicating organisms.

\textit{Mycoplasma genitalium} is a newly discovered species isolated in 1980 from the urethras of males with nongonococcal urethritis (22, 23). Recently, it was also identified in the throat swab specimens of pneumonia patients as a coaggregate of \textit{Mycoplasma pneumoniae} (2). Morphological, biochemical, serological, and genetic studies indicate that these two mycoplasma species share common biological features. Both organisms require cholesterol, ferment glucose, exhibit immunologic cross-reactivity, and are flask shaped with a distinct tiplike organelle (12, 22, 23). The major cytoadhesin, a 170-kilodalton (kDa) protein in \textit{M. pneumoniae} (1, 19), and a corresponding 140-kDa protein in \textit{M. genitalium} (5, 16) concentrate at the tip (1, 7, 8, 9), share epitopes (15), and possess homologous sequences at the DNA and protein levels (5, 6). However, differences between these two mycoplasmas have also been observed. Electrophoretic patterns of digested genomic DNA from each species indicate genetic heterogeneity (3). The G+C content of the 170-kDa adhesin gene of \textit{M. pneumoniae} is 53.5\%, while that of the 140-kDa adhesin gene of \textit{M. genitalium} is 40\% (5). Comparisons of codon usage between the two mycoplasmas reveal a preferential usage of A- and T-rich codons by \textit{M. genitalium} (5). Furthermore, these organisms differ dramatically in their acquisition of human lactoferrin, as reflected in the saturable and specific binding of lactoferrin by \textit{M. pneumoniae} and the total absence of such binding by \textit{M. genitalium} (20).

Mycoplasmas are considered the smallest free-living organisms and have a circular double-stranded DNA with a molecular size of about 500 megadaltons (13, 14, 16). Their genomes are also relatively low in guanine and cytosine (G+C) content, and \textit{M. pneumoniae} and \textit{M. genitalium} have G+C levels of 40 and 32\%, respectively (5).

The genome of \textit{M. pneumoniae} has been selected as a model for studying the minimal amount of genetic information required for a self-replicating biological system (13). Wenzel and Herrmann (24) screened an \textit{M. pneumoniae} cosmid library, analyzed 32 overlapping cosmid clones covering a continuous DNA stretch of 720 kilobases (kb), and estimated the total genomic length of \textit{M. pneumoniae} to be about 800 kb. Other physical mapping approaches such as the use of infrequent-cutting restriction enzymes and pulsed-field gel electrophoresis analysis have provided similar estimates (D. C. Krause and C. B. Mawn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989, G31, p. 153). These data are comparable with earlier results based on DNA renaturation kinetics and electron microscopy (14, 16).

Because of the biological-chemical similarities and virulence potential of \textit{M. pneumoniae} and \textit{M. genitalium}, we examined the genome structure of \textit{M. genitalium} G37. To find infrequent-cutting restriction enzymes, we digested 8 \mu g of \textit{M. genitalium} chromosomal DNA with various restriction enzymes and then separated the DNA by electrophoresis on 0.75\% agarose gel. This technique also allowed the accurate size estimate of small DNA fragments. Figure 1 presents selected restriction enzyme digestion patterns representing the \textit{M. genitalium} genome. \textit{BamHI} generated fragments mostly in the 8- to 50-kb range. \textit{EcoRI} digested more often, and fragments ranged from 2.5 to 30 kb. \textit{HindIII} generated small fragments, all below 9.5 kb. In contrast, three other enzymes with GC-rich recognition sequences, \textit{SacI}, \textit{SacII}, and \textit{SmaI}, all cut \textit{M. genitalium} DNA infrequently. \textit{SacI} generated at least two small fragments of 3.5 and 9.5 kb, with the rest of the fragments in the high-molecular-weight range. \textit{SacII} produced two small fragments of 5 and 12.2 kb and several high-molecular-weight bands. All fragments generated by \textit{SmaI} were larger than 20 kb.

On the basis of the initial screening results, restriction enzymes \textit{ApaI}, \textit{MluI}, \textit{NarI}, \textit{NraI}, \textit{SacI}, \textit{SacII}, and \textit{SmaI} that cut the \textit{M. genitalium} DNA infrequently were used to analyze the genome size. \textit{M. genitalium} was grown in 32-oz (950-ml) glass prescription bottles containing 70 ml of SP4 medium (21) at 37°C for 3 days before harvesting (15). Cells were scraped from the glass, pelleted by centrifugation, washed twice with phosphate-buffered saline buffer (10 mM sodium phosphate [pH 7.2]-0.1 M NaCl), and frozen at −70°C. The methods of Jackson and Cook (11) were used to prepare agarose-encapsulated intact genomic DNA. Mycoplasma pellets from 12 bottles were suspended in 0.8 ml of phosphate-buffered saline buffer and warmed to 37°C. Then, 0.4 ml of 1.6\% agarose (SeaKem low-gelling-temperature agarose; FMC Corp., Rockland, Maine) prewarmed at 42°C was added and mixed, 0.4 ml of prewarmed mineral oil was added, and the mixture was vortexed for 30 s at top speed and chilled in ice for 5 min to allow the agarose to gel. The mycoplasma-containingagarose beads were washed extensively to remove mineral oil and unencapsulated mycoplasmas. The beads were suspended in 0.25 M EDTA (pH 8.0)-1% sodium dodecyl sulfate and digested with 2 mg of protease K (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml at 50°C for 24 h. Then, the beads were washed extensively with buffer (10 mM Tris hydrochloride [pH 8.0]-10 mM EDTA) containing 2 mM of phenylmethylsulfonyl fluoride, rinsed three times with the same buffer, and kept at 4°C. Agarose-encapsulated \textit{M. pneumoniae} organisms were prepared similarly for comparative purposes.

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Restriction enzymes were purchased either from Bethesda Research Laboratories, Gaithersburg, Md. or from New England BioLabs, Inc., Beverly, Mass. DNA-containing agarose beads were equilibrated with appropriate buffers, digested with various restriction enzymes, and separated on 0.8% agarose gels in 0.5× TBE buffer (1× TBE is 90 mM Tris–45 mM boric acid–0.5 mM EDTA). A programmable power inverter (PPI-200; M.J. Research Inc., Cambridge, Mass.) with a preset program was used to regulate electrophoresis. Gels were run at room temperature at 7 V/cm for 20 to 24 h with buffer recirculation. High-molecular-weight DNA markers and megabase DNA standards were purchased from Bethesda Research Laboratories. λ DNA concatamers were prepared by ligating λ bacteriophage DNA with T4 DNA ligase (4). Figure 2 shows DNA band patterns after digestion with selected restriction enzymes and separation by using preset program 4 of the power inverter.

Under these experimental conditions, the program permitted clear separation of DNA fragments from 10 to 200 kb. Fragments larger than 200 kb required program 5 or 7 to obtain correct molecular sizing (data not shown). ApaI cut the M. genitalium genome into 3 pieces of 500, 65, and 18 kb, indicating a total genome size of about 583 kb. MluI cut the genome into 10 pieces, and NraI cut the genome into 3 pieces. The results of the analysis are summarized in Table 1. Thus, from the size of restriction fragments generated by different enzymes, we calculated the genome of M. genitalium to be about 577 to 590 kb (4×10^6 daltons). Using similar methodology and restriction enzymes Rsrl, ApaI, NarI, and NraI, we calculated the genome of M. pneumoniae to be about 780 kb (data not shown), which is consistent with earlier reports (14, 16; Krause and Mawn, Abstr. Annu. Meet. Am. Soc. Microbiol. 1989). Therefore, the M. genitalium genome is one-fourth smaller than that of M. pneumoniae and is at the low end of the minimal genome size for a free-living organism (13, 14). The difference in genome size between M. pneumoniae and M. genitalium could be explained in part by the existence of multiple copies of many repeated sequences in M. pneumoniae (17, 25). Some of these repeated gene families share extensive homology with the major cytadhesin gene (P1) of M. pneumoniae. In contrast, the corresponding M. genitalium cytadhesin gene (140-kDa protein) has many fewer copies of repeat sequences (unpublished results). These data further support the distinct species designation of M. genitalium and its uniqueness as a human pathogen.

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### LITERATURE CITED
