Relationships between Members of the *Mycoplasma mycoides* Cluster as Shown by DNA Probes and Sequence Analysis

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A gene probe, CAP-21, which demonstrated interrelationships between the members of the *Mycoplasma mycoides* cluster was developed. The probe easily differentiated mycoplasmas in this cluster by clear and predictable hybridization patterns in Southern blots and separated the cluster into four groups. Strains of *M. mycoides* subsp. *mycoides* which were capable of causing contagious bovine pleuropneumonia composed one group. Strains of *M. mycoides* subsp. *mycoides* which did not cause contagious bovine pleuropneumonia together with strains of *M. mycoides* subsp. *capri* composed the second group. *Mycoplasma capricolum* and the *F38* mycoplasmas formed a third group, while the bovine group 7 mycoplasmas composed a separate, fourth group. Further support for the above grouping of the cluster was obtained when amplified DNA analogous to the probe from one representative strain of each of the cluster members was sequenced and these data were used to construct a phylogenetic tree. Contagious caprine pleuropneumonia is recognized as an important disease, and the etiological agent of this disease is now known to be the F38 mycoplasma. The CAP-21 probe did not differentiate between *M. capricolum* and the closely related F38 mycoplasma. A second probe, F38-12, which was capable of distinguishing these two mycoplasmas was made.

There are six mycoplasmas that make up the group known as the *Mycoplasma mycoides* cluster. The mycoplasmas in this group are pathogens of cattle, sheep, and goats. *M. mycoides* subsp. *mycoides* small colony (SC) is primarily a pathogen of cattle and causes contagious bovine pleuropneumonia (CBPP). *M. mycoides* subsp. *mycoides* large colony (LC) is usually found in goats, causing septicaemia, arthritis, and pneumonia. *M. mycoides* subsp. *capri* causes pneumonia and arthritis in goats, and *Mycoplasma capricolum* causes septicaemia, arthritis, and mastitis in goats and sheep. Two other groups of unnamed mycoplasmas complete the cluster. These are mycoplasmas represented by the F38 mycoplasma, which is now recognized as the agent of contagious caprine pleuropneumonia (CCPP), and bovine group 7 mycoplasmas, which cause arthritis and mastitis in cattle (8).

A number of difficulties have been identified in the classification of this group of organisms. Intraspecific varieties of *M. mycoides* subsp. *mycoides* (SC and LC forms) have been recognized. The two types are serologically indistinguishable but are quite different in pathogenicity; the SC form causes CBPP in cattle, while the LC form does not. Cottew and Yeats (9) showed that the two types could be separated when they correlated differences in cultural characteristics with differences in heat stability and variation in proteolytic activities. The presence of two enzymes, α-glucosidase and ornithine transcarbamylase, was demonstrated in LC types but not in SC types (17). The ability to produce mycoplasmaemia in mice has also been used to separate LC and SC types (18).

*M. mycoides* comprises two subspecies, *M. mycoides* subsp. *mycoides* and *M. mycoides* subsp. *capri*. They can be differentiated by serological techniques but not by biochemical tests, as the reaction of the LC type of *M. mycoides* subsp. *mycoides* is identical to that of *M. mycoides* subsp. *capri*. Although the type strains of the two subspecies are serologically distinct, significant cross-reactivity can occur between the subspecies with some antisera (7). Establishing the correct taxonomic position of the LC type of *M. mycoides* subsp. *mycoides* relative to *M. mycoides* subsp. *mycoides* SC and *M. mycoides* subsp. *capri* has been made difficult because of conflicting results. DNA homology studies have shown that *M. mycoides* subsp. *mycoides* LC was more closely related to *M. mycoides* subsp. *mycoides* SC than to *M. mycoides* subsp. *capri* (2). Protein studies have indicated the opposite (6, 15, 16).

There have also been problems in classifying the two unnamed mycoplasmas belonging to the cluster. Some field isolates of the F38 mycoplasma have been shown to cross-react with *M. capricolum* and bovine group 7 mycoplasma antisera (4). Also, DNA studies have shown an 80% relatedness between the type strains of the F38 mycoplasma and *M. capricolum* which indicated that the F38 mycoplasmas should become a subspecies of *M. capricolum* (5). One-dimensional (1D)- and 2D-polyacrylamide gel electrophoresis (PAGE) analyses of proteins also group these two mycoplasmas together (6, 16).

The bovine group 7 mycoplasmas have remained an unclassified group for many years. Very strong serological cross-reactions have been demonstrated between bovine group 7 and F38 mycoplasmas, and on this basis it was proposed that bovine group 7 mycoplasmas form a biovar of the F38 subspecies of *M. capricolum* (4). DNA homology studies, on the other hand, suggest that bovine group 7 mycoplasmas are equally related to the *M. mycoides* species and *M. capricolum* and that because of this, they should form a separate species (5).

Many different techniques have been used to try to clarify the relationships between these organisms. These techniques have shown a very complicated range of relationships with conflicting results, and as a result an ad hoc committee established by the Subcommittee on the Taxonomy of the Mollicutes has not been able to reach agreement on the classification of the members of the *M. mycoides* cluster but...
TABLE 1. List of the members of the M. mycoides cluster used in this study as well as their host species of origin and country of origin

<table>
<thead>
<tr>
<th>Mycoplasma Strain</th>
<th>Species of origin</th>
<th>Country of origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. mycoides subsp. mycoides SC</td>
<td>Bovine</td>
<td>Australia</td>
</tr>
<tr>
<td>M. mycoides subsp. mycoides LC</td>
<td>Caprine</td>
<td>Australia</td>
</tr>
<tr>
<td>M. capricolum F38</td>
<td>Caprine</td>
<td>Nigeria</td>
</tr>
<tr>
<td>M. capricolum</td>
<td>Caprine</td>
<td>Turkey</td>
</tr>
<tr>
<td>M. capricolum</td>
<td>Caprine</td>
<td>United States</td>
</tr>
<tr>
<td>M. capricolum</td>
<td>Caprine</td>
<td>Australia</td>
</tr>
<tr>
<td>M. capricolum</td>
<td>Caprine</td>
<td>United States</td>
</tr>
<tr>
<td>F38 mycoplasma</td>
<td>Bovine</td>
<td>Australia</td>
</tr>
<tr>
<td>Bovine group 7 mycoplasma</td>
<td>Bovine</td>
<td>Australia</td>
</tr>
</tbody>
</table>

emphasized the need for further DNA studies. This paper describes the use of gene probes and nucleic acid sequencing to further differentiate and classify this group of mycoplasmas and provides the basis of a test for distinguishing the etiological agents of CBPP and CCPP.

MATERIALS AND METHODS

Organisms. Table 1 lists the mycoplasma species used in this study as well as their host species of origin and country of origin. Other organisms used were laboratory strains of Mycoplasma hyorhinis, Mycoplasma orale, Acholeplasma laidlawii, Escherichia coli, and Pasteurella multocida.

Cloning and sequencing of probes. Two probes were prepared. One probe, CAP-21, was used in the differentiation of members of the M. mycoides cluster. M. mycoides subsp. capri was digested with the restriction endonuclease EcoRV.

TABLE 2. Primers used in sequencing and PCR

<table>
<thead>
<tr>
<th>Primer no.</th>
<th>Nucleotide no.a</th>
<th>Primer sequence (5’→3’b)</th>
<th>Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>TTAGAGTTACCCATAGTGTCCAGG</td>
<td>Forward</td>
</tr>
<tr>
<td>2</td>
<td>207</td>
<td>AAGAGATTTGGTATCGGAGTCC</td>
<td>Forward</td>
</tr>
<tr>
<td>3</td>
<td>281</td>
<td>GTGAAAAGAGGAAATGGGC</td>
<td>Forward</td>
</tr>
<tr>
<td>4</td>
<td>649</td>
<td>TAATCGAATTAGAAAAGG</td>
<td>Forward</td>
</tr>
<tr>
<td>5</td>
<td>837</td>
<td>CTAGAGGAGAAGAAGCGT</td>
<td>Forward</td>
</tr>
<tr>
<td>6</td>
<td>1000</td>
<td>GAATTGGGTTGGACCG</td>
<td>Forward</td>
</tr>
<tr>
<td>7</td>
<td>1223</td>
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<td>1422</td>
<td>TTAGATGCTGATTTTCTGCC</td>
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</tr>
<tr>
<td>9</td>
<td>1509</td>
<td>GATATGCTAAGGTTGATG</td>
<td>Reverse</td>
</tr>
<tr>
<td>10</td>
<td>237c</td>
<td>TTTCTCTGTGTTTTTTATTGGC</td>
<td>Forward</td>
</tr>
</tbody>
</table>

a The numbers correspond to the nucleotide positions of the first base of the primer relative to the total sequence in Fig. 4A for CAP-21.
b Oligonucleotides were synthesized by using a DNA synthesizer (Applied Biosystems) according to manufacturer’s instructions and purified by using the oligonucleotide purification cartridge columns supplied.
c For primer 10 the nucleotide number corresponds to the first base of primer relative to the total sequence in Fig. 5A for F38-12.

Several fragment size classes were inserted into M13mp8 at the SmaI site. Clones produced were screened for the presence of an insert, and several clones with inserts of 500 to 2,000 bp were chosen for further investigation. These were tested as probes by using the DNA of SC (Gladysdale) and LC (Y Goat) strains of M. mycoides subsp. mycoides by Southern hybridization. One clone CAP-21 was sequenced by using Sequenase version 2.0 (United States Biochemical Corp.) with primers 2, 4, 6, and 8 (Table 2) and M13 forward primer (supplied with kit).

The second probe, F38-12, was used to differentiate F38 mycoplasma and M. capricolum. Genomic DNA from F38 mycoplasma and M. capricolum was digested with TaqI and probed with 32P-labelled CAP-21. Hybridization bands can be seen with all organisms.
mycoplasma was digested with the restriction endonuclease EcoRI. Fragments were randomly inserted into M13mp18 at the EcoRI site. Clones produced were screened for the presence of an insert. Several clones were chosen for testing with F38 mycoplasma and M. capricolum (California Kid) by Southern hybridization. F38-12 was chosen and sequenced by using Sequenase version 2.0 (United States Biochemical Corp.) with primer 10 (Table 2) and M13 forward primer.

**DNA extraction, PCR amplification, and sequencing.** Representative members of the *M. mycoides* cluster selected were *M. mycoides* subsp. *mycoides* SC strain Gladysdale, *M. mycoides* subsp. *mycoides* LC strain Y Goat, *M. mycoides* subsp. *capri* ZZ, M. capricolum California Kid, F38 mycoplasma original isolate, and bovine group 7 mycoplasma strain R2222. Template DNA from these organisms was extracted after treatment with proteinase K by using phenol as described in reference 19. Regions analogous to CAP-21 were amplified by polymerase chain reaction (PCR) using primers 1 and 9 (Table 2). Amplification was for 30 cycles in a total volume of 50 μl of reaction mixture consisting of 10 mM Tris-HCl (pH 8.3); 50 mM KCl; 1.5 mM MgCl₂; 0.001% (wt/vol) gelatin; 200 μM (each) dATP, dGTP, dTTP, and dCTP; and 1.5 U of *Taq* polymerase (Perkin-Elmer Cetus). Each primer was used at 50 pmol. Each amplification cycle consisted of annealing at 50°C for 1 min, extension at 72°C for 2 min, and denaturation at 94°C for 1 min. PCR product (5 μl) was then electrophoresed on a 0.8% agarose gel, and the PCR product was visualized by staining with ethidium bromide. The DNA band was excised and purified by using GeneClean (Bio 101, La Jolla, Calif.) and resuspended in 5 μl of H₂O. This was used to make single-stranded DNA by using asymmetric PCR and conditions as described above except that amplification was for 40 cycles and the primer concentrations were made so that one was in excess (50 pmol) and one was in a limiting amount (0.5

**FIG. 2.** Comparison of DNA from strains of *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* *TaqI* digested and probed with 3²P-labelled CAP-21. LC strains are Y Goat, KH1, COV2, LB2, 801, M243/67, OSB42, EZG, and F30. PG3, BQT, YC, ZZ, and N108 are strains of *M. mycoides* subsp. *capri.* *M. mycoides* subsp. *mycoides* SC strain Gladysdale is used as a comparison. The relative positions of size markers are indicated; the sizes are in kilobase pairs. F30, EZG, and N108 deviate from the general hybridization pattern.

**FIG. 3.** Comparison of DNA from strains of *M. capricolum* (California Kid and 3220) and bovine group 7 mycoplasmas (N29 and R2222) *TaqI*-digested and probed with 3²P-labelled CAP-21. *M. mycoides* subsp. *mycoides* SC strain Gladysdale is used as a reference.
M. capricolum coides agarose gels, and transferred to a nitrocellulose membrane precipitated with isopropanol and resuspended in 7.5 µl of F38 mycoplasma (original isolate), by using the DNAIST and KITSCH programs of reference hybridization bands. Sizes are indicated in kilobase pairs.

32P-labelled F38-12. Strains used were the H,O (14). This was then used in a sequencing reaction using endonuclease (when F38-12 probe was used), electrophoresed in 0.8% Sequenase version 2.0 (United States Biochemical Corp.) pg) was digested overnight with 50 U of the restriction enzyme (when CAP-21 probe was used) or RsaI (when F38-12 probe was used), and the film was developed by standard methods.

Southern hybridization sequence accession numbers. The sequence of the F38-12 insert was also submitted to GenBank. They have been given the accession numbers which are listed below. The sequence for the F38-12 insert was also submitted, and the accession number for this sequence is also given: M96586, M. mycoides subsp. capri; M96587, bovine group 7 mycoplasma; M96588, M. capricolum; M96589, F38 mycoplasma; M96590, M. mycoides subsp. mycoides SC; M96591, M. mycoides subsp. mycoides LC; and M96592, F38 mycoplasma.

RESULTS

Southern hybridization. Southern hybridization with the CAP-21 probe was used to study the interrelationships of the M. mycoides cluster. This probe in combination with TaqI digestion resulted in a very simple hybridization pattern of one or two bands which facilitated the easy identification of the members of the M. mycoides cluster. The probe differentiated the M. mycoides cluster into groups and separated the two subspecies of M. mycoides represented by PG1 and PG3. PG3, the type strain of M. mycoides subsp. capri, and Y Goat, the representative strain of M. mycoides subsp. mycoides LC, both gave similar hybridization patterns. The probe grouped M. capricolum and F38 mycoplasma together, while the bovine group 7 mycoplasmas made a separate, distinct grouping (Fig. 1).

A total of five strains of M. mycoides subsp. capri and nine strains of M. mycoides subsp. mycoides LC were tested. Eleven of the 14 strains produced similar hybridization patterns. Among the three strains which showed different hybridization patterns were one strain of M. mycoides subsp. capri, N108, and two strains of M. mycoides subsp. mycoides LC, EZG and F30. Strains EZG and N108 appeared to be the same as each other and different from F30. All 3 differed from the other 11 strains in having an additional hybridization band and presumably contained an extra TaqI site (Fig. 2). The bovine group 7 mycoplasmas, strain R2222, and the representative strain N29, along with strains of M. capricolum, California Kid and 3220, were tested with CAP-21 using the Gladysdale strain of M. mycoides subsp. mycoides SC as a comparison. The two strains of the bovine group 7 mycoplasmas hybridized in identical positions, as did the two strains of M. capricolum (Fig. 3). To differentiate M. capricolum from F38 mycoplasmas, a second probe, F38-12, was developed. Genomic DNAs of representative strains of each member of the M. mycoides cluster were
was 464 bases in length (Fig. 6). It had a base composition of 17.7% G (271 bases), 38.9% A (81 bases). Plots in all six frames showed two possible open reading frames. The nucleotide sequence of the F38-12 insert was distinct in pattern from those of the F38 mycoplasma. The bands were well separated and differentiated the two organisms (Fig. 4).

Sequence analysis. The CAP-21 insert was determined to be 1,525 bases in length (Fig. 5), and the base composition was 17.7% G (271 bases), 38.9% A (596 bases), 31.5% T (475 bases), and 11.9% C (183 bases). Plots of potential open reading frames in all six frames showed two possible open reading frames. The nucleotide sequence of the F38-12 insert was 464 bases in length. It had a base composition of 12.7% G (59 bases), 26.3% A (122 bases), 43.5% T (202 bases), and 17.5% C (81 bases). Plots in all six frames did not show any open reading frames of significant length.

Specificity of probe. Experiments were done to determine the specificity of the two probes. TaqI digests of genomic DNA from the mycoplasmas A. laidlawii, M. orale, M. hyorhinis, and M. arginini and two representative bacteria, P. multocida and E. coli, together with TaqI digests of members of the M. mycoides cluster were tested by using CAP-21. Hybridization bands were produced with all of these organisms; however, signals that resulted from distantly related organisms were much weaker and had patterns distinctly different from those from mycoplasmas belonging to the cluster (results not shown).

When RsaI digests of DNA from the same organisms were hybridized with the F38-12 probe, only F38, M. capricolum, and E. coli produced hybridization bands. The signal from E. coli was distinct in pattern from those of the F38 mycoplasma and M. capricolum (results not shown).

Sequence data. Regions homologous to the CAP-21 insert were amplified from representative strains of each member of the cluster and sequenced. The sequence data are shown together with the respective restriction enzyme digests of genomic DNA from these organisms; however, signals that resulted from distantly related organisms were much weaker and had patterns distinctly different from those from mycoplasmas belonging to the cluster (results not shown).

FIG. 5. Nucleotide sequence of the insert in the recombinant M13 clone CAP-21. The start codons of the two possible coding regions are boxed, and the stop codon of the first is underlined.

FIG. 6. Nucleotide sequence of the insert in the recombinant M13 clone F38-12.
F38 mycoplasma are more closely related to each other than to the other members of the cluster, *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC are very closely related, and bovine group 7 mycoplasmas form a separate group.

Several changes were observed between the sequence of the CAP-21 insert and that derived from the genome of *M. mycoides* subsp. *capri* directly by PCR and asymmetric PCR. These changes were minor and were C-to-T transitions at nucleotides 1130, 1403, and 1406.
The organisms assigned to the *M. mycoides* cluster have been shown to be related to each other by a variety of tests, but classification of individual members within the cluster has proved to be difficult. We have studied the genetic differentiation of strains of this organism capable of causing CBPP from those which did not produce the disease (19). CBPP from those which did not produce the disease (19). Members of the cluster have been shown to be related to each other by a variety of tests, but classification of individual members within the cluster has proved to be difficult. We have studied the genetic differentiation of strains of this organism capable of causing CBPP from those which did not produce the disease (19). CBPP from those which did not produce the disease (19). Members of the cluster have been shown to be related to each other by a variety of tests, but classification of individual members within the cluster has proved to be difficult. We have studied the genetic differentiation of strains of this organism capable of causing CBPP from those which did not produce the disease (19).

DNA-DNA hybridization studies estimated that DNA of the type strain *M. mycoides* subsp. *mycoides* SC, PG1, had a 90% homology with DNA from *M. mycoides* subsp. *mycoides* LC strain Y Goat (2). When PG1 was compared with the type strain of *M. mycoides* subsp. *capri*, PG3, 80% homology was found. These figures indicated Y Goat was more closely related to PG1 than was PG3. Several workers using 2D PAGE of acidic cellular proteins concluded the opposite (15, 16). TaqI-restricted DNA from Y Goat, when probed with CAP-21, produced a single band; PG3 produced a band in a

**DISCUSSION**

The organisms assigned to the *M. mycoides* cluster have been shown to be related to each other by a variety of tests, but classification of individual members within the cluster has proved to be difficult. We have studied the genetic differentiation of strains of this organism capable of causing CBPP from those which did not produce the disease (19). When genomic DNA from each member of the *M. mycoides* cluster was digested with the restriction endonuclease TaqI and probed with CAP-21, a pattern of hybridization which indicated that this probe could be useful in the classification of the members of the *M. mycoides* cluster resulted.

The classification of *M. mycoides* subsp. *mycoides* LC within the cluster has been a difficult problem. DNA-DNA hybridization studies estimated that DNA of the type strain of *M. mycoides* subsp. *mycoides* SC, PG1, had a 90% homology with DNA from *M. mycoides* subsp. *mycoides* LC strain Y Goat (2). When PG1 was compared with the type strain of *M. mycoides* subsp. *capri*, PG3, 80% homology was found. These figures indicated Y Goat was more closely related to PG1 than was PG3. Several workers using 2D PAGE of acidic cellular proteins concluded the opposite (15, 16). TaqI-restricted DNA from Y Goat, when probed with CAP-21, produced a single band; PG3 produced a band in a
similar position. PG1 also produced a single band but in a position different from those produced by Y Goat and PG3. This shows that Y Goat and PG3 are more closely related to each other than to PG1, and these results agree with those of the 2D-PAGE studies. We have compared eight strains of \textit{M. mycoides} subsp. \textit{mycoides} SC and found them to be a very homogeneous group (19). Further evidence of this differentiation has been shown by Costas and associates (6), who, using 1D PAGE, found that strains of \textit{M. mycoides} subsp. \textit{mycoides} SC made up a distinct phenon quite separate from a phenon comprising both \textit{M. mycoides} subsp. \textit{mycoides} LC and \textit{M. mycoides} subsp. \textit{capri}.

We have compared nine strains of \textit{M. mycoides} subsp. \textit{mycoides} LC and five strains of \textit{M. mycoides} subsp. \textit{capri} (Fig. 2). Some variability in mobility of the hybridizing band is seen, indicating generally minor variations in the genomes of these organisms. Most (i.e., 11) of the 14 strains are similar to \textit{M. mycoides} subsp. \textit{capri} PG3 and may belong to a common group. However, three of the strains tested (N108, EZG, and F30) appear to be different. It is likely that strains N108 and EZG are the same organism, although serologically N108 has been previously identified as \textit{M. mycoides} subsp. \textit{capri} while EZG was identified as \textit{M. mycoides} subsp. \textit{mycoides} LC. A result similar to this was reported by Leach and associates (13), who found that strain N108 was one of two strains that fell outside a large phenon that comprised all the \textit{M. mycoides} subsp. \textit{mycoides} LC and \textit{M. mycoides} subsp. \textit{capri} strains tested. The F30 strain appears to be unrelated to either the \textit{M. mycoides} subsp. \textit{mycoides} LC or \textit{M. mycoides} subsp. \textit{capri} group. All three of these variant strains share some homology, as evidenced by the similar positions of one of the bands of hybridization.

The bovine group 7 mycoplasmas have remained an unclassified group within the \textit{M. mycoides} cluster. Hybridization studies of this group using CAP-21 clearly separated them from all the other members of the \textit{M. mycoides} cluster. This separation has previously been proposed on the basis of isoenzyme studies (17). Costas and colleagues (6), by 1D PAGE, also found that this group formed a phenon separate from all the other members of the \textit{M. mycoides} cluster at a 75% similarity level. Christiansen and Erns (5), using DNA homology studies, found that bovine group 7 mycoplasmas were equally related to \textit{M. mycoides} and \textit{M. capricolum} with homologies of 60% and on this basis should be recognized as a separate species.

The CAP-21 probe was unable to distinguish between \textit{M. capricolum} and the F38 mycoplasma, indicating that the organisms are very similar. Further evidence that these two mycoplasmas have a close relationship has been shown serologically (3, 4); also, in DNA homology studies they showed an 80% relatedness (5). Rodwell (16) and Andersen and coworkers (1) found a 42% protein congruence for F38 and \textit{M. capricolum} which was consistent with the 80% DNA homology value from reference 5. CCPP, a disease of economic importance in Africa, is caused by the F38 mycoplasma, and there is a need to have a reliable method for its diagnosis and to monitor its spread. Problems in the identification of the organism have been compounded by serological cross-reactions with other members of the cluster. The F38 mycoplasma produces very strong cross-reactions with the bovine group 7 mycoplasma (4, 11). F38 field strains that cross-react with \textit{M. capricolum} have also been isolated (4). Confusing variations in biochemical characteristics of an isolate which was identified as \textit{M. capricolum} but serologically resembled F38 mycoplasma were also reported (4). A second probe which could specifically differentiate between these two organisms was developed. With these two probes is it possible not only to classify all the members of the \textit{M. mycoides} cluster but also for a diagnostic laboratory to identify specifically the agents which cause CBPP and CCPP.

Comparison of nucleotide sequences of each member of the \textit{M. mycoides} cluster over the region delineated by the CAP-21 probe enabled the construction of a phylogenetic tree which demonstrates relationships between them. The results are similar to those obtained when the cluster was studied by hybridization analysis of whole cellular DNA. The dendrogram showed a maximum variation between members of 4%, with \textit{M. mycoides} subsp. \textit{mycoides} SC and LC and \textit{M. mycoides} subsp. \textit{capri} being closely related and with \textit{M. mycoides} subsp. \textit{mycoides} LC being more closely related to \textit{M. mycoides} subsp. \textit{capri} than to \textit{M. mycoides} subsp. \textit{mycoides} SC. \textit{M. capricolum} and the F38 mycoplasmas were also grouped with a closer relationship to each other than to the other members of the cluster. Bovine group 7 mycoplasmas are most closely related to \textit{M. mycoides} subsp. \textit{mycoides} SC but are more distantly related to the other members of the cluster.

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REFERENCES


