

Physical Mapping of the *Mycoplasma gallisepticum* S6 Genome with Localization of Selected Genes

TIMOTHY S. GORTON, M. STEPHANIE GOH, AND STEVEN J. GEARY*

Department of Pathobiology, University of Connecticut, Storrs, Connecticut 06269-3089

Received 6 June 1994/Accepted 22 October 1994

We report the construction of a physical map of the *Mycoplasma gallisepticum* S6 genome by field-inversion gel electrophoresis of DNA fragments generated by digestion of genomic DNA with rare-cutting restriction endonucleases. The size of the *M. gallisepticum* S6 genome was calculated to be approximately 1,054 kb. The loci of several genes have been assigned to the map by Southern hybridization utilizing specific gene probes.

Phylogenetic studies indicate that mycoplasmas have undergone degenerative evolution arising from a clostridial ancestry (38). The reduced genome of mycoplasmas results in a loss of cellular structures, the most notable of which being the absence of a cell wall. Although their apparent simplicity makes the mycoplasmas attractive subjects for genetic analysis, classical approaches have proven relatively difficult to apply. Their complex nutritional requirements and microscopic growth on solid medium have hindered isolation of auxotrophic mutants useful in establishing metabolic pathways. Their use of the universal termination codon UGA as a tryptophan codon (15, 39) has complicated the utility of *Escherichia coli* for expression of cloned mycoplasma DNA sequences. For these reasons, a more guided approach to the examination of the mycoplasma genome is appropriate.

Pulsed-field electrophoretic techniques have proven useful in constructing physical maps of several mycoplasmas (2, 4, 6, 16, 18, 25–27, 35). In an organism in which the isolation and characterization of induced mutants is difficult, physical mapping establishes points of reference to analyze sites of insertional mutagenesis (8, 9, 24, 35, 37) and enables the identification of genes involved in pathogenesis.

Several studies comparing DNA cleavage patterns and random amplified polymorphic DNA and fingerprint analysis have demonstrated that a genotypic heterogeneity exists among strains of *Mycoplasma gallisepticum* (13, 28, 30). We have chosen *M. gallisepticum* S6 for our studies on the basis of the premise that it is widely studied throughout the world. Here we describe the construction of a physical genomic map of *M. gallisepticum* S6 which provides a foundation for continued genetic analysis of this organism.

M. gallisepticum S6 was cultured at 37°C in Frey's medium (12). The cells (2×10^9 /ml) were pelleted by centrifugation at $12,000 \times g$ for 5 min at 4°C and washed twice with an equal volume of phosphate-buffered saline (130 mM NaCl–10 mM Na₂HPO₄ and 130 mM NaCl–10 mM NaH₂PO₄). DNA was prepared in agarose plugs according to a modification of the procedure described by Krause and Mawn (16). Three milliliters of pelleted mycoplasma culture (6×10^9 cells) was suspended in 125 µl of TE buffer (10 mM Tris base, 1 mM EDTA, pH 8.0). Then 125 µl of degrading solution (1 mg of proteinase K per ml in 100 mM EDTA, pH 8.0) and 250 µl of 1.5% low-melting-point agarose (Gibco BRL, Gaithersburg, Md.),

melted and equilibrated at 42°C, were added. This mixture was transferred to a plastic mold to form 60-µl agarose plugs. The plugs were transferred to 3.0 ml of degrading solution containing sodium dodecyl sulfate (SDS) at a final concentration of 2.5% and incubated at 42°C for 18 h with rocking. The plugs were then rinsed twice in wash solution (50 mM Tris HCl, 50 mM EDTA) for 15 min at room temperature with rocking. The proteinase K was inactivated with phenylmethylsulfonyl fluoride, at a final concentration of 10 mM, in wash solution at 37°C for 1 h with rocking. After two additional washes in wash solution, the plugs were stored at 4°C in storage solution (5 mM Tris HCl, 5 mM EDTA).

Restriction endonuclease digestions were performed on agarose-embedded DNA in microcentrifuge tubes. The restriction enzymes used were *EagI* and *I-CeuI* (New England BioLabs, Beverly, Mass.) and *SmaI* (Gibco BRL). Each plug was sliced in half and soaked for 2 h at 4°C in reaction buffer, according to the manufacturer's recommendations, at a final volume of 150 µl. The buffer was replaced with complete reaction mixture containing a restriction endonuclease, and the mixture was incubated at 4°C for an additional 2 h. *EagI* and *SmaI* digestions were performed with 30 U of enzyme. *I-CeuI* digestions were performed with 3 U of enzyme and were supplemented with 100 µg of bovine serum albumin per ml. The reactions were incubated at the appropriate temperature for 16 to 20 h. The plugs were soaked twice in 0.5 ml of storage solution for 30 min at 4°C. Double digestions were performed by repeating the procedure described above with the second enzyme.

DNA fragments were separated by field-inversion gel electrophoresis (FIGE) into 1% low electroendosmosis agarose (Gibco BRL) in 0.5× Tris buffer (TBE) (89 mM Tris-borate–89 mM boric acid–2 mM EDTA). Electrophoresis was performed at 4°C in horizontal gels at 6.9 V/cm in 0.5× TBE with field-inversion parameters programmed into the Switchback Pulse Controller (Hofer Scientific Instruments, San Francisco, Calif.). Multiple-run parameters were programmed with a 3:1 forward-reverse pulse ratio in the reverse mode, an initial run-in time of 5 min, and one of the following pulse time settings: a 1- to 20-s pulse time for 8 h followed by a 1- to 6-s pulse time for 16 h or a 1- to 30-s pulse time for 10 h followed by a 1- to 8-s pulse time for 12 h, depending on the size range of DNA fragments to be separated. Mid Range PFG Marker I and Lambda Ladder PFG Marker (New England BioLabs) were used as size markers. The average of at least three separate analyses was used to determine fragment sizes (Table 1). On the basis of *SmaI* and *EagI* single and double digests, we estimate the *M. gallisepticum* S6 genome to be 1,054 kb.

DNA fragments resolved by FIGE were transferred to BA-

* Corresponding author. Mailing address: Department of Pathobiology, University of Connecticut, 61 N. Eagleville Rd., Storrs, CT 06269-3089. Phone: (203) 486-0835. Fax: (203) 486-2794.

TABLE 1. *M. gallisepticum* S6 DNA fragments generated by single and double digestion of agarose-embedded DNA with *Sma*I and *Eag*I

Fragment(s)	<i>Sma</i> I		<i>Eag</i> I		<i>Sma</i> I- <i>Eag</i> I size (kb)
	Size ^a (kb)	Fragment(s)	Size (kb)	Fragment(s)	
Sm1	356 ± 14	Ea1, Ea2	268 ± 10		226 ± 9
Sm2	298 ± 8	Ea3	176 ± 5		172 ± 2
Sm3	130 ± 1	Ea4	129 ± 5		69 ± 2 ^b
Sm4	125 ± 4	Ea5	122 ± 2		64 ± 3 ^b
Sm5, Sm6	50 ± 1	Ea6	59 ± 2		61 ± 3 ^c
Sm7	33 ± 1	Ea7	35		50 ± 1
Sm8	10				35 ± 1
					33
					27
					10

^a Sizes are averages of at least three determinations for each fragment and are given with standard deviations from the mean. The total size of the *M. gallisepticum* S6 genome, on the basis of *Sma*I, *Eag*I, and *Sma*I-*Eag*I digests, was estimated to be 1,052, 1,057, and 1,052 kb, respectively.

^b Band contains two comigrating fragments.

^c Band contains three comigrating fragments.

S85 supported nitrocellulose (Schleicher and Schuell, Inc., Keene, N.H.) by a modified method of Southern (32). To enhance the transfer of large DNA fragments, electrophoresed DNA was partially depurinated by treatment with 0.25 M HCl for 10 min prior to alkali denaturation, according to the manufacturer's recommendations. Southern transfer was carried out for 20 to 24 h, and the blots were baked at 80°C for 1.5 h.

Blots were prehybridized for 2.5 h at 42°C in prehybridization fluid containing 6× SSC (900 mM NaCl, 90 mM sodium citrate), 5× Denhardt's solution (7), 10 mM EDTA, 0.5% SDS, and 100 μg of denatured salmon sperm DNA per ml, followed by hybridization overnight under stringent conditions (prehybridization fluid with 45% formamide at 42°C), moderately stringent conditions (prehybridization fluid with 45% formamide at 32°C), or conditions of only low stringency (prehybridization fluid with 22.5% formamide at 32°C). Post-hybridization washes were performed twice with 2× SSC-0.1% SDS at room temperature for 3 min each time and twice with 0.2× SSC-0.1% SDS at room temperature for 3 min each time. Nitrocellulose filters were dried, overlaid with Fuji RX film (Fisher Scientific, Pittsburgh, Pa.), and exposed for 12 to 16 h at -70°C.

Gene loci were determined by Southern blot hybridization analysis. The following plasmids and PCR-amplified product were used as hybridization probes: the cloned *Mycoplasma pneumoniae* *tuf* gene, encoding elongation factor Tu (40); plasmid pSC833 (5), containing the gyrase B gene and the 5' end of the gyrase A gene in a 2.7-kb *Sal*I fragment from *M. pneumoniae* (a gift from K. F. Bott, University of North Carolina, Chapel Hill); plasmid pMC5 (1), containing a portion of the 16S rRNA genes and the complete 23S and 5S rRNA genes of *Mycoplasma capricolum*; plasmid pMpP1 (14), containing a 5.0-kb fragment of the P1 cytoadhesin gene of *M. pneumoniae*; and a 0.3-kb fragment of the *M. gallisepticum* S6 *recA* gene amplified by PCR using degenerative primers (a gift from K. Dybvig, University of Alabama, Birmingham) as described by Dybvig et al. (10). Plasmids were maintained within transformed *E. coli* strains and extracted by the alkaline lysis method (17).

PCR was performed with AmpliTaq DNA polymerase (Applied Biosystem/Perkin Elmer, Norwalk, Conn.) in the Perkin Elmer Cetus DNA Thermal Cycler 480 (Applied Biosystem/Perkin Elmer). Amplification reactions for both the *M. galli-*

septicum S6 and *Acholeplasma laidlawii* (as a positive control) *recA* fragments were performed in 100-μl final volumes containing 2.5 U of AmpliTaq DNA polymerase, each nucleotide at 250 μM (dATP, dCTP, dGTP, and dTTP), 1.5 mM MgCl₂, 400 ng of each degenerate primer, and either 25 ng of S6 genomic DNA or 200 ng of 0.8-kb *A. laidlawii* *recA* fragment (11) (gift of K. Dybvig). Reaction mixtures were subjected to three cycles of the following: 1 min at 94°C, 1 min at 37°C, and 1 min at 72°C, with a ramp time of 2 min for adjustment of the temperature from 37°C to 72°C. After these initial cycles, the reaction mixtures were subjected to 30 cycles of the following: 1 min at 94°C, 1 min at 55°C, and 1 min at 72°C, with a final extension for 10 min at 72°C. *M. gallisepticum* S6 *recA* fragment identity was confirmed by sequencing (GenBank accession number, L36389) and hybridization (data not shown) using the positive control *A. laidlawii* *recA* fragment as a probe under stringent conditions.

Primers flanking the P1 gene of *M. pneumoniae* were synthesized from a published nucleotide sequence (34) to amplify a 5.0-kb fragment of the P1 gene. The oligonucleotide primer sequences are 5'-CAA AAA AAC TGC CCT GTC CA-3' (forward) and 5'-GGG CGT GTA GGG GGT GAG GG-3' (reverse). Amplification reactions for the *M. pneumoniae* P1 gene were performed in a 100-μl final volume containing 2.5 U of AmpliTaq DNA polymerase, each nucleotide at 250 μM (dATP, dCTP, dGTP, and dTTP), 2.5 mM MgCl₂, 350 ng of each primer, and 50 ng of *M. pneumoniae* genomic DNA. Reaction mixtures were subjected to 20 cycles of the following: 1 min at 94°C, 30 s at 60°C, and 2 min at 72°C, with a final extension for 10 min at 72°C. The amplified *M. pneumoniae* P1 gene was then cloned into the pCRII TA cloning vector and transformed into *E. coli* INVαF' according to the manufacturer's instructions (Invitrogen Corporation, San Diego, Calif.). The identity of the P1 gene was confirmed by nucleotide se-

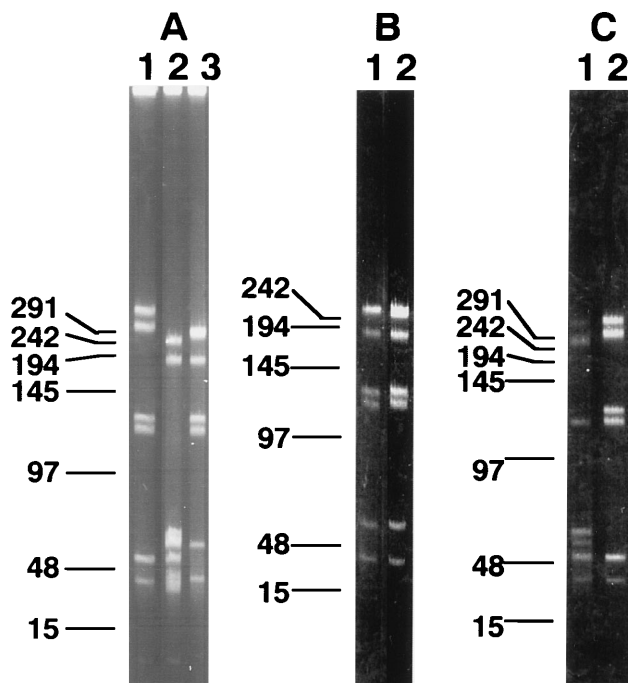


FIG. 1. *M. gallisepticum* S6 DNA separated by FIGE. (A) Lane 1, *Sma*I; lane 2, *Sma*I plus *Eag*I; lane 3, *Eag*I. (B) Lane 1, *Eag*I plus I-*Ceu*I; lane 2, *Eag*I. (C) Lane 1, *Sma*I plus I-*Ceu*I; lane 2, *Sma*I. For visual comparison, the lanes were reordered via computer imaging with Adobe Photoshop.

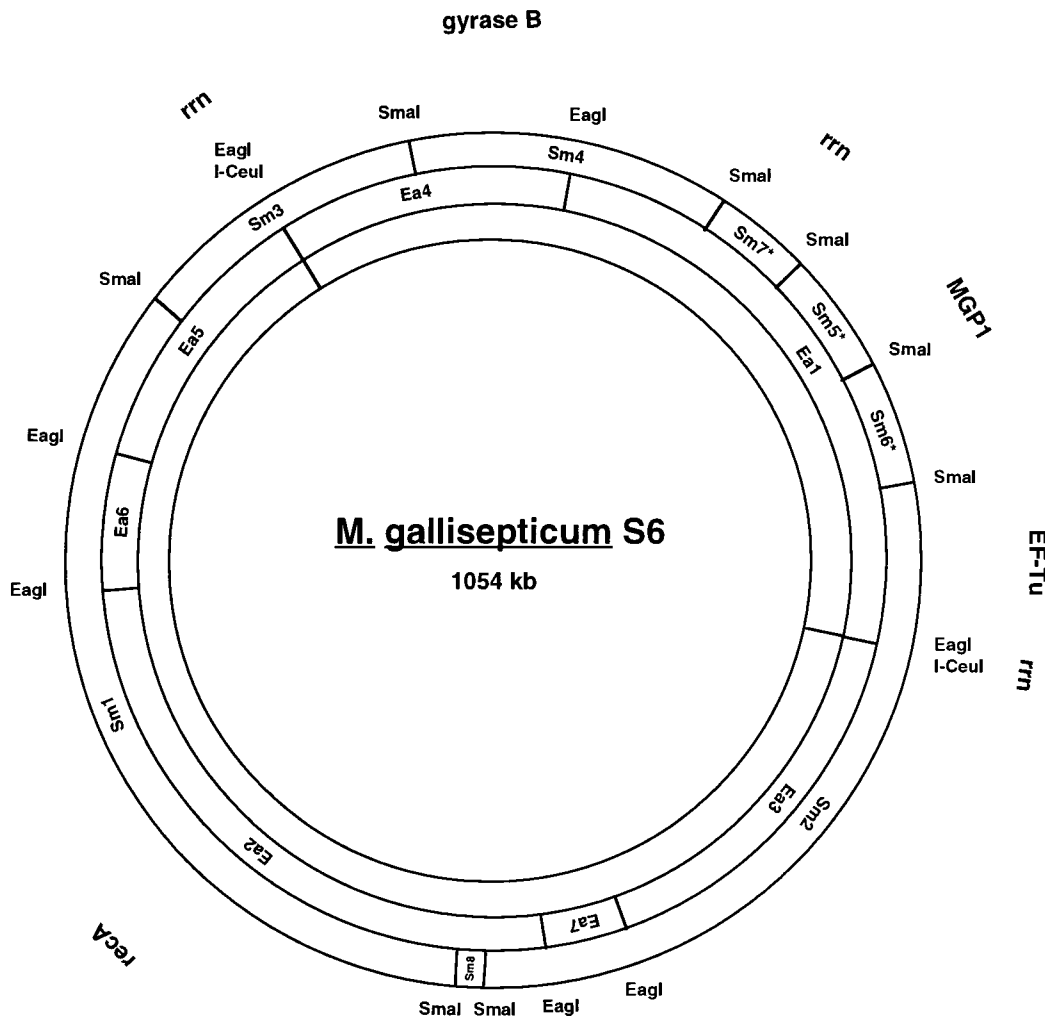


FIG. 2. Physical genomic map of *M. gallisepticum* S6. The loci of mapped restriction enzyme sites and genes are indicated outside the circle. The order of fragments generated from digests using individual restriction enzymes is indicated in the inner circles. Fragments Sm5, Sm6, and Sm7 are marked with asterisks to indicate that their relative order is presently unclear.

quence analysis of 0.3 kb of each end of the cloned PCR product.

All DNA probes were labeled by the incorporation of [³²P]dATP (DuPont, NEN Research Products, Boston, Mass.) by standard nick translation procedures (29).

M. pneumoniae and *M. gallisepticum* S6 genomic DNAs were digested with *EcoRI* and electrophoresed in a 0.8% agarose gel. Southern blot analysis was performed as described above with the 5.0-kb P1 cytoadhesin gene as a probe (14). The hybridization pattern for *M. pneumoniae* (14) was consistent with published data (33).

Goh et al. (14) have shown, by hybridization analysis, that the cloned P1 fragment, pMpP1, recognizes a single *EcoRI* fragment of *M. gallisepticum* S6. This *EcoRI* fragment has been cloned, sequenced, and shown to be the *M. gallisepticum* homolog of the *M. pneumoniae* P1 gene. This homolog, termed MGP1, hybridized with the same single *M. gallisepticum* *EcoRI* fragment, suggesting that the P1 homolog in *M. gallisepticum* S6 exists as a single-copy gene, as it does in *M. pneumoniae* and *Mycoplasma genitalium*. MGP1, however, lacks the repeat characteristics of its *M. pneumoniae* and *M. genitalium* counterparts, P1 and MgPa, respectively. As the role of the repeat

sequences in P1 and MgPa is currently undefined, the lack of such repeats in MGP1 is of interest, but its significance has yet to be determined.

Digestion of *M. gallisepticum* S6 DNA in agarose plugs with *SmaI* generated eight fragments (Table 1). Seven bands were resolved by FIGE (Fig. 1A, lane 1). An increased intensity of the 50-kb band (Fig. 1A, lane 1) upon staining with ethidium bromide suggested that it was two bands. It was confirmed that Sm5 and Sm6 comigrated as one band by excising the band and digesting it with *SalI* (data not shown). Digestion with *EagI* generated seven fragments (Table 1). An increased intensity of the 270-kb band (Fig. 1A, lane 3) upon staining with ethidium bromide suggested that two bands were migrating as one. Double digestion with *EagI* and *SmaI* confirmed that Ea1 and Ea2 were in fact comigrating.

EagI and *SmaI* single and double digests (Fig. 1A, lane 2) were analyzed to determine the relative positions of the respective enzyme sites. Upon double digestion of agarose plugs, only nine bands were resolved by FIGE. With *SmaI* and *EagI* single digests generating eight and seven bands, respectively, double digests should produce fifteen bands. The third and fourth bands from the top in lane 2 (Fig. 1A) exhibited much

greater intensities upon staining with ethidium bromide, suggesting that these bands consisted of several fragments. On the basis of the sizes of the single-digest fragments and the resulting sizes of the double-digest fragments, we predicted that seven fragments were comigrating between approximately 61 and 69 kb. Fragments generated upon double digestion are shown in Table 1. Fragments Sm5, Sm6, Sm7, Sm8, Ea3, Ea6, and Ea7 remained uncut following double digestion. Fragments Sm1, Sm2, Sm3, and Sm4 were absent in double digests, indicating internal *EagI* sites. Fragments Ea1, Ea2, Ea4, and Ea5 were absent in double digests and therefore contain *SmaI* sites.

The endonuclease I-*CeuI* has been shown to cleave within the 23S rRNA genes of *E. coli*, *Salmonella* spp., and other eubacteria (19–22). Restriction enzyme site analysis of the DNA sequence for the 23S rRNA genes of *Mycoplasma hyopneumoniae* and *M. pneumoniae* (23) identifies an I-*CeuI* site, suggesting conservation of this sequence among mycoplasmas. *SmaI* and I-*CeuI* double digestion of S6 DNA (Fig. 1C, lane 1) identifies an I-*CeuI* site within both the Sm2 and Sm3 fragments. These data suggest that two copies of the 23S rRNA gene exist within the genome, located within the Sm2 and Sm3 fragments. These data are consistent with previous reports (3, 31) that two copies of the rRNA operon exist in *M. gallisepticum* S6: one complete copy of the 16S-23S-5S rRNA genes and one copy of the 16S rRNA gene separated from the 23S-5S rRNA genes. Scamrov and Beabealashvili (31) also suggest the existence of a third 16S rRNA gene.

Fragments were aligned in a physical map (Fig. 2) on the basis of fragment size estimates and Southern hybridization using selected genes. The cloned *M. pneumoniae* *tuf* gene hybridized, under conditions of moderate stringency, with fragments Ea1 and Sm2. The genes encoding *M. pneumoniae* DNA gyrase subunit B and the N-terminal portion of subunit A hybridized, under conditions of moderately low stringency, with fragments Sm4 and Ea4. We indicate on the map (Fig. 2) the location of the gyrase B subunit, as there are currently no published data showing that the S6 gyrase subunits A and B are linked. However, preliminary data within our laboratory indicate that these two genes are indeed linked. The 0.3-kb fragment of the *M. gallisepticum* *recA* gene hybridized, under conditions of high stringency, with fragments Sm1 and Ea2. The *M. pneumoniae* P1 cytoadhesin gene hybridized, under conditions of low stringency, with fragment Ea1 and either Sm5 or Sm6. On the basis of fragment size estimations and these hybridization data both Sm5 and Sm6 are located within Ea1, but their relative positions have not yet been determined. The *Mycoplasma capricolum* *rrn* genes hybridized, under conditions of moderate stringency, with fragments Sm2, Sm3, Sm7, Ea1, Ea3, Ea4, and Ea5. These hybridization data, the I-*CeuI* restriction enzyme digest data, and a previous report of the *tuf* gene being located adjacent to the complete rRNA operon in *M. gallisepticum* S6 (31) suggest that the complete 16S-23S-5S rRNA operon exists within the Sm2 fragment, that the separated 16S and 23S-5S rRNA genes exist within the Sm3 fragment, and that the third 16S rRNA gene lies within the Sm7 fragment.

Little information exists on *M. gallisepticum* regarding the gene locations, operon organizations, or nucleic acid sequences of genes encoding proteins involved in either metabolism or pathogenicity. Here we report the construction of a physical genomic map of *M. gallisepticum* S6 identifying *SmaI*, *EagI*, and I-*CeuI* restriction sites and the loci of the genes encoding elongation factor Tu, gyrase B, rRNA, RecA, and MGP1. The total genome size is estimated to be 1,054 kb. While the manuscript was in review, an article describing the

physical maps of *M. gallisepticum* R and ATCC 19610 was published (36). These maps show *EagI* and *SmaI* restriction sites, transposon insertion sites, and the locations of the 16S rRNA genes. The results from these two studies support the findings of earlier studies (13, 28, 30) that demonstrated genotypic heterogeneity among *M. gallisepticum* strains. The physical genomic map of *M. gallisepticum* S6 reported here provides a useful foundation for further genetic analysis of genes involved in the pathogenicity of this strain. This map will prove useful for the placement of loci of other genes, as well as for future transposon mutagenesis studies of *M. gallisepticum* S6 to identify genes, such as those involved with adherence.

We thank Mark Forsyth for helpful discussions and critical review of the manuscript.

This research was supported in part by a research grant from the University of Connecticut Research Foundation to S.J.G. (grant 440114) and a Grant-in-Aid of Research from Sigma Xi, The Scientific Research Society, to T.S.G.

REFERENCES

- Amikam, D., S. Razin, and G. Glaser. 1982. Ribosomal RNA genes in mycoplasma. *Nucleic Acids Res.* **10**:4215–4222.
- Bautsch, W. 1988. Rapid physical mapping of the *Mycoplasma mobile* genome by two-dimensional field inversion gel electrophoresis techniques. *Nucleic Acids Res.* **16**:11461–11467.
- Chen, X., and L. R. Finch. 1989. Novel arrangement of rRNA genes in *Mycoplasma gallisepticum*: separation of the 16S gene of one set from the 23S and 5S genes. *J. Bacteriol.* **171**:2876–2878.
- Cocks, B. G., L. E. Pyle, and L. R. Finch. 1989. A physical map of the genome of *Ureaplasma urealyticum* 960T with ribosomal RNA loci. *Nucleic Acids Res.* **17**:6713–6719.
- Colman, S. D., P.-C. Hu, and K. F. Bott. 1990. *Mycoplasma pneumoniae* DNA gyrase genes. *Mol. Microbiol.* **4**:1129–1134.
- Colman, S. D., P.-C. Hu, W. Litaker, and K. F. Bott. 1990. A physical map of the *Mycoplasma genitalium* genome. *Mol. Microbiol.* **4**:683–687.
- Denhardt, D. 1966. A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* **23**:641–652.
- Dybvig, K., and J. Aldrette. 1988. Transformation of *Mycoplasma pulmonis* and *Mycoplasma hyorhinis*: transformation of Tn916 and formation of cointegrate structures. *Plasmid* **20**:33–41.
- Dybvig, K., and G. H. Cassell. 1987. Transposition of gram-positive transposon Tn916 in *Acholeplasma laidlawii* and *Mycoplasma pulmonis*. *Science* **253**:1392–1394.
- Dybvig, K., S. K. Hollingshead, D. G. Heath, D. B. Clewell, F. Sun, and A. Woodard. 1992. Degenerative oligonucleotide primers for enzymatic amplification of *recA* sequences from gram-positive bacteria and mycoplasmas. *J. Bacteriol.* **174**:2729–2732.
- Dybvig, K., and A. Woodard. 1992. Cloning and DNA sequence of a mycoplasma *recA* gene. *J. Bacteriol.* **174**:778–784.
- Frey, M. L., R. P. Hanson, and D. R. Anderson. 1968. A medium for the isolation of avian mycoplasmas. *Am. J. Vet. Res.* **29**:2163–2171.
- Geary, S. J., M. H. Forsyth, S. Aboul Saoud, G. Wang, D. E. Berg, and C. M. Berg. 1994. *Mycoplasma gallisepticum* strain differentiation by arbitrary primer PCR (RAPD) fingerprinting. *Mol. Cell. Probes* **8**:311–316.
- Goh, M. S., M. H. Forsyth, T. S. Gorton, and S. J. Geary. 1994. Cloning and sequence analysis of a *Mycoplasma gallisepticum* cytoadhesin gene, abstr. P 252, p. 669. In IOM letters, vol. 3. Abstracts of the 10th International Congress of the International Organization for Mycoplasmaology 1994. International Organization for Mycoplasmaology, Bordeaux, France.
- Inamine, J. M., K. C. Ho, S. Loechel, and P.-C. Hu. 1990. Evidence that UGA is read as a tryptophan codon rather than as a stop codon by *Mycoplasma pneumoniae*, *Mycoplasma genitalium*, and *Mycoplasma gallisepticum*. *J. Bacteriol.* **172**:504–506.
- Krause, D. C., and C. B. Mawn. 1990. Physical analysis and mapping of the *Mycoplasma pneumoniae* chromosome. *J. Bacteriol.* **172**:4790–4797.
- Krieg, P., and D. Melton. 1991. Rapid isolation of plasmid DNA, p. 106. In D. E. Titus (ed.), *Promega protocols and applications guide*. Promega Corporation, Madison, Wis.
- Ladefoged, S. A., and G. Christiansen. 1992. Physical and genetic mapping of the genomes of five *Mycoplasma hominis* strains by pulsed-field gel electrophoresis. *J. Bacteriol.* **174**:2199–2207.
- Liu, S.-L., A. Hessel, H.-Y. M. Cheng, and K. E. Sanderson. 1994. The *XbaI*-*BlnI*-*CeuI* genomic cleavage map of *Salmonella paratyphi* B. *J. Bacteriol.* **176**:1014–1024.
- Liu, S.-L., A. Hessel, and K. E. Sanderson. 1993. Genomic mapping with I-*CeuI* and intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. *Proc. Natl.*

- Acad. Sci. USA **90**:6874–6878.
21. Liu, S.-L., A. Hessel, and K. E. Sanderson. 1993. *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella typhimurium* LT2, determined by double digestion, end labelling, and pulsed-field electrophoresis. *J. Bacteriol.* **175**:4104–4120.
 22. Liu, S.-L., A. Hessel, and K. E. Sanderson. 1994. The *Xba*I-*Bln*I-*Ceu*I genomic cleavage map of *Salmonella enteritidis* shows an inversion relative to *S. typhimurium* LT2. *Mol. Microbiol.* **10**:655–664.
 23. Ludwig, W., G. Kirchof, N. Klugbauer, M. Weizenegger, D. Betzl, M. Ehrmann, C. Herter, S. Jilg, R. Tatzel, H. Zitzelsberger, S. Liebl, M. Hochberger, J. Shah, D. Lane, and P. R. Wallnoef. 1992. Complete 23S ribosomal RNA sequences of gram-positive bacteria with a low DNA G+C content. *Syst. Appl. Microbiol.* **15**:487–501.
 24. Mahairas, G. G., and F. C. Minion. 1989. Random insertion of the gentamicin resistance transposon Tn4001 in *Mycoplasma pulmonis*. *Plasmid* **21**: 43–47.
 25. Miyata, M., L. Wang, and T. Fukumura. 1991. Physical mapping of the *Mycoplasma capricolum* genome. *FEMS Microbiol. Lett.* **79**:329–334.
 26. Pyle, L. E., and L. R. Finch. 1988. A physical map of the genome of *Mycoplasma mycoides* subspecies *mycoides* Y with some functional loci. *Nucleic Acids Res.* **16**:6027–6039.
 27. Pyle, L. E., T. Taylor, and L. R. Finch. 1990. Genomic map of some strains of the *Mycoplasma mycoides* cluster. *J. Bacteriol.* **172**:7265–7268.
 28. Razin, S., J. G. Tully, D. L. Rose, and M. F. Barile. 1983. DNA cleavage patterns as indicators of genotypic heterogeneity among strains of *Acholeplasma* and *Mycoplasma* species. *J. Gen. Microbiol.* **129**:1935–1944.
 29. Rigby, P. W. J., M. Dieckmann, C. Rhodes, and P. Berg. 1977. Labeling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. *J. Mol. Biol.* **113**:237–251.
 30. Santha, M., K. Lukacs, K. Burg, S. Bernath, I. Rasko, and L. Stipkovits. 1988. Intraspecies genotypic heterogeneity among *Mycoplasma gallisepticum* strains. *Appl. Environ. Microbiol.* **54**:607–609.
 31. Scamrov, A., and R. Beabealashvilli. 1991. *Mycoplasma gallisepticum* strain S6 genome contains three regions hybridizing with 16S rRNA and two regions hybridizing with 23S and 5S rRNA. *FEBS Lett.* **291**:71–74.
 32. Southern, E. M. 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**:503–517.
 33. Su, C. J., A. Chavoya, and J. B. Baseman. 1988. Regions of *Mycoplasma pneumoniae* cytoadhesin P1 structural gene exist as multiple copies. *Infect. Immun.* **56**:3157–3161.
 34. Su, C. J., V. V. Tryon, and J. B. Baseman. 1987. Cloning and sequence analysis of cytoadhesin gene (P1) from *Mycoplasma pneumoniae*. *Infect. Immun.* **55**:3023–3029.
 35. Tigges, E., and F. C. Minion. 1994. Physical map of the genome of *Acholeplasma oculi* ISM1499 and construction of a Tn4001 derivative for macrorestriction chromosomal mapping. *J. Bacteriol.* **176**:1180–1183.
 36. Tigges, E., and F. C. Minion. 1994. Physical map of *Mycoplasma gallisepticum*. *J. Bacteriol.* **176**:4157–4159.
 37. Whitley, J. C., and L. R. Finch. 1989. Location of sites of transposon Tn916 insertion in the *Mycoplasma mycoides* genome. *J. Bacteriol.* **171**:6870–6872.
 38. Woese, C. R., J. Maniloff, and L. B. Zablen. 1980. Phylogenetic analysis of the mycoplasmas. *Proc. Natl. Acad. Sci. USA* **77**:494–498.
 39. Yamao, F., A. Muto, Y. Kaqauchi, M. Iwami, S. Iwagami, Y. Azumi, and S. Osawa. 1985. UGA is read as tryptophan in *M. capricolum*. *Proc. Natl. Acad. Sci. USA* **82**:2306–2309.
 40. Yogeve, D., S. Sela, H. Bercoquier, and S. Razin. 1990. Nucleotide sequence and codon usage of the elongation factor Tu(EF-Tu) gene from *Mycoplasma pneumoniae*. *Mol. Microbiol.* **4**:1303–1310.