Restriction enzyme digestion and field inversion gel electrophoresis were used to analyze the chromosomes of strains of *Mycoplasma hyopneumoniae* and the related organism *Mycoplasma flocculare*. The chromosome size for the *M. hyopneumoniae* type strain was calculated from individual fragments to be 1,011.3 ± 32.9 kbp. The chromosomes of *M. hyopneumoniae* field strains were approximately the same size. The restriction patterns obtained for the chromosomes of phenotypically similar *M. hyopneumoniae* strains were quite different. Therefore, the species *M. hyopneumoniae* seems to be very heterogeneous. A field inversion gel electrophoresis analysis of the entire chromosomes allowed us to distinguish *M. hyopneumoniae* strains easily and hence to characterize further the species *M. hyopneumoniae*. The chromosome size for *M. flocculare* was calculated to be 988.3 ± 39.5 kbp. Restriction enzyme *XhoI*, which statistically should cut the *M. hyopneumoniae* chromosome frequently, did not cut the DNA of any of the *M. hyopneumoniae* strains but did digest *M. flocculare* DNA, indicating that there is a site-specific modification at CTCGAG which probably belongs to a restriction modification system in *M. hyopneumoniae* and is absent in *M. flocculare*.

*Mycoplasma hyopneumoniae* and *Mycoplasma flocculare* are commonly isolated from porcine respiratory tracts. *M. hyopneumoniae* is the primary agent of mycoplasmal pneumonia in swine (enzoic porcine pneumonia) (11, 19). This disease is common all over the world and causes large economic losses through retarded growth, poor food conversion, and increased susceptibility of swine to infection by other organisms (27). On the other hand, *M. flocculare* does not seem to be implicated directly in porcine mycoplasmal pneumonia (10). Studies on the taxonomic relationship between *M. hyopneumoniae* and *M. flocculare* have revealed only a few biochemical or cultural differences between these two mycoplasmas (26); the differentiation of these species is based on the results of serological tests, which are hampered by immunological cross-reactions between the species (1, 23). The two species are clearly different at the genetic level, since DNA-DNA hybridization has revealed levels of sequence identity of only approximately 10% (10). No heterogeneity has been reported previously within the species *M. hyopneumoniae*, probably because of the limited tests which are available for differentiation of strains.

Most information on *M. hyopneumoniae* has been obtained from analyses of type strain ATCC 25934 (= NCTC 10110 = J) (11, 26), which has been reported to be indistinguishable from field strains on the basis of the results of growth inhibition, metabolic inhibition, and direct immunoelectrophoresis tests (24, 26). However, very little is known about possible differences in the chromosome structure, immunogenic factors, and virulence of *M. hyopneumoniae* strains. Although immunoblot analyses performed with rabbit hyperimmune serum or serum from experimentally infected pigs do not distinguish *M. hyopneumoniae* strains, Ro and Ross (23) have detected antigenetic diversity among various *M. hyopneumoniae* strains by two-dimensional immunoelectrophoresis. Stipkovits et al. (31) have reported that an early and strongly immunogenic protein, protein P36, is specific to *M. hyopneumoniae* and is conserved in many *M. hyopneumoniae* strains obtained from various geographic areas. Therefore, this protein is a useful marker for identification of *M. hyopneumoniae* strains and infections. It is essential to have a better understanding of the degree of genetic relatedness or variability among *M. hyopneumoniae* strains for studies on the taxonomy, epidemiology, and pathogenicity of this organism. Any analysis of genetic variability should be practical and reliable. In addition, fundamental knowledge concerning the structure and sizes of the chromosomes of the *M. hyopneumoniae* and the related nonpathogenic organism *M. flocculare* will be necessary to conduct further genetic studies on these species.

Restriction endonuclease fingerprinting analysis is a powerful technique for comparing DNAs of phenotypically and genetically similar organisms (13, 14, 17). However, this method is often hampered by the large number of DNA bands which have to be resolved or by the fact that only bands in a certain size range, representing only a part of the genome, can be analyzed. Field inversion gel electrophoresis (FIGE) (29) is an ideal tool for separating large DNA fragments in analyses of bacterial chromosomes. In this study our objective was to use restriction enzyme digestion and FIGE to analyze the chromosome of the *M. hyopneumoniae* type strain and to use this methodology to compare this type strain with various *M. hyopneumoniae* field strains and *M. flocculare* strains. We took advantage of the relatively low G+C contents (33 mol%) of the chromosomes of these two *Mycoplasma* species (30) and used restriction enzymes with GC-rich recognition sites to cut the chromosomes into approximately 20 large restriction fragments. This analysis permitted us to accurately calculate the sizes of the chromosomes of *M. hyopneumoniae* and *M. flocculare* and to investigate the genetic heterogeneity in various *M. hyopneumoniae* field strains.

**MATERIALS AND METHODS**

**Strains and growth conditions.** The porcine *Mycoplasma* strains used in this study are shown in Table 1. The strains were grown in Friis medium (10) at 37°C to the end of the exponential growth phase, which occurred when the pH of the medium was 6.8 (corresponding to a concentration of

* Corresponding author.
approximately $10^8$ cells per ml). Chloramphenicol was then added to the growth medium at a concentration of 80 $\mu$g/ml, and the cells were incubated for another 2 h before they were harvested in order to let replication forks terminate without the initiation of new rounds of replication (16, 22). The cells were harvested by centrifugation at 20,000 $\times g$ for 20 min, washed three times in phosphate-buffered saline (10 mM sodium phosphate [pH 7.0], 0.9% NaCl), resuspended in phosphate-buffered saline at a concentration of $3 \times 10^9$ cells per ml, and kept frozen until they were used. The field isolates of \textit{M. hyopneumoniae} were identified by using the growth inhibition test described by Clyde (7) and antisera against strain J\textsuperscript{T} (T = type strain) (11). The strains were also tested for the presence of protein P36, a common and specific antigenic protein of \textit{M. hyopneumoniae} (31).

**Southern hybridization of genomic DNA.** Genomic DNAs of \textit{M. hyopneumoniae} and \textit{M. flocculare} were isolated as described previously (32). Aliquots (500 ng) of DNA were digested with EcoRI, separated on a 0.7% agarose gel in TBE buffer (28), and blotted onto a nitrocellulose membrane (Bio-Rad Laboratories, Richmond, Calif.), by using a Pharmacia model LKB 1962 VacuGene system according to the instructions of the supplier. The probes which we used were chromosomal DNAs of \textit{M. hyopneumoniae} NCTC 10110\textsuperscript{T} and \textit{M. flocculare} Ms 42\textsuperscript{T} that were digested with EcoRI and labeled with $^{32}$PdCTP (3,000 Ci/mmol; Amersham) by using the random priming method (8). Hybridizations were carried out at 37°C in 5 x SSC (1x SSC is 0.15 M NaCl plus 0.015 M trisodium citrate [pH 7.0]) containing 5% polyethylene glycol 6000, 0.5% sodium dodecyl sulfate, 50% formamide, and 100 $\mu$g of denatured and sonified salmon sperm DNA per ml. The filters were washed twice in 0.1x SSC-0.1% sodium dodecyl sulfate at 50°C, a temperature that corresponded to the melting temperature of DNA-DNA duplexes having approximately 85% sequence identity. The filters were exposed for 1 day at -80°C on Fuji RX film by using an intensifying screen.

**Preparation of chromosomal DNA in agarose blocks and FIGE.** Mycoplasma cells were molded into blocks (1.5 by 5.5 by 4 mm) of low-gelling low-melting-point (LGLM) agarose (Biofinex, Praroman, Switzerland) at a concentration of 4 x $10^9$ cells per ml as described previously (22). The Mycoplasma cells were then lysed by placing the blocks in ETS buffer (10 mM Tris-HCl, 500 mM EDTA, 1% N-lauroylsarcosine [catalog no. L-5125; Sigma]) containing 1 mg of proteinase K (Sigma) per ml (pH 8.0) at 50°C for 18 h, and then they were washed three times for 2 h in TE buffer (10 mM Tris-HCl, 1 mM EDTA; pH 8.0). DNA restriction digestion was performed by incubating the agar blocks in 250 $\mu$l of the appropriate assay buffer (as recommended by the producer) containing 20 U of restriction endonuclease (Boehringer Mannheim). The DNA was methylated in 250 $\mu$l of TaqI methylase buffer (Biolabs, Beverly, Mass.) containing 2 mM dithiothreitol, 0.1 mM S-adenosylmethionine, and 30 U of TaqI methylase.

We produced multimers of bacteriophage $\lambda$ CR875Sam7 DNA (Boehringer Mannheim) to use as molecular size markers for DNA fragments by using the method of Bancroft and Wolk (2), taking care to thaw the DNA only once, just before multimerization and molding into agarose blocks. In addition, intact chromosomes of \textit{Saccharomyces cerevisiae} YD148 (21) which were prepared by using the method of Bellis et al. (3) were used as size standards.

**FIGE was performed as described by Carle et al. (5).** Agarose gels (0.8%) were run for 40 h in TBE buffer (44.5 mM Tris, 44.5 mM sodium borate, 1 mM EDTA; pH 8.0) cooled to 15°C by using a voltage gradient of 4.0 V/cm. Pulse times were generated by using a model PFI-200 power inverter (MJ Research, Cambridge, Mass.). Preprogrammed pulse time ramps varying from 0.15 to 75 s forward and 0.05 to 25 s backward (according to the size range of DNA fragments to be analyzed) were generated by following the supplier's instructions. After electrophoresis, the gels were stained in ethidium bromide (1 $\mu$g/ml) for 30 min, and then they destained in 1 mM MgSO\textsubscript{4} for 60 min before photography under UV light. DNA fragments less than 20 kbp long were analyzed by conventional agarose gel electrophoresis (28), using bacteriophage $\lambda$ DNA digested with HindIII as the size standard.

**RESULTS**

**Chromosome sizes for \textit{M. hyopneumoniae} and \textit{M. flocculare.** Enzymatic digestion of the chromosomal DNA of \textit{M. hyopneumoniae} ATCC 25934\textsuperscript{T} with 35 restriction enzymes with recognition sequences of 6 bp revealed that the enzymes that have only G and C residues in their recognition sequences generated 12 to 25 different restriction fragments (Table 2), while the numbers of fragments that were generated by the other enzymes were more than 50. Restriction enzyme XhoI did not cut \textit{M. hyopneumoniae} DNA. Digestion with 

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
Species & Strain & Origin & Reference or source* \\
\hline
\textit{M. hyopneumoniae} & ATCC 25934\textsuperscript{T} (= J\textsuperscript{T}) & United Kingdom & ATCC \\
\textit{M. hyopneumoniae} & NCTC 10110\textsuperscript{T} (= J\textsuperscript{T}) & United Kingdom & NCTC \\
\textit{M. hyopneumoniae} & EP-184\textsuperscript{a} (= J\textsuperscript{T}) & United Kingdom & 12 \\
\textit{M. hyopneumoniae} & EP-29 & Canada (C. L'Ecuyer) & Field isolate \\
\textit{M. hyopneumoniae} & BQ 14 & France (M. Kobisch) & Field isolate \\
\textit{M. hyopneumoniae} & EP-S 924 & Switzerland & Field isolate (our laboratory) \\
\textit{M. hyopneumoniae} & EP-S 938 & Switzerland & Field isolate (our laboratory) \\
\textit{M. hyopneumoniae} & EP-S 939 & Switzerland & Field isolate (our laboratory) \\
\textit{M. hyopneumoniae} & EP-S 946 & Switzerland & Field isolate (our laboratory) \\
\textit{M. hyopneumoniae} & EP-S 223 & Switzerland & Field isolate (our laboratory) \\
\textit{M. flocculare} & NCTC 10143\textsuperscript{T} (= Ms 42\textsuperscript{T}) & Denmark & NCTC \\
\hline
\end{tabular}
\caption{Mycoplasma strains}
\end{table}

* ATCC, American Type Culture Collection, Rockville, Md.; NCTC, National Collection of Type Cultures, London, United Kingdom.
ATCC 25934T and M. flocculare NCTC 10143T chromosomal DNAs that were generated with *ApaI* were subjected to analysis on different FIGE gels by using various FIGE parameters in order to obtain high resolution of the sizes of all of the fragments which are shown in Table 3. The fragment sizes were calculated from the results of 10 independent experiments, and the accuracy of the size determinations was estimated to be 5% or better. Addition of the lengths of the different fragments indicated that the sizes of the *M. hyopneumoniae* ATCC 25934T and *M. flocculare* NCTC 10143T chromosomes are 1,011.3 ± 32.9 and 988 ± 39.5 kbp, respectively.

**Chromosomal divergence.** In order to estimate roughly the genetic similarity of the chromosomes of *M. hyopneumoniae* ATCC 25934T, the field isolates, and *M. flocculare*, chromosomal DNAs of the strains were digested with *EcoRI*, separated by agarose gel electrophoresis, and analyzed by Southern blots and *EcoRI*-digested 32P-labeled chromosomal DNAs were digested with restriction enzymes which contain the same "core" sequence (TCGA) in their recognition sequence as *M. hyopneumoniae* or *M. flocculare* treated with XhoI or a chromosome treated with XhoI or a chromosome DNAs of all *M. hyopneumoniae* field strains produced hybridization patterns on autoradiograms that could not be distinguished from the pattern of the homologous type strain. In addition, no clear differences in the *EcoRI* restriction patterns of the various strains were observed because of the high number of bands.

Since *M. flocculare* DNA had only a few bands that hybridized with *M. hyopneumoniae*, the level of sequence identity between the chromosomal DNAs of these two species was estimated to be less than 10%.

A FIGE analysis of chromosomal DNAs that were digested with *ApaI* showed that there were clear differences in the restriction patterns of the various *M. hyopneumoniae* strains (Fig. 1). This method also showed that the restriction pattern for type strain J was identical to the patterns for the two type strains obtained from culture collections, strains ATCC 25934 and NCTC 10110 (Fig. 1). Interestingly, strain JF 184aT which was a clone of strain JT (received from P. Whittlestone) but had undergone a different number of passages in culture medium than the culture collection strains, contained an additional 125-kb *ApaI* fragment (Fig. 1) but lacked the 8-kb *ApaI* fragment (not visible on Fig. 1). Digestion with BstEII confirmed that strain JF 184aT contained additional DNA fragments compared with strains ATCC 25934T and NCTC 10110T. The various field strains produced different *ApaI* restriction patterns and could all be distinguished by the FIGE analysis. However, the field strains from Switzerland that were isolated by workers from our laboratory seemed to produce more homogeneous restriction patterns than the strains that had different geographical origins. The chromosome sizes of the different field strains were very similar to the chromosome size of the type strain (close to 1,000 kb) (Fig. 1). The same results were obtained when we used restriction enzyme BstEII.

**Site-specific modification of *M. hyopneumoniae* DNA.** The DNAs of all of the *M. hyopneumoniae* strains that were tested in our study were completely resistant to digestion by *XhoI*, while *M. flocculare* DNA was fully digested and produced a large number of small fragments. *XhoI* restriction was sensitive to methylation of the C residue in position 3 and the A residue in position 5 (CTC'TGAG). Therefore, we used the restriction enzymes which contain the same "core" sequence (TCGA) in their recognition sequence as *XhoI* (CTCGAG) and which are sensitive to A residue methylation. Both *M. hyopneumoniae* and *M. flocculare* DNAs were digested with restriction enzymes BstBI (TTCCGAA), CiaI (ATCGAT), SalI (GTCGAC), and TaqI (TCGA). DNAs from *M. hyopneumoniae* and *M. flocculare* that were methylated by using *TaqI* methylase and then incubated with any of the restriction enzymes mentioned above (data not shown). It is worthwhile to note that an *M. hyopneumoniae* chromosome treated with *XhoI* or a chromosome from *M. hyopneumoniae* or *M. flocculare* treated with *TaqI* methylase and then incubated with any of the restriction enzymes mentioned above on FIGE analysis produced a band at approximately 1,000 kb that corresponded to the full-size chromosome. This might have represented chromosomal DNA that was randomly linearized once.

**TABLE 3. ApaI fragments of *M. hyopneumoniae* and *M. flocculare***

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Size (kbp) in:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>M. hyopneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>ATCC 25934T</td>
</tr>
<tr>
<td>A</td>
<td>278.3 ± 6.2</td>
</tr>
<tr>
<td>B</td>
<td>154.0 ± 4.3</td>
</tr>
<tr>
<td>C</td>
<td>144.5 ± 4.3</td>
</tr>
<tr>
<td>D</td>
<td>117.0 ± 2.2</td>
</tr>
<tr>
<td>E</td>
<td>106.6 ± 2.3</td>
</tr>
<tr>
<td>F</td>
<td>72.3 ± 5.4</td>
</tr>
<tr>
<td>G</td>
<td>63.0 ± 3.7</td>
</tr>
<tr>
<td>H</td>
<td>69.3 ± 3.2</td>
</tr>
<tr>
<td>I</td>
<td>49.0 ± 3.7</td>
</tr>
<tr>
<td>J</td>
<td>47.7 ± 2.1</td>
</tr>
<tr>
<td>K</td>
<td>22.0 ± 1.1</td>
</tr>
<tr>
<td>L</td>
<td>8.3 ± 0.4</td>
</tr>
</tbody>
</table>

* The G+C content of both strains was 33 mol%. The mean ± standard deviation chromosome sizes of *M. hyopneumoniae* ATCC 25934T and *M. flocculare* NCTC 10143T are 1,011.3 ± 32.9 and 988.3 ± 39.5 kbp, respectively. The means and standard deviations were calculated from 10 independent experiments for *M. hyopneumoniae* and from 3 independent experiments for *M. flocculare*. 

**TABLE 2. Restriction enzymes that cut infrequently**

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Site</th>
<th>No. of fragments obtained with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><em>M. hyopneumoniae</em> ATCC 25934T</td>
</tr>
<tr>
<td>ApaI</td>
<td>GGGCGG</td>
<td>12</td>
</tr>
<tr>
<td>BsrHII</td>
<td>GGGCGG</td>
<td>18</td>
</tr>
<tr>
<td>EagI</td>
<td>CCGCGG</td>
<td>12</td>
</tr>
<tr>
<td>NaeI</td>
<td>CCCGGG</td>
<td>18</td>
</tr>
<tr>
<td>NarI</td>
<td>GGGCGG</td>
<td>16</td>
</tr>
<tr>
<td>SacI</td>
<td>CCGCGG</td>
<td>23</td>
</tr>
<tr>
<td>SmaI</td>
<td>CCCGGG</td>
<td>25</td>
</tr>
</tbody>
</table>

* The G+C content of both strains was 33 mol%. The mean ± standard deviation number of fragments for *M. hyopneumoniae* ATCC 25934T was 18 ± 4. ND, not determined.
ApaI, and the fragments were separated by FIGE on a 0.8% agarose gel (length, 25 cm) by using model PPI-200 power inverter program 4. The sizes are indicated for the left and were determined by using bacteriophage lambda multimers. The calculated chromosome sizes for the M. hyopneumoniae strains were as follows: strain ATCC 25934T, 1,011.3 ± 32.9 kb; strain NCTC 10110T, 1,011.0 ± 41.9 kb; strain JF 184aT, 1,142.4 ± 38.2 kb; strain EP 29, 1,025.0 ± 58.3 kb; strain BO 14, 1,069.2 ± 66.2 kb; strain EP-S 924, 981.7 ± 31 kb; strain EP-S 938, 970.0 ± 29 kb; strain EP-S 939, 970 ± 29.5 kb; strain EP-S 946, 992.5 ± 33.4 kb; strain EP-S 223, 955.7 ± 30.6 kb; and M. flocculare NCTC 10143, 988 ± 39.5 kb. Mhp, M. hyopneumoniae; Mf, M. flocculare; Kb, kilobase pairs.

However, so far there have been no experimental data which prove that the low G+C contents affect the migration of DNA fragments during PFGE. In contrast, various workers have successfully used PFGE techniques to estimate the sizes of full-length Mycoplasma and Acholeplasma chromosomes that were linearized by gamma irradiation (20) or by partial restriction enzyme digestion (25).

The chromosome size for M. hyopneumoniae JT (= ATCC 25934T), as calculated from 12 ApaI restriction fragments, is 1,011.3 ± 32.9 kbp (Table 3) and corresponds well to the result obtained by Robertson et al. (25), who measured the linearized nonfragmented chromosome of M. hyopneumoniae ATCC 25095 (= VMRI-11). The size of the M. flocculare chromosome is 988.3 ± 39.5 kbp (calculated from 11 ApaI fragments) (Table 3). This is 100 kbp larger than the value determined by Robertson et al. (25) for the nonfragmented M. flocculare Ms 42T (= ATCC 27716T) chromosome. The difference in the two values could have been due to the fact that the strain used by Robertson et al. (25) (strain ATCC 27716T) had been propagated and recloned under different growth conditions and for a different number of generations than the strain used in our study (strain NCTC 10143T [= ATCC 27399T]); such differences could have caused chromosome rearrangements in one of the strains.

The different restriction enzymes with 6-bp recognition sequences consisting of exclusively G and C residues which we used cut the M. hyopneumoniae chromosome an average of 18 times (Table 2). Assuming a G+C content of 33 mol% (30), we expected that the M. hyopneumoniae 1,000-kbp chromosome would be cut 18 times by enzymes that recognize a 6-bp sequence that is composed of only G and C residues (number of cuts = chromosome size [in bp] × [G+C]○ × [0.5])°. This value correlates well with the mean number of fragments that were obtained experimentally (Table 2).

The restriction patterns shown in Fig. 1 were different for most M. hyopneumoniae strains. Although the strains that were isolated by workers from our laboratory in Switzerland could be distinguished, these strains shared a number of fragments. The differences among the strains isolated in different countries were much larger. The sizes of the chromosomes of most of the M. hyopneumoniae strains that were analyzed were close to 1,000 kbp; the exception was strain JF 184aT, whose chromosome was approximately 100 kbp larger (Fig. 1). It is interesting that the cultures of strain JF that were obtained from two culture collections (strains ATCC 25934T and NCTC 10110T) produced identical restriction patterns, while strain JF 184aT, which is strain JF culture that was obtained directly from P. Whittlestone, had a 125-kbp ApaI fragment instead of the 8.3-kbp fragment. We assumed that the chromosome of strain JF 184aT had undergone a duplication of a 117-kbp chromosomal fragment or that the strain JF cultures that were deposited in the culture collections had undergone a deletion of 117-kb during passage or recloning. A similar chromosomal rearrangement might also have occurred in one of the two culture collection cultures of type strain Ms 42T, and this could have led to the difference in chromosome sizes described above. The finding that the chromosomes of M. hyopneumoniae strains are heterologous is surprising. Some variations, such as the differences among strains NCTC 10110T, JF 184aT, and ATCC 25934T, which all originate from the strain JF isolate, indicate that the chromosome of M. hyopneumoniae undergoes frequent chromosomal rearrangements. These arrangements could be due to repetitive (insertion sequence-like) elements that have recently been detected in M. hyopneumoniae (9, 12) and might play a role in control of gene expression and host or medium adaptation. However, other variations in the chromosomal structure seem to be due to larger phylogenetic differences since we observed a regional homogeneity of the strains that were isolated in Switzerland,
a small geographical area, and rather strong heterogeneity of strains that originated from distant places. However, the chromosome differences do not seem to affect the few phenotypes that are currently used for identification of *M. hyopneumoniae*. It should be noted that strains ATCC 25934 and BQ 14, which produce very different *ApaI* restriction patterns, seem to have very different virulence properties when they are used in experimental infections (13a). A similar situation has been observed with *Leptospira* infections of pigs, in which differences in virulence are associated with differences in the chromosomal restriction patterns of strains which could not be distinguished by other methods (7a).

Restriction enzyme *XhoI* does not cut the chromosomal DNAs of the *M. hyopneumoniae* strains which we analyzed, although on the basis of statistics we expected it to cut the chromosome 114 times. In contrast, *M. flocculare* DNA was digested by *XhoI*. Therefore, believe that *M. hyopneumoniae* DNA is modified (probably methylated) at the *XhoI* site. Our results show that the modification must be specific to the *XhoI* recognition site. Therefore, *M. hyopneumoniae* might have its own restriction modification system. This modification is different from the more frequent site-specific methylation at *GATC* which has been described in *M. hyopneumoniae* (6) and resembles the methylation induced by the *Escherichia coli dam* gene. It should be noted that Pyle et al. (21) have found that the chromosome of *M. hyopneumoniae* is cut only once by *XhoI*, resulting in a 1,140-kbp fragment. On the basis of our results, we believe that the single 1,140-kbp fragment might have been created by linearization of the chromosome at random during an incubation reaction and not by site-specific cleavage.

In summary, we found that the chromosomes of *M. hyopneumoniae* strains are quite different. Restriction enzyme digestion with infrequently cutting enzymes and subsequent analysis by FIGE is a simple and reliable method for distinguishing different *M. hyopneumoniae* strains. This finding is important for studies of the taxonomy of *M. hyopneumoniae*, as well as for epidemiological studies of *M. hyopneumoniae* outbreaks. In view of the genetic diversity of *M. hyopneumoniae* strains, specific diagnostic tools, such as polymerase chain reaction amplification based on nucleotide sequences of randomly chosen DNA fragments, need to be evaluated with a large number of strains before they can be proposed as safe methods for detecting *M. hyopneumoniae* infections. Site-specific modification at CTCGAG, which is probably a component of a restriction modification system, seems to be common to *M. hyopneumoniae* strains and is not found in *M. flocculare*.

ACKNOWLEDGMENTS

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REFERENCES


