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Physical and Genetic Mapping of the Genomes of Five *Mycoplasma hominis* Strains by Pulsed-Field Gel Electrophoresis

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We present the complete maps of five *Mycoplasma hominis* genomes, including a detailed restriction map and the locations of a number of genetic loci. The restriction fragments were resolved by field inversion gel electrophoresis or by the contour-clamped homogeneous-electric-field system of pulsed-field gel electrophoresis. All the *Apa*I, *Sma*I, *Bam*HI, *Xho*I, and *Sal*I restriction sites (total of 21 to 33 sites in each strain) were placed on the physical map, yielding an average resolution of 26 kb. The maps were constructed using three different approaches: (i) size determination of DNA fragments partially or completely cleaved with one or two restriction enzymes, (ii) hybridization analysis with purified restriction fragments and specific probes, and (iii) use of linking clones. A genetic map was constructed by hybridization with gene-specific probes for *rpoA*, *rpoC*, *rrn*, *tuf*, *gyrB*, *hup*, *ftsY*, the *unc* operon, the genes for two *M. hominis*-specific antigenic membrane proteins, and one gene encoding a protein with some homology to *Escherichia coli* alanyl-tRNA synthetase. The positions of mapped loci were partially conserved in the five strains except in one strain in which a 300-kb fragment was inverted. The numbers and order of mapped restriction sites were only partly conserved, and this conservation was restricted to certain regions. The gene order was compared with the gene order established for other bacteria and was found to be identical to that of the phylogenetically related *Clostridium perfringens*. The genome size of the *M. hominis* strains varied from 704 to 825 kb.

Members of the class *Mollicutes* are considered the simplest self-replicating cellular organisms known with the smallest recorded genome size. *Mollicutes* are unusual in several respects, most notably by the absence of a cell wall and the low guanine-and-cytosine (G+C) content. Despite their apparent simplicity, genetic analysis of these organisms is hampered by difficulties in isolation of stable mutants and the lack of an appropriate gene transfer system. Furthermore, the use by mollicutes of the otherwise generally universal termination codon UGA as a codon for tryptophan (44) complicates attempts to express cloned DNA in *Escherichia coli*. To facilitate the genetic analysis of the organism at the molecular level, construction of a physical map based on localization of restriction sites would be advantageous. To date, physical-genetic maps of procaryotes of various complexities have been constructed for *E. coli* (40), *Haemophilus influenzae* (8, 21, 24), *Clostridium perfringens* (9), *Pseudomonas aeruginosa* (35, 38), *Caulobacter crescentus* (18), *Anabaena* sp. strain PCC 7120 (3), *Thermococcus celer* (29), *Campylobacter jejuni* (10), *Bacillus subtilis* (42), and various mollicutes: *Mycoplasma pneumoniae* (22), *Mycoplasma genitalium* (16), *Mycoplasma mycoides* (32, 33), *Ureaplasma urealyticum* (15), and *Mycoplasma mobile* (5).

In this paper, we report the construction of combined physical and genetic maps of five *Mycoplasma hominis* strains (PG21, 132, 93, 4195, and 7488) (1, 12). *M. hominis* is commonly found as part of the normal flora in the female genital tract, but several studies have shown that it is potentially pathogenic and may be associated with a variety of disorders, especially urogenital and neonatal infections. However, assessment of the pathogenic potential of *M. hominis* is complicated by the fact that this species constitutes a relatively heterogeneous group of organisms as judged by serological studies, nucleic acids hybridization, comparison of protein patterns, and analysis of restriction enzyme cleavage patterns (for a review, see reference 36). The genetic background for this heterogeneity has not yet been clarified.

A complete physical and genetic map of the *M. hominis* chromosome makes it possible to assess the polymorphisms within the species and to compare *M. hominis* with other species. The objective of the present study was to determine whether the heterogeneity in the *M. hominis* species was reflected in dissimilarities in the genome structure in terms of gene localization and restriction enzyme cleavage sites of infrequently cutting enzymes.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. Exponentially growing cultures of *M. hominis* strains were grown in BEA medium (19) modified to contain only 0.3% arginine. The five strains examined in detail were PG21 (from the lower genital tract); 132, 4195, and 93 (all from the vagina); and 7488 (a clinical isolate from the cervix). PG21 is the type strain for *M. hominis* (1, 12). An additional 10 strains (DC63, V2785, M1449, SC4, 183, 10, W2, P2, P7, and P71) were included for determination of genome size (1). *E. coli* XL1-blue (Stratagene, La Jolla, Calif.) was used as host for the phagemid pBluescript SK+ (Stratagene) and their derivatives. The strains were cultivated in Luria-Bertani broth or Terrific broth (39).

DNA preparation. *M. hominis* DNA for conventional agarose gel electrophoresis and cloning was isolated by the methods of Trevino et al. (41).

Preparation and restriction endonuclease digestion of DNA in agarose blocks. Chloramphenicol (final concentration, 80 mg/liter) was added to growing cultures of *M. hominis* 1.5 h before harvest (31) to allow DNA replication to reach completion while inhibiting the initiation of new rounds of replication. The organisms were harvested by centrifugation

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at 20,000 × *g* for 30 min and washed twice with phosphate-buffered saline (PBS) (20 mM sodium phosphate, 250 mM NaCl, pH 7.4). Pelleted mycoplasmas were resuspended in PBS and mixed with an equal volume of 2% low-melting-point agarose (Incert agarose; FMC BioProducts, Rockland, Maine) in PBS which had been melted and equilibrated to 43°C. The suspension was transferred to a plastic mold (20 by 9 by 1.2 mm) (Bio-Rad Laboratories, Richmond, Calif.) which had been cooled down on ice to allow instant hardening to prevent sedimentation of the mycoplasmas. The blocks were transferred from the mold to a sterile tube containing lysis buffer (1% Sarkosyl, 0.5 M EDTA, 10 mM Tris hydrochloride, pH 9.5) with 1 mg of proteinase K (Sigma Chemical Co., St. Louis, Mo.) per ml and incubated at 50°C overnight. This step was repeated, and the agarose blocks were then washed twice in lysis buffer without proteinase K for 2 h. The agarose blocks were stored in lysis buffer at 4°C. Before restriction endonuclease digestion, blocks were washed in several changes of TE buffer (10 mM Tris HCl, 1 mM EDTA, pH 8.0) for 6 h at 4°C and equilibrated in 1 ml of the appropriate restriction enzyme buffer at 4°C for 30 min. The blocks were then incubated overnight in 120 μl of restriction enzyme buffer with 10 to 40 U of restriction enzyme and 100 μg of bovine serum albumin (Boehringer Mannheim Biochemicals, Indianapolis, Ind.) per ml. Before electrophoresis, the blocks were washed for 30 min in TE buffer. The restriction enzymes *Apa*I, *Sma*I, *Bam*HI, *Xho*I, and *Sal*I were purchased from Boehringer Mannheim. The restriction enzyme buffers and incubation temperatures were as specified by the supplier except for the *Sal*I restriction enzyme buffer, in which the NaCl concentration was increased to 150 mM to avoid the decrease in sequence specificity which is seen under nonoptimal incubation conditions (star activity).

Cleavage of genomic DNA by irradiation. Agarose blocks containing genomic DNA were washed twice in PBS, placed in 200 μl of PBS in Eppendorf tubes, and irradiated with a ^{137}Cs gamma-ray source (Gamma cell 2000 RH; Danish Atomic Energy Commission Research Establishment, Risø, Denmark) at a dose rate of 3.3 Gy/min (45). Irradiation time was 12 s, corresponding to a gamma-ray dose of 0.7 Gy. Following irradiation, the DNA was electrophoresed as described below.

Electrophoresis. Pulsed-field gel electrophoresis was performed either by the contour-clamped homogeneous-electric field (CHEF) technique or by field inversion gel electrophoresis (FIGE) using the CHEF-DR II system (Bio-Rad) and the Sub-Cell electrophoresis cell (Bio-Rad), respectively. Agarose gels (1%) were run for 19 to 24 h in 0.5× TBE buffer (44.5 mM Tris-borate, 44.5 mM boric acid, 1 mM EDTA, pH 8) at 14°C and 200 V. Pulse times varied between 0.7 and 100 s, with different extents of ramping depending on the size range of molecules to be resolved. Gels were stained with ethidium bromide after completion of electrophoresis. The sizes of DNA fragments were determined by measuring distances of band migration compared with the DNA standards of a lambda DNA ladder (FMC), lambda DNA cut with *Hin*dIII, and *Saccharomyces cerevisiae* YNN295 chromosome marker (Bio-Rad).

Southern hybridization. Preceding the alkali denaturation, partial hydrolysis in 0.25 M HCl for 20 min was done to enhance the transfer of large DNA fragments (39). DNA transfer to Hybond-N membrane (Amersham International plc, Amersham, United Kingdom) was carried out as described by Sambrook et al. (39). Hybridization at high stringency was carried out at 62°C in a buffer containing 2× SSC (1× SSC is 0.15 M NaCl and 0.015 M sodium citrate), 0.5% sodium dodecyl sulfate (SDS), 100 μg of yeast RNA per ml, and 5× Denhardt solution (1× Denhardt solution contains 0.02% Ficoll, 0.02% bovine serum albumin, and 0.02% polyvinylpyrrolidone). Hybridization at low stringency was carried out at 55°C with the same buffer but with 6× SSC. The filters were washed twice for 15 min in 2× SSC–0.5% SDS at room temperature and for 2.5 h at the same temperature as was used for hybridization. DNA fragments were labeled with [α-^{32}P]dATP by nick translation using standard methods (39). To strip the probe from the blots, the filters were washed for 1.5 h in 0.5× Denhardt solution–25 mM Tris HCl (pH 7.5)–0.1% SDS at 80°C. Blots were reused up to 12 times.

Construction of a *Bam*HI linking library. One method applied to determine the order of the restriction fragments was to generate a linking library (3) for *Bam*HI fragments of *M. hominis* PG21. *M. hominis* PG21 DNA was cleaved with the restriction enzyme *Hin*dIII. The DNA was diluted to 20 μg/ml and self-ligated into circles with T4 DNA ligase (Boehringer Mannheim) at 14°C for 16 h. The ligated fragments which contained a *Bam*HI restriction site were then linearized with *Bam*HI and ligated to pBluescript SK+ opened with *Bam*HI. The plasmids were transformed into *E. coli* XL1-blue by electroporation (Gene pulser and controller unit; Bio-Rad) and plated on Luria-Bertani agar (39) containing ampicillin, tetracycline, 5-bromo-4-chloro-3-indoyl-β-D-galactopyranoside (X-Gal), and isopropyl-β-thiogalactopyranoside (IPTG). Plasmid DNA was isolated from the Lac$^-$ transformants and checked for the presence of a single *Hin*dIII site within a *Bam*HI insert and for hybridization to only one fragment of *Hin*dIII-digested *M. hominis* PG21 DNA.

DNA sequencing. Single-stranded DNA was produced by use of the helper phage R408 (Stratagene). Single-stranded DNA was sequenced by the dideoxynucleotide triphosphate chain termination method with [α-^{32}P]dATP and Sequenase version 1 (United States Biochemical) as recommended by the supplier. DNA sequencing of denatured double-stranded DNA was done by the methods of Hattori and Sakaki (20).

Sequence analysis and comparison. The sequence data were compiled and analyzed with the Genetics Computer Groups Sequence Analysis Software Package Version 6.2 on VAX/VMS (17). A data base search was performed on the National Biomedical Research Foundation (NBRF) DNA/RNA Sequence Database and NBRF Protein Database.

Hybridization with gel-purified genomic restriction fragments. For recovery of restriction fragments for nick translation, electrophoresis was performed through a 1.0% low-gelling-temperature agarose (SeaPlaque GTG Agarose; FMC BioProducts) by using CHEF or FIGE at appropriate pulse times. Bands were visualized with ethidium bromide. Gel slices containing the appropriate restriction fragment were excised and melted at 62°C before purification by phenol-chloroform extraction and ethanol precipitation. The DNA recovered was labeled with [α-^{32}P]dATP by nick translation.

RESULTS

The strains examined in this study included *M. hominis* type strain PG21 and four other genital-tract isolates.

Size of the *M. hominis* genome determined by gamma irradiation. By gamma irradiation of the circular *M. hominis* chromosome, it was possible to introduce, on average, one double-strand break resulting in a single full-size linear DNA molecule. The DNA was run on a CHEF gel, resulting in a

TABLE 1. Sizes of restriction fragments of five *M. hominis* strains

Restriction enzyme and fragment	Fragment size (kb) in strain:				
	PG21	132	4195	93	7488
*Sma*I					
A	100	102	104	104	106
B	74	94	74	33	190
C	1.7	1.7	1.7	44	124
D	1.4	1.4	1.4	1.7	0.5
E	0.5	0.5	0.5	1.4	1.4
F	124	122	130	0.5	1.7
G	156	162	20	170	138
H	136	37	300	162	62
I	102	50	19	145	120
J	6.5	66	84	94	6.5
K	1.7	50	6.5	6.5	1.7
L	1.4	82	1.7	1.7	1.4
M	0.5	6.5	1.4	1.4	0.5
N		1.7	0.5	0.5	
O		1.4			
P		0.5			
Total	706	779	745	768	754
*Bam*HI					
A	269	289	38	—[a]	39
B	29	124	240		84
C	94	9.4	41		90
D	64	94	125		32
E	130	66	44		28
F	90	28	90		238
G	25	50	44		128
H		15	15		0.96
I		75	74		15
J		26	29		74
K					26
Total	701	776	740		755
*Xho*I					
A	278	300	286	334	245
B	34	34	66	33	34
C	150	316	256	260	132
D	118	128	134	140	216
E	128				128
Total	703	778	742	767	755
*Sal*I					
A	104	122	721	212	60
B	186	514		556	21
C	414	132			669
Total	704	768	721	768	750
*Apa*I					
A	—	—	660	765	750
B			66		
Total			726	765	750
Avg size	704	775	735	767	753
Full-size linear chromosomes[b]	696	761	720	753	745

[a] —, no cleavage by the restriction enzyme.
[b] Determined by gamma irradiation of *M. hominis* DNA (see text).

single sharp band. The chromosome size was determined by comparing mobilities of these bands with those of DNA size standards (lambda ladders and *S. cerevisiae* chromosomes). The genome sizes of the five *M. hominis* strains examined in detail are shown in Table 1. The range of genome sizes for all 15 strains examined was 696 kb (strain PG21) to 825 kb (strain V2785).

Digestion of *M. hominis* DNA by low-frequency-cleaving restriction endonucleases. *M. hominis* PG21 DNA was screened for cleavage with different restriction enzymes to identify restriction fragment patterns suitable for mapping the genome. The criterion for the selection of appropriate enzymes was the DNA base composition of the organism. In G+C-poor *M. hominis* (G+C = 29 mol%) (28), restriction enzymes with recognition sequences rich in G and C were promising candidates for enzymes which will cleave the *M. hominis* genome infrequently. The restriction enzymes *Not*I and *Sfi*I, which have 8-bp recognition sequences (GC↓GGCCGC and GGCCNNNN↓NGGCC), did not cut the *M. hominis* chromosome at all. Many other restriction enzymes tested cleaved *M. hominis* DNA into fragments too small and numerous for genome mapping, and some did not digest the DNA properly. However, five restriction enzymes, *Apa*I (GGGCC↓C), *Bam*HI (G↓GATCC), *Sal*I (G↓TCGAC), *Sma*I (CCC↓GGG), and *Xho*I (C↓TCGAG), cleaved *M. hominis* into a small number of DNA fragments and proved useful for construction of the genome maps (Fig. 1). The size of each restriction fragment generated by the five restriction enzymes was determined by using conventional gel electrophoresis as well as FIGE and CHEF separation systems. The restriction fragments obtained by *Apa*I, *Bam*HI, *Sal*I, *Sma*I, and *Xho*I digestions were sized and numbered as summarized in Table 1. Different pulse and electrophoresis times were used so that each restriction fragment size could be determined under optimal electrophoretical conditions. The average genome size based on the sum of the sizes of the restriction enzyme fragments is shown in Table 1.

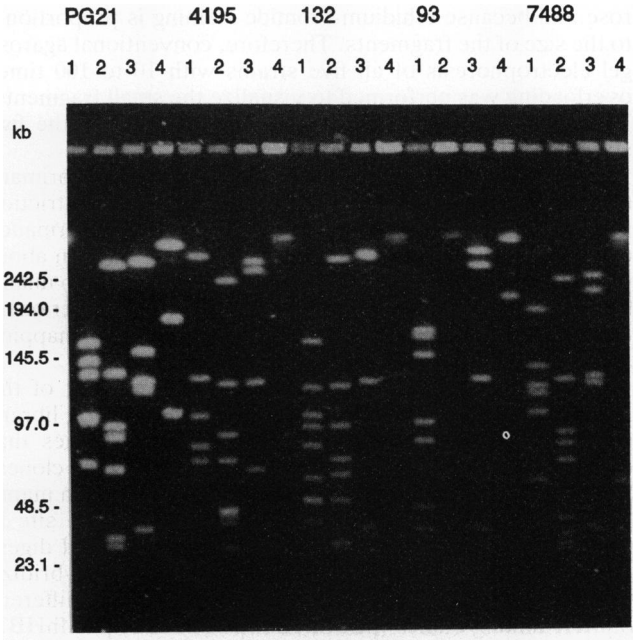


FIG. 1. Analysis of *M. hominis* DNA by CHEF. (A) *M. hominis* PG21; (B) *M. hominis* 4195; (C) *M. hominis* 132; (D) *M. hominis* 93; (E) *M. hominis* 7488. Lanes show digestion with *Sma*I (lanes 1), *Bam*HI (lanes 2), *Xho*I (lanes 3), and *Sal*I (lanes 4). CHEF gel was run for 20 h at 200 V with a pulse time ramped from 1 to 15 s through a 1% agarose gel. Bacteriophage lambda DNA oligomers and *Saccharomyces cerevisiae* strain YNN295 chromosomes were used as molecular weight markers.

Small fragments are difficult to detect by pulsed-field gel electrophoresis because they readily diffuse out of the aga-

rose and because ethidium bromide staining is proportional to the size of the fragments. Therefore, conventional agarose gel electrophoresis of all five strains with 10 to 100 times overloading was performed to visualize the small fragments. Table 1 shows that *Sma*I digestion of DNA from the five strains resulted in 13 to 16 fragments.

Physical mapping of the *M. hominis* genome. Our primary method for determining the relative order of the restriction fragments generated by each enzyme was the performance of double digests, which yielded valuable information about physical relationships. However, it was impossible to unambiguously allocate all fragments, especially the smaller ones, to a precise location. Therefore, complementary mapping approaches were employed.

The second method used to determine the order of the restriction fragments was the generation of a linking library (3) for *M. hominis* PG21 *Bam*HI fragments. Clones that contain the rare restriction site are called linking clones. Such clones are useful for construction of restriction maps. When a linking clone containing a particular rare site is used to probe a blot of a pulsed-field gel-separated digest generated with the same enzyme, the probe will hybridize to two adjacent DNA fragments. We obtained six different *Bam*HI linking clones (pBMhHB1, pBMhHB3, pBMhHB7, pBMhHB14, pBMhHB15, and pBMhHB23) of seven possible such clones as judged from the *Bam*HI digest of *M. hominis* PG21. The results of hybridizations involving the linking clones are summarized in Table 2. This approach established the order of all *Bam*HI fragments in the type strain *M. hominis* PG21 (Table 2). Only *Bam*HI fragments B and C could not be linked. Clone pBMhHB23 gave an unexpected hybridization pattern, as it hybridized to three *Bam*HI fragments, A, F, and G, suggestive of the involvement of a repeated element. Restriction enzyme and hybridization analyses revealed that the clone contained part of the 23S and 5S subunits of the *rrn* operon, which explains the hybridization pattern. Restriction analysis of the linking clones revealed that pBMhHB1 contained, besides the *Bam*HI site, an *Xho*I site and a *Sma*I site, thus linking two *Xho*I and *Sma*I sites as indicated in Table 2. Likewise, pBMhHB3 contained a *Bam*HI site and a *Sal*I site, and pBMhHB23 contained two *Sma*I sites. It is known from sequencing data that the *rrn* genes of many procaryotes contain a highly conserved *Sma*I site in the 16S region. Previously, the *Eco*RI and *Hin*dIII restriction map for the *rrn* genes of the *M. hominis* strains used in this study was determined (12). By using three probes specific for different parts of the *rrn* operon (Table 3), the map was complemented with the *Sma*I sites. This examination demonstrates that each of the two *rrn* genes present in *M. hominis* contains four *Sma*I sites: 5′-*Sma*I-1.7 kb-*Sma*I-1.4 kb-*Sma*I-0.5 kb-*Sma*I-3′. An additional *Sma*I site was located in the region 6.5 kb upstream from *rrnB* (Fig. 2). The *rrn* clones were thus used as linking clones for some of the *Sma*I fragments (Table 2).

The linking clones were likewise used as probes in hybridization analyses for the remaining four *M. hominis* strains. The results are shown in Table 2.

By these two approaches, it was possible for each of the strains to allocate all but two or three fragments which failed to hybridize with the linking clones and contained no internal restriction sites.

The third approach was to isolate restriction fragments of the chromosomes, label them with [^{32}P]ATP by nick translation, and hybridize the labeled fragments to blots of DNA digests. A purified restriction fragment should produce a single band if it was hybridized to digests obtained with the same restriction enzyme. One, two, or more bands could theoretically be produced if the purified restriction fragment was hybridized with DNA fragments cleaved with another restriction enzyme. The presence of two or more hybridization bands indicates overlap and was used to construct the physical map. However, using this approach, duplications in the genome (e.g., *rrn* genes) may obscure the results of the procedure. In *M. hominis* 7488, *Bam*HI fragment B (Fig. 2) was used to allocate the small *Sal*I fragment B to its appropriate position. In *M. hominis* 132, *Bam*HI fragment G was used as probe to assign the position of the K-L *Sma*I site, the C-D *Xho*I site, and the B-C *Sal*I site, and *Bam*HI fragment D was used to confirm the position of *Sma*I fragment H.

The final approach used to locate the *Bam*HI E fragment in *M. hominis* 4195 was partial digestion. Complete digestion with *Xho*I followed by partial digestion with *Bam*HI and hybridization with pBMhHB7 determined the position of the fragment.

The restriction maps of the chromosomes of *M. hominis* PG21, 132, 4195, 93, and 7488 constructed from the data are shown in Fig. 2. The maps confirm that the genomes must be circular. The circular genomes were represented as linear maps, with the zero coordinate for each map localized at the conserved *Xho*I site identified by the linking clone pBMhHB3.

Sequence analysis of linking clones. The six *Bam*HI linking clones were further investigated by DNA sequencing. Each clone was sequenced approximately 500 bp in each direction from the *Bam*HI site, and the sequence was examined for open reading frames. The putative reading frames were translated to putative polypeptides. By computer search of the NBRF Protein Database, the deduced amino acid sequence was compared with published sequences to identify possible homologies.

An open reading frame in clone pBMhHB1 showed 48% similarity (with conservative substitutions) and 29% identity with the *Clostridium pasteurianum* DNA binding protein Hu and a similar degree of homology to the *E. coli* and *Salmonella typhimurium* DNA binding protein Hu-1. Clone pBMhHB3 encodes the 230 C-terminal amino acids of a putative polypeptide that showed 61% identity with the C-terminal part of the β chain of *E. coli* DNA gyrase. The putative protein encoded by an open reading frame in pBMhHB14 had 42% identity with the 354 C-terminal amino acids of the FtsY protein in *E. coli*. The sequenced parts of pBMhHB7, pBMhHB15, and pBMhHB23 revealed no significant homology to sequences in the data base.

Assignment of genetic markers to the physical map. The chromosomal locations of 12 genes or operons were mapped. We used Southern hybridization to place a number of cloned genes on the restriction map. The probes used are listed in Table 3. The map positions are shown above the maps in Fig. 2.

Three clones (pMhX3-91, pMhX1-28, and pMhS3-1) were isolated in our laboratory, as described in reference 14. An *M. hominis* DNA library was cloned in the expression vector pEX. By immunoscreening with a polyclonal hyperimmune serum produced by rabbit immunization with *M. hominis* PG21 or 7488, immunoreactive recombinants were obtained. pMhX3-91 contains part of the gene encoding a 98-kDa hydrophilic protein, in which the amino acid sequence deduced from the DNA sequence showed some homology to the distal part of *E. coli* alanyl-tRNA synthetase. pMhS3-1 contains part of the gene for a 108-kDa protein which is a

TABLE 2. Hybridization of *M. hominis* PG21 linking clones

Clone	*M. hominis* strain	Fragment[a]				
		*Sma*I	*Bam*HI	*Xho*I	*Sal*I	*Apa*I
pBMhHB3	PG21	A	G/A	A	C/A	—
	132	A	J/A	A	C/A	—
	4195	A	J/A	A	A/A	A
	93	A	—	A	B/A	A
	7488	A	K/A	A	C/A	A
pBMhHB1	PG21	F/G	A/B	A/B	B	—
	132	F/G	A/B	A/B	B	—
	4195	F/G	B/C	A/B	A	A
	93	G/H	—	A/B	B	A
	7488	B/C	E/F	B/C	C	A
pBMhHB7	PG21	G	C/D	C	C	—
	132	G	B/C	C	B	—
	4195	H	D	C	A	A
	93	H	—	C	B	A
	7488	B	C	A	C	A
pBMhHB15	PG21	H	D/E	D	C	—
	132	I	D/E	C	B	—
	4195	H	E/F	C	A	A
	93	I	—	C	B	A
	7488	G	F/G	D	C	A
pBMhHB14	PG21	I	E/F	E	C	—
	132	L	G/H	D	C	—
	4195	J	G/H	D	A	A
	93	J	—	D	B	A
	7488	I	G/H/I	E	C	A
pBMhHB23	PG21	D/E/F-L/M/A	A-F/G	A-E	B-C	—
	132	D/E/F-O/P/A	A-I/J	A-D	B-C	—
	4195	D/E/F-M/N/A	B-I/J	A-D	A-A	A-A
	93	E/F/G-M/N/A	—	A-D	A-B	A-A
	7488	C/D/E-L/M/A	F-J/K	C-E	C-C	A-A
pMYC575	PG21	E/F-M/A	A-F	A-E	B-C	—
	132	D/E-P/A	A-I	A-D	B-C	—
	4195	E/F-N/A	B-I	A-D	A-A	A-A
	93	F/G-N/A	—	A-D	A-B	A-A
	7488	C/D-M/A	F-J	C-E	C-C	A-A
pMYC147	PG21	C/D/E-K/L/M	A-F	A-E	B-C	—
	132	C/D/E-N/O/P	A-I	A-D	B-C	—
	4195	C/D/E-L/M/N	A-I	A-D	A-A	A-A
	93	D/E/F-L/M/N	—	A-D	A-B	A-A
	7488	D/E/F-K/L/M	F-J	C-E	C-C	A-A
pMYC318	PG21	B/C-J/K	A-F	A-E	B-C	—
	132	B/C-M/N	A-I	A-D	B-C	—
	4195	B/C-K/L	A-I	A-D	A-A	A-A
	93	C/D-K/L	—	A-D	A-B	A-A
	7488	F/G-H/J	F-J	C-E	C-C	A-A

[a] Symbols: /, linkage of the fragments is given on each side of the slash; -, fragments on each side are not continuous on the map; —, no restriction site in the strain.

surface-located antigen and has amphiphilic properties. pMhX1-28 contains part of the gene for a 124-kDa surface-located membrane protein. The DNA sequence of this gene contains repeated elements which are found at two locations in the genomes of *M. hominis* PG21, 93, 4195, 132, and 7488.

Comparison of the five restriction maps. Comparison of the genomic maps of the five *M. hominis* strains showed some conservation in the locations of mapped restriction sites (Fig. 2). *M. hominis* 93 showed the distinctive feature that all the *Bam*HI sites were missing. The order of and distances between the restriction sites in the regions of the *gyrB* gene and the *hup* gene were completely conserved. Likewise, identical positions for the four *Sma*I sites in the *rrnA* operon and the five *Sma*I sites and one *Bam*HI site in *rrnB* were seen. The *M. hominis* 7488 chromosome showed some remarkable features concerning the positions of some restriction sites and genes. From position 120 kb to position 410 kb from zero (Fig. 2), the order of mapped

TABLE 3. Probes used for localizing genes

Gene	Corresponding protein	Biological source	Clone	Reference or source
cp-91	94-kDa hydrophilic protein, cytoplasmic	*M. hominis* PG21	pMhX3-91	This laboratory
ftsY	*E. coli* cell division protein homolog	*M. hominis* PG21	pBMhHB14	This laboratory
gyrB	*E. coli* DNA gyrase, subunit B homolog	*M. hominis* PG21	pBMhHB3	This laboratory
hup	*E. coli* DNA binding protein Hu-1 homolog	*M. hominis* PG21	pBMhHB1	This laboratory
lmp-1	124-kDa surface-exposed membrane protein	*M. hominis* PG21	pMhX1-28	14
pap	108-kDa surface-exposed membrane protein	*M. hominis* 7488	pMhs3-1	This laboratory
rpoA	*E. coli* RNA polymerase α-subunit homolog	*M. hominis* PG21	pBL2812-3	This laboratory
rpoC	RNA polymerase β′ subunit	*Mycoplasma* strain PG50	pMYC708	34
rrnA-rrnB	23S and 5S rRNAs	*Mycoplasma* strain PG50	pMYC575	12
rrnA-rrnB	16S rRNA	*Mycoplasma* strain PG50	pMYC318	12
rrnA-rrnB	16S and 23S rRNAs	*Mycoplasma* strain PG50	pMYC147	11
tuf	EF-Tu	*M. hominis* PG21	pBMhH3-3	Ladefoged et al.
unc operon	Membrane-bound ATP synthetase subunits α, γ, and β	*Mycoplasma* strain PG50	pMYC405	34

restriction sites was *Bam*HI-(*Bam*HI*)-*Xho*I-*Bam*HI-*Sma*I-*Xho*I-*Bam*HI-*Sma*I-*Sma*I-*Sma*I-*Sma*I. The order of restriction sites in the same region of *M. hominis* PG21 was *Sma*I-*Sma*I-*Sma*I-*Sma*I-*Bam*HI-*Xho*I-*Sma*I-(*Sal*I*)-*Bam*HI-*Xho*I-*Bam*HI. This can be explained by an inversion in *M. hominis* 7488 involving the loss of the *Bam*HI* and *Sal*I* sites, as indicated by the inverse gene order.

DISCUSSION

The sizes of the chromosomes of all 15 *M. hominis* strains estimated by electrophoresis of full-size linear DNA prepared by gamma irradiation ranged from 696 kb (PG21) to 825 kb (V2785). These were in good agreement with the sizes of the five mapped *M. hominis* chromosomes for which the sizes were also determined from the sums of the sizes of the restriction endonuclease fragments (Table 1). The size of *M. hominis* PG21 is within the range expected from renaturation kinetics (4.5×10^8 Da, ~681 kb) as determined by Bak et al. (2). The size variation implies some heterogeneity within the species *M. hominis* although not as profound a variation as that observed for *U. urealyticum*, another genus of the family *Mollicutes*, in which sizes range from 760 to 1,140 kb (37).

The theoretical number of restriction sites for a restriction enzyme with a 6-base recognition sequence can be calculated on the basis of base composition (G+C = 29 mol% in *M. hominis* [28]) and genome size (*M. hominis* genome, ~700 kb). *Bam*HI, *Sal*I, and *Xho*I were thus calculated to cleave the genomes 39 times, and *Sma*I and *Apa*I cleaved it 7 times. However, *Bam*HI, *Sal*I, *Xho*I, and *Apa*I should cleave the five genomes only 0 to 11, 1 to 3, 4 or 5, and 0 to 2 times, respectively. Contrary to this, *Sma*I digestion resulted in more fragments than expected, i.e., 13 to 16 sites.

This discrepancy can be accounted for in two ways. One is that the G+C mole percent differs in different parts of the genome. The other is that some oligonucleotide sequences may be more infrequent than others. Supporting the first statement is the hypothesis of Muto (27) that the G+C level of functionally less important parts of the mycoplasma genome has during evolution become lower than the levels of more important parts. An example of this is the essential *tuf* gene in *M. hominis* PG21, which has a G+C content of 38% (23) compared with the overall content of 29 mol%.

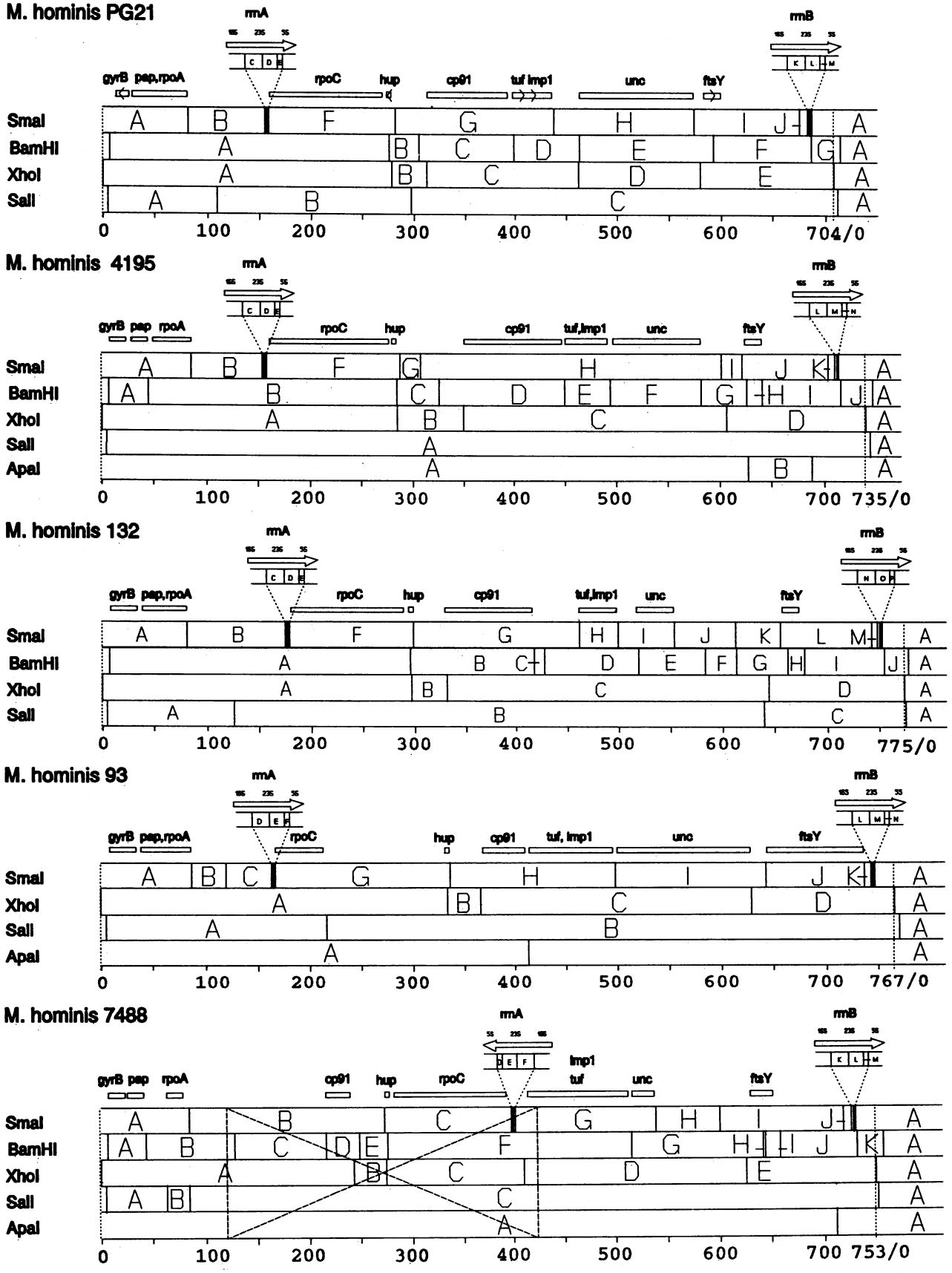
By analyzing all available sequencing data, McClelland et al. (26) found a nonrandom DNA sequence arrangement in di- and trinucleotides. They found, for instance, that CCG and CGG are the rarest trinucleotides in genomes with G+C contents of less than 45%. This is, however, not consistent with our findings for *M. hominis*, since *Sma*I (CCCGGG) cuts significantly more than *Apa*I (GGGCCC) in each of the five strains.

As revealed in Table 1, there are no *Bam*HI sites at all in *M. hominis* 93, in contrast to the other strains, in which *Bam*HI cuts at least seven times. The resistance of *M. hominis* 93 DNA to *Bam*HI may be due to the presence of a restriction modification system. Further investigation will be needed to clarify the issue if this is the case.

With the recent progress in electrophoretic techniques for the separation of large DNA fragments such as pulsed-field gel electrophoresis and variations of it, it has become possible to construct restriction maps of bacterial chromosomes. Such maps accurately measure genome sizes and reveal various aspects of genome complexity. One of the methods used in this study to align the restriction enzyme fragments of *M. hominis* into a map was cross-hybridization with purified fragments generated by restriction endonuclease digestion of the *M. hominis* genome. However, this method is not generally applicable in studies of *M. hominis*, as the genome contains repeated DNA elements. Beside the rRNA operons, genes encoding some surface-exposed membrane antigens are partly duplicated in most strains (14). The restriction map supports the observation by Bode and Morowitz (6) that *M. hominis* possesses a single circular chromosome.

Restriction maps are of greater genetic use if the locations of known genes can be identified relative to the restriction sites. The locations of 12 genes and their operons are indicated on the maps (Fig. 2). Several species of mollicutes, including *M. hominis*, display pronounced intraspecies heterogeneity as judged from serological studies, nucleic acid hybridization, comparison of protein patterns, and analysis

FIG. 2. Physical and genetic maps of five *M. hominis* strains. Restriction sites for *Sma*I, *Bam*HI, *Xho*I, *Sal*I, and *Apa*I are indicated. Restriction fragments are designated alphabetically as they appear on the map. Map units are in kilobases, starting from the *Xho*I site common to all strains. For presentation, each of the circular genomes is shown linearized from the common *Xho*I site. The positions of the functional loci are indicated precisely as given from the resolution of the map. X-marked area, region with the postulated inversion in *M. hominis* 7488. Arrows indicate directions of transcription.


M. hominis PG21
rrnA
rrnB
gyrB pap,rpoA
rpoC
hup
cp91
tuf imp1
unc
ftsY
SmaI
BamHI
XhoI
SalI
0 100 200 300 400 500 600 704/0
M. hominis 4195
rrnA
rrnB
gyrB pap rpoA
rpoC
hup
cp91
tuf,imp1
unc
ftsY
SmaI
BamHI
XhoI
SalI
ApaI
0 100 200 300 400 500 600 700 735/0
M. hominis 132
rrnA
rrnB
gyrB pap,rpoA
rpoC
hup
cp91
tuf,imp1
unc
ftsY
SmaI
BamHI
XhoI
SalI
0 100 200 300 400 500 600 700 775/0
M. hominis 93
rrnA
rrnB
gyrB pap,rpoA
rpoC
hup
cp91
tuf, imp1
unc
ftsY
SmaI
XhoI
SalI
ApaI
0 100 200 300 400 500 600 700 767/0
M. hominis 7488
rrnA
rrnB
imp1
gyrB pap rpoA
cp91
hup
rpoC
tuf
unc
ftsY
SmaI
BamHI
XhoI
SalI
ApaI
0 100 200 300 400 500 600 700 753/0

of conventional restriction enzyme cleavage pattern (1, 4, 12, 13, 25). We therefore anticipated that the chromosomal organization of different *M. hominis* strains would show a similar heterogeneity.

When the restriction maps of the five *M. hominis* strains are compared (Fig. 2), it is striking that the numbers and relative positions of restriction sites are conserved in some regions but quite different in others. The gene orders of *M. hominis* PG21, 132, 4195, and 93 are surprisingly identical. Thus, the study shows that in spite of considerable congruence in the genetic maps, the restriction patterns of different strains may be quite dissimilar. These differences in the restriction maps may conceivably result from a single base substitution, small insertions, or deletions.

The positions of restriction sites and genetic markers in *M. hominis* 7488 indicate a rearrangement in the region from about 120 to 410 kb in this strain. This rearrangement can be explained by one large inversion constituting 40 to 50% of the genome (Fig. 2).

From phylogenetic studies (43), it is known that strains belonging to the class *Mollicutes* and the genus *Clostridium* form a phylogenetically related group. Extending the comparison of the *M. hominis* maps to *C. perfringens* (9), a striking similarity was evident. The gene order in *M. hominis* PG21 was *rrn-gyrB-rpoA-rrn-rpoC-tuf-uncAGD*, and in *C. perfringens*, the order was *rrn-gyrB-rrn-rpoC-rpoA-tuf-uncAGD*. The only difference is the location of *rpoA*. Moreover, in *E. coli* K-12, these genes are all located in a region of about 765 kb surrounding the origin of replication (30). It would be interesting to compare the gene order in *M. hominis* with those of other published mycoplasmal maps (5, 15, 16, 22, 32, 33). However, this is not possible, since previously published maps were obtained either without or with other genetic markers.

As seen from the five maps, there is a tendency to accumulation of certain restriction enzyme cleavage sites. Nine of 13 *Sma*I sites in *M. hominis* PG21 are located in the regions of the *rrn* genes constituting less than 2% of the genome. One *Sma*I, one *Xho*I, and one *Bam*HI site are located in the region of the *hup* gene, and one *Bam*HI, one *Xho*I, and one *Sal*I site are located in the region of the *gyrB* gene, comprising less than 0.5 and 1% of the genomes, respectively. This is probably due to different G+C contents in different parts of the genome.

G+C-rich restriction sites may be indicative of the functional importance of the DNA in which they are situated (27). Occurrence of the *Sma*I sites within the *rrn* operons may be examples of such functional importance. This point led us to carry out sequence analysis of the *Bam*HI linking clones, by which we identified three genes (Table 3). One linking clone, pBMhHB23, was shown by restriction enzyme cleavage and hybridization analysis to contain part of the *rrn* operon, but this was located more than 500 bp from the *Bam*HI site and therefore was not in the sequenced region. Thus, this cloning strategy may be suitable for cloning essential genes with a conserved structure in other organisms with low G+C contents.

In many procaryotes, the location of a putative origin of replication can be inferred from the positions and directions of transcription of genes and operons (7). Efficient transcription of essential genes is facilitated by their clustering around the origin of replication and their transcription following the direction of each replication fork. Furthermore, in *E. coli*, *B. subtilis*, and *Salmonella typhimurium*, the origin of replication is linked to the DNA gyrase B gene, *gyrB*. On the *M. hominis* PG21 map, the directions of transcription of seven genes, including the two *rrn* genes, and the position of *gyrB* have been determined (Fig. 2). Assuming a bidirectional replication as seen in *M. mycoides* (32), one can make no parallel interpretation for the location of the origin in *M. hominis*, since there is no common point from which the directions of transcription of the mapped genes diverge.

Comparison of chromosomal maps of heterogeneous organisms as in this study shows that the level of information is greatly enhanced by supplementing the physical maps with genetic maps, since this makes it possible to obtain a more-detailed analysis of genomic organization and rearrangements. Such detailed physical and genetic maps not only have important ramifications for bacterial genetics but may also have considerable impact on the understanding of phylogeny and molecular evolution.

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