Genetic and Serological Analysis of Lipoprotein LppA in
*Mycoplasma mycoides* subsp. *mycoides* LC and
*Mycoplasma mycoides* subsp. *capri*

MARIE-PIERRE MONNERAT,1 FRANÇOIS THIAUCOURT,2 JOSE B. POVEDA,3
JACQUES NICOLET,1 AND JOACHIM FREY1*

Institute for Veterinary Bacteriology, University of Berne, CH-3012 Berne, Switzerland; CIRAD-EMVT, Campus International de Baillarguet, F-34032 Montpellier, France; and Facultad de Veterinaria, Universidad de Las Palmas, E-35071 Las Palmas, Spain

Received 29 June 1998/Returned for modification 6 November 1998/Accepted 4 December 1998

The genes encoding the 62-kDa lipoproteins from the *Mycoplasma mycoides* subsp. *mycoides* large-colony type (LC) strain Y-goat and the *M. mycoides* subsp. *capri* strain PG3 were cloned and analyzed by sequencing. These two lipoproteins have been named LppA[MmyLC] and LppA[Mmyca], and their corresponding genes have been named *lppA* [MmyLC] and *lppA* [Mmyca], respectively. The nucleotide and deduced amino acid sequences of these two lipoproteins showed a very high degree of similarity between these two mycoplasmas. Given the sequence data, LppA seems to fulfill the same structural functions as the previously described major lipoproteins P72 of *M. mycoides* subsp. *mycoides* small-colony type and P67 of the *Mycoplasma* species bovine group 7. Based on *lppA* gene sequences of *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* type strains, a specific PCR assay was developed so that it amplified this gene in all field strains of the two species analyzed in this study but not in the other members of the *M. mycoides* cluster. Analysis of the PCR-amplified *lppA* genes with frequently cutting restriction enzymes showed a certain degree of genetic variability which, however, did not cluster the two subspecies. This PCR therefore allows a rapid identification of *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* but does not distinguish between these two closely related subspecies. LppA was expressed in *Escherichia coli* K-12 and used for the production of polyclonal mouse antiserum. Antibodies against recombinant LppA[MmyLC] reacted with a 62-kDa protein in all *M. mycoides* subsp. *mycoides* LC strains and field strains tested but not with the other members of the *M. mycoides* cluster, thus showing the antigenic specificity of LppA and further supporting the concept that a close relationship exists between these two mycoplasmas.

The *Mycoplasma mycoides* subsp. *mycoides* large-colony type (LC) and *M. mycoides* subsp. *capri* strains belong to the *M. mycoides* cluster, a group of six closely related mycoplasmas (13). *M. mycoides* subsp. *mycoides* LC causes mastitis, keratoconjunctivitis, polyarthritis, pneumonia, and septicemia in goats (13, 14, 34, 36). It has also been isolated, rarely, from cattle (20, 27) and sheep (25). *M. mycoides* subsp. *capri* is reported to cause a pattern of diseases similar to those induced by *M. mycoides* subsp. *mycoides* LC specifically in goats, including mastitis, arthritis, and pulmonary diseases (13, 21, 23, 28, 40, 41). From an epidemiological point of view, differential identification of the subspecies or subtypes of mycoplasmas of the *M. mycoides* cluster is prerequisite for the differentiation, since the different members of this cluster show very strong differences in virulence and epidemiological impact. However, many methods fail in specificity because they are hampered by strong serological cross-reactions between the different members of the *M. mycoides* cluster (7, 12, 15, 17, 32, 39).

It has been reported that *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* are antigenically very similar as assessed by numerical analysis of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein patterns (11, 22). Identical results have been obtained by two-dimensional PAGE protein patterns, which confirm that *M. mycoides* subsp. *mycoides* LC strains are more closely related to *M. mycoides* subsp. *capri* strains than to *M. mycoides* subsp. *mycoides* small-colony type (SC) strains (26, 35).

A DNA probe based on a randomly chosen genomic fragment was developed for the differentiation of the different members of the *M. mycoides* cluster into four groups. This DNA probe grouped *M. mycoides* subsp. *mycoides* LC strains together with *M. mycoides* subsp. *capri* and distinguished them from the other members of the *M. mycoides* cluster. However, it did not allow differentiation between these two mycoplasmas (38). DNA-DNA hybridization studies revealed variable values for DNA homology between *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* (75 to 94%), between *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *mycoides* SC (88 to 93%), and between *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* SC (75 and 93%) depending on experimental conditions (2, 9). Phylogenetic studies based on sequence analysis of 16S rRNA genes (*rrs*) revealed 99.9% similarity between *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri*. These results suggested that these two mycoplasmas could be grouped into a single subspecies, one distinct from *M. mycoides* subsp. *mycoides* SC (29).

With the objective of getting a better insight into the antigenic and genetic differences among the different members of the *M. mycoides* cluster, the major surface-located lipoprotein antigens of *M. mycoides* subsp. *mycoides* SC, P72, and of *Mycoplasma* sp. bovine group 7, P67, had been characterized (8,
Immuno blot analysis of type and field strains of the different species from the M. mycoides cluster by using monospecific polyclonal antibodies against each of these proteins revealed that these two proteins were antigenically species specific.

Southern blot hybridization with a gene probe for P72 revealed that the M. mycoides subsp. capri strain PG3 and the M. mycoides subsp. mycoides LC strain Y-goat as well contained an analogous gene. The aim of the present study was to clone, sequence, and analyze the genes encoding the major lipoproteins from M. mycoides subsp. capri and M. mycoides subsp. mycoides LC.

### MATERIALS AND METHODS

#### Strains and growth conditions

Mycoplasma strains used in this study and their origins are listed in Table 1. The Mycoplasma species were cultured in standard mycoplasma medium at 37°C. Cells were pelleted by centrifugation at 20,000 x g for 20 min, washed in TES buffer (10 mM Tris, 1 mM EDTA, 0.5% NaCl [pH 8.0]) and resuspended in TES buffer to reach a cell concentration of 10^9 cells ml^-1. Escherichia coli XL1-Blue MRF (Δ(mcrA)183 Δ(mcrCB-hsdS-mrr)173 endA1 supE44 thi-1 recA1 gye496 relA1 lac [F proAB lacZAM15 Tn10 (Tetr)] and XLOLR (Δ(mcrA)183 Δ(mcrCB-hsdS-mrr)73 endA1 thi-1 recA1 gye496 relA1 lac [F proAB lacZAM15 Tn10 (Tetr)]), Stragen (La Jolla, Calif.) were grown on Luria-Bertani broth at 37°C (3). E. coli YN2980 (hisG, leu, G615U, thyA, trp, g496505, leu, ttr, h6924, tlethy, metB, argH, proB, rpsL, pfrB3 [pISMs30]) was used as a suppressor strain for UGA^r+ and was grown as described (37). Expression vector pBH-CMV phagemid (Stratagene) was propagated in XLOLR strain.

DNA manipulation, construction and screening of gene library, and PCR. Genomic DNA was extracted by the guanidinium thiocyanate method as indicated (31). DNA from the M. mycoides subsp. mycoides LC strain Y-goat and the M. mycoides subsp. capri strain PG3 was partially digested with Sau3A1 and used to construct A ZAP Express phage banks (Stratagene) as described (8). The gene libraries were screened with a digoxigenin-11-dUTP-labeled DNA probe, and positive clones were transformed to phagemids as indicated (16). All standard techniques of molecular biology have been described previously (3). PCR amplifications were carried out as indicated (16), by using the oligonucleotide primers listed in Table 2.

### TABLE 1. Strains of mycoplasmas used and signals of PCRs for the lppA gene

| Strain | Species | Origin | Host | PCR (MMMLC2-L/ MMMLC1-R)* |
|--------|---------|--------|------|---------------------------|
| Y-goat | M. mycoides subsp. mycoides LC | Type strain | Goat | Pos. |
| LC0655 | M. mycoides subsp. mycoides LC | France | Goat | Pos. |
| D 2503 | M. mycoides subsp. mycoides LC | Bern, Switzerland | Goat | Pos. |
| 2482/91 | M. mycoides subsp. mycoides LC | Bern, Switzerland | Goat | Pos. |
| D 2083/91 | M. mycoides subsp. mycoides LC | Bern, Switzerland | Goat | Pos. |
| B671/93 | M. mycoides subsp. mycoides LC | Portugal | Cattle | Pos. |
| 266/94 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 6P | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 293 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 152/93 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 153/93 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 80x3 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| 83/93 | M. mycoides subsp. mycoides LC | Gran Canaria | Goat | Pos. |
| CP271 | M. mycoides subsp. mycoides LC | Portugal | Goat | Pos. |
| 9096-C9415 | M. mycoides subsp. mycoides LC | Nigeria | Goat | Pos. |
| 8756-13 | M. mycoides subsp. mycoides LC | United States | Goat | Pos. |
| 8794-Inde | M. mycoides subsp. mycoides LC | India | Goat | Pos. |
| PG3 | M. mycoides subsp. capri | Type strain | Goat | Pos. |
| Capri N108 | M. mycoides subsp. capri | Nigeria | Goat | Pos. |
| Capri L | M. mycoides subsp. capri | Turkey | Goat | Pos. |
| 9139-11/91 | M. mycoides subsp. capri | Switzerland | Cattle | Neg. |
| WK354/80 | M. mycoides subsp. capri | Turkey | Cattle | Neg. |
| PG1 | M. mycoides subsp. mycoides SC | Type strain | Cattle | Neg. |
| Afaqé | M. mycoides subsp. mycoides SC | Chad | Cattle | Neg. |
| L2 | M. mycoides subsp. mycoides SC | Italy | Cattle | Neg. |
| B17 | M. mycoides subsp. mycoides SC | Chad | Zebu | Neg. |
| T1/44 | M. mycoides subsp. mycoides SC | Tanzania | Cattle | Neg. |
| PG50 | Mycoplasma sp. bovine group 7 | Reference strain | Cattle | Neg. |
| PAD3186 | Mycoplasma sp. bovine group 7 | India | Goat | Neg. |
| CP291 | Mycoplasma sp. bovine group 7 | Portugal | Goat | Neg. |
| California kid | M. capricolum subsp. capricolum | Type strain | Goat | Neg. |
| 173/87 | M. capricolum subsp. capricolum | Greece | Sheep | Neg. |
| F38 | M. capricolum subsp. capripneumoniae | Type strain | Goat | Neg. |
| Gabés | M. capricolum subsp. capripneumoniae | Tunisia | Goat | Neg. |
| 9081-487p | M. capricolum subsp. capripneumoniae | Oman | Goat | Neg. |
| KS1 | M. putrefaciens | Type strain | Goat | Neg. |

* Taxonomic identification unclear; originally characterized as Mycoplasma sp. bovine group 7.  
* Fragment of smaller size amplified.  
* Complement fixation test antigen.  
* Vaccine strain (30), passage 44.  
* Pos., positive; Neg., negative.
was measured by the method of Bradford (6) and reached 400 at an output of 3 for 1 min. The protein concentration of the suspension obtained of TES buffer. The resuspended cells were sonicated on ice with a Branson were then harvested, washed with TES buffer, and resuspended in a 0.1 volume

subsp. M. mycoides polyclonal mouse anti-LppA[MmymyLC] serum. The

and NBRF databases were performed with the BLAST programs (1). Computer Group, Madison, Wis.). Sequence comparisons with GenBank/EMBL

was done according to standard protocols (3, 8). The mouse serum was diluted 1:2,000, and reaction products were visualized with affinity-purified goat phosphatase-labeled anti-mouse immunoglobulin G (heavy and light chains) (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) diluted 1:2,000, and used to immunize mice. After 3 weeks the

mixed with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) as described (18) and used to immunize mice. After 3 weeks the

was then harvested. Washed with TES buffer, and resuspended in a 0.1 volume of TES buffer. The resuspended cells were sonicated on ice with a Branson Sonifier 250 (Branson Ultrasonics, Danbury, Conn.) equipped with the Micropipet at an output of 3 for 1 min. The protein concentration of the suspension obtained was measured by the method of Bradford (6) and 400 μg ml⁻¹

In order to obtain polyclonal anti-LppA[MmymyLC] serum, 150 μl of the suspension described above containing 60 μg of protein per administration was mixed with an equal volume of Freund's complete adjuvant (Difco Laboratories, Detroit, Mich.) as described (18) and used to immunize mice. After 3 weeks the mice were booster immunized with the same amount of protein, but Freund's incomplete adjuvant was used. Blood for serum was taken 2 weeks later. The serum was adsorbed with E. coli YN2980 carrying the empty vector pBK-CMV. The antigen for adsorption was prepared as indicated (8). Immunoblot analysis was done according to standard protocols (3, 8). The mouse serum was diluted 1:2,000, and reaction products were visualized with affinity-purified goat phosphatase-labeled anti-mouse immunoglobulin G (heavy and light chains) (Kirkegaard & Perry Laboratories, Gaithersburg, Md.) diluted 1:2,000.

Nucleotide sequence accession numbers. The GenBank/EMBL sequence accession numbers for the lpp4[MmymyLC] gene from M. mycoides subsp. mycoides subsp. capri are AF072714 and AF072715, respectively.

**RESULTS**

**Cloning of the lpp4 genes of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri.** Southern blot analysis of HindIII-digested genomic DNA from the type and reference

strains of all members of the M. mycoides cluster was performed with a digoxigenin-labeled gene probe for the P72 (lipoprotein) gene of M. mycoides subsp. mycoides SC (8). These hybridization results showed the presence of genes analogous to the P72 gene in all members of the M. mycoides cluster.

Gene libraries of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri containing about 10⁶ phage clones ml⁻¹ were constructed with λ phage ZAP Express vector and screened. From both libraries, plasmids which were shown to contain the entire genes of the lipoproteins named lpp4[MmymyLC] and lpp4[Mmyca], as assessed from sequencing data of the extremities of the inserts, were retained. Plasmid pJFFMMMLC2 contained a 2.0-kb insert from M. mycoides subsp. mycoides LC, and plasmid pJFFMMCA4 contained a 4.5-kb insert from M. mycoides subsp. capri (Fig. 1). The integrity of the inserts of these plasmids was verified by PCR amplification of the corresponding fragments from genomic DNA of M. mycoides subsp. mycoides LC and from M. mycoides subsp. capri by using primers matching the sequenced extremities of the inserts (Table 2).

**DNA sequence analysis of the lipoprotein genes and of flanking genes.** The inserts of plasmids pJFFMMMLC2 and pJFFMMCA4 were sequenced in both directions. The nucleotide sequence of the insert pJFFMMMLC2 contained an open reading frame (ORF) encoding the lipoprotein LppA[MmymyLC] precursor of 526 amino acid (aa) residues with a predicted molecular mass of 60,288 kDa (Fig. 2). It is preceded by a consensus sequence for a ribosome binding site (RBS) located five nucleotides (nt) upstream of the initiation codon, AUG. The analogous ORF in plasmid pJFFMMCA4 encoded the lipoprotein LppA[Mmyca] precursor, which shows 523 aa residues and a predicted molecular mass for LppA[Mmyca] of

- **TABLE 2. Primer pairs used in PCR assays and for DNA sequencing**

| Primer pair | Nucleotide position | Sequence | Annealing temperature (°C) | Fragment size (bp) |
|------------|---------------------|----------|---------------------------|--------------------|
| MMMLC2-L   | 608–689             | 5'-CAATTCCAGATCAAGACCT-3' | 49                   | 1,049              |
| MMMLC1-R   | 1716–1997           | 5'-TCCTCTATATCCCCTTAGAA-3' | 47                   | 4,403              |
| JFFMMCA4-L | 141–160             | 5'-GATTATTTGAATGCAAC-3'    |                      |                    |
| JFFMMCA4-R | 4543–4522           | 5'-AAGTTACAAATGAAATTTA-3'  |                      |                    |
| T3         | T3 promoter         | 5'-GGCCCGAATTACCACTCATAAG-3' |                      |                    |
| T7         | T7 promoter         | 5'-GTAATACGATATCTAGGGGC-3' |                      |                    |

* The primer pairs were used as follows: MMMLC2-L and MMMLC1-R, for specific lpp4[MmymyLC] and lpp4[Mmyca] gene amplification; JFFMMCA4-L and JFFMMCA4-R, for amplification of genomic DNA for the verification of the integrity of the insert of pJFFMMCA4; and T3 and T7, for DNA sequencing.

* For MMMLC2-L and MMMLC1-R, the nucleotide positions correspond to those in the pJFFMMMLC2 insert according to GenBank accession no. AF072714, and for JFFMMCA4-L and JFFMMCA4-R, they correspond to those in the pJFFMMCA4 insert according to GenBank accession no. AF072715.

![FIG. 1. Locations of the lpp4 gene (black boxes) and surrounding genes on plasmids pJFFMMMLC2 containing a 2.0-kb insert cloned from the DNA of the M. mycoides subsp. mycoides LC strain Y-goat (a) and pJFFMMCA4 containing a 4.5-kb insert cloned from the DNA of the M. mycoides subsp. capri strain PG3 (b). The ORFs are represented by white boxes, and arrowheads indicate the direction of transcription and translation. The locations of the oligonucleotide primers are shown by arrows. Filled triangles indicate the location of p auc promoter of the cloning vector.](image-url)
FIG. 2. Amino acid sequence comparison of LppA precursors from the M. mycoides subsp. mycoides LC strain Y- goat and from the M. mycoides subsp. capri strain PG1. Underlining amino acids correspond to the consensus sequence for the signal peptidase II recognition site. The arrow indicates the potential cleavage site for this peptide. Vertical bars show identical amino acids, and dots between sequences show similar amino acids.

Expression and serological specificity of LppA of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri. The expression and the antigenic specificity of LppA in M. mycoides subsp. mycoides LC and M. mycoides subsp. capri were analyzed on immunoblots by using adsorbed polyclonal mouse antibodies directed against recombinant LppA[MmymyLC]. Whole cells of the M. mycoides subsp. mycoides LC and M. mycoides subsp. capri type strains and field strains, as well as the type and reference strains of the other members of the M. mycoides cluster (Table 1), were solubilized in SDS sample buffer and used as antigens on the immunoblots. The mouse anti-LppA[MmymyLC] serum strongly reacted with a 62-kDa band or a 60- and 62-kDa doublet for all strains of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri tested except strain WK354/80. The apparent molecular mass of LppA on SDS polyacrylamide gels (62 kDa) is slightly higher than the expected molecular mass as calculated from its DNA sequence-deduced amino acid composition (60 kDa). Strain WK354/80, for which the taxonomic classification is not clear, showed weak reactions with a doublet band of 58 and 60 kDa. The other mycoplasmas of the M. mycoides cluster did not react with anti-LppA[MmymyLC] antibodies, thus showing the antigenic specificity of LppA[MmymyLC] to M. mycoides subsp. mycoides LC and M. mycoides subsp. capri. The presence of lppA[MmymyLC] and lppA[MmymyLC] in M. mycoides subsp. mycoides LC and M. mycoides subsp. capri was studied in M. mycoides subsp. mycoides LC and M. mycoides subsp. capri strains and in the other members of the M. mycoides cluster by PCR amplification with the primer pair MMLC2-L and MMLC1-R (Table 2) by using as the templates chromosomal DNA from all mycoplasma strains used in this study (Table 1). All strains of M. mycoides subsp. mycoides LC and M. mycoides subsp. capri showed the expected DNA fragment of 1.05 kb with the exception of strain WK354/80, which amplified a smaller fragment (0.85 kb). The PCR product obtained from WK354/80 was analyzed further by DNA sequencing. DNA sequencing results showed that the edges of the PCR fragment but not the central part of the segment were identical to lppA[MmymyLC] and lppA[MmymyLC]. The sequence analysis of both plasmids pJFFMMLC2 and pJFFMMMC4 revealed a part of an ORF, ORF1, upstream of lppA. Its function is currently unknown. However, ORF1 in both M. mycoides subsp. mycoides LC and M. mycoides subsp. capri showed a high similarity to the ORF upstream of the gene encoding P72 in M. mycoides subsp. mycoides SC (8). The ORF1 of M. mycoides subsp. mycoides LC showed 52% identical amino acids and 69% identical nucleotides at the DNA level with the ORF1 of M. mycoides subsp. capri. In both plasmid clones, the lppA gene is followed by a hairpin structure representing a potential transcriptional termination signal (Fig. 1). In pJFFMMMC4, the hairpin structure was followed by two ORFs that sequenced in the opposite direction; one, ORFX, encodes a polypeptide with unknown function, and the other, representing a potential transcriptional termination signal, is part of the potential DNA methylase gene we found a hairpin structure that acts as a transcription stop signal and is part of the open reading frame that shows 39% identical nucleotides to the mtID (which encodes mannitol-1-phosphate dehydrogenase) gene analogue found in M. mycoides subsp. mycoides SC (8) (Fig. 1). In pJFFMMLC2, an ORFX was found downstream of the transcription stop signal following lppA. It showed 89% identical nucleotides to the analogous location in M. mycoides subsp. capri (Fig. 1).
coides cluster analyzed showed any amplification product (Fig. 4 and Table 1). These results confirmed that the primer pair MMMLC2-L and MMMLC1-R amplified a specific 1.05-kb fragment from \textit{M. mycoides} subsp. \textit{mycoides} LC and \textit{M. mycoides} subsp. \textit{capri}. For further analysis, the PCR amplification products of the \textit{lppA}\_[MmymyLC] and \textit{lppA}\_[Mmyca] genes were digested with the restriction enzyme \textit{AluI} and examined (Fig. 5). Profiles obtained from the different strains showed some variations, indicating a certain heterogeneity of the \textit{lppA} gene within the different strains. The profiles show that the same differences are found within strains of the same subspecies as well as among strains of different subspecies and hence do not differentiate \textit{M. mycoides} subsp. \textit{mycoides} LC strains from \textit{M. mycoides} subsp. \textit{capri} strains.

**DISCUSSION**

Sequence data show that the lipoproteins LppA\_[MmymyLC] and LppA\_[Mmyca] form a family together with the major lipoproteins of \textit{M. mycoides} subsp. \textit{mycoides} SC, P72, and of \textit{Mycoplasma} sp. bovine group 7, P67, and are hence suggested to have a function which is analogous to the two latter lipoproteins. Accordingly, we propose that P72 and P67 be renamed lipoproteins LppA\_[MmymySC] and LppA\_[Mbgr7], respectively.

Analysis of LppA from both \textit{M. mycoides} subsp. \textit{mycoides} LC and \textit{M. mycoides} subsp. \textit{capri} showed that the potential transmembrane region located in the leader sequence of LppA\_[Mmyca] is identical to that found in LppA\_[MmymySC] of \textit{M. mycoides} subsp. \textit{mycoides} SC and in LppA\_[Mbgr7] of \textit{Mycoplasma} sp. bovine group 7 (8, 16) and differs only in a single amino acid (residue 11) from the leader sequence of LppA\_[MmymyLC]. The corresponding gene fragment is also highly conserved and could therefore serve as a valuable probe for the cloning of other mycoplasmal lipoprotein genes.

DNA sequence analysis of the \textit{lppA} genes of \textit{M. mycoides} subsp. \textit{mycoides} LC and \textit{M. mycoides} subsp. \textit{capri} revealed a very high degree of similarity which is also reflected antigenically, as shown on immunoblots. Minor variations, which can be observed in the \textit{lppA} genes of various field strains, are not specific to the two subspecies. The differences seem to have
only minor phenotypic impact since they are not reflected anti-
genically as revealed by immunoblots. Genetic variation among
different field isolates from M. mycoides subsp. mycoides LC and
M. mycoides subsp. capri has also been detected in other gene loci (40a).
Hence, LppA seems to be a common and specific antigen of the two closely related mycoplasmas M. mycoides subsp. mycoides LC and M. mycoides subsp. capri. The chromosomal location of the lppA genes seems to be conserved in the vicinity of the 16S rRNA gene (which encodes mrmntitol-1-
phosphate dehydrogenase).

The specific PCR using the primer pair MMMLC2-L and
MMMLC1-R amplified the lppA genes from M. mycoides subsp. mycoides LC and M. mycoides subsp. capri field strains of various geographic origins but not those from other members of the M. mycoides cluster. We therefore propose that this PCR be used for the identification of the phylogenetic and antigenic entity M. mycoides subsp. mycoides LC/M. mycoides subsp. capri.

Interestingly, for strain WK354/80, PCR amplification of the
lppA gene resulted in a shorter-than-expected fragment which was
shown to be most similar to the lppA[Mbrg7] (P67) gene of
Mycoplasma sp. bovine group 7. WK354/80 strain was first
described in the literature as Mycoplasma sp. bovine group 7 (10) and was later retyped by us as M. mycoides subsp. capri. Se-
quence analysis of the rrs gene of strain WK354/80, on the
other hand, showed its strong similarity to that of M. mycoides subsp. mycoides LC. These results reflect the ambiguous taxo-
monic status of WK354/80, which must be located intermediate
between those of Mycoplasma sp. bovine group 7 and M. my-
coides subsp. mycoides LC/M. mycoides subsp. capri. It illus-
trates further the good discriminatory potential of the lppA
genes, which encode the major lipoproteins, in the differenti-
ation of the members of the M. mycoides cluster.

ACKNOWLEDGMENTS

We are grateful to Yvonne Schlatter for technical assistance
with DNA sequence analysis and PCR and to Margrit Krawinkler for expert
help with identification and cultivation of mycoplasmas. We thank Chris Minion, Ames, Iowa, for the kind gift of strain YN29/80, which
proved to be most helpful for the expression of cloned mycoplasmal
genes, and Shmuel Razin, Jerusalem, Israel, Kevin Dybvig, Birming-
ham, Alabama, and Karl-Erik Johanson, Uppsala, Sweden, for their
helpful suggestions in naming lipoproteins.

This study is part of European COST action 826 on ruminants’
mycoplasmas and was supported by grant C96.0073 of the Swiss
Ministry of Education and Science and by the Swiss Federal Veterinary
Office.

REFERENCES

1. Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman.
1990. Basic local alignment search tool. J. Mol. Biol. 215:403–410.
2. Askaa, G., H. Ernø, and M. O. Ojo. 1978. Bovine mycoplasmas: classification of
groups related to Mycoplasma mycoides. Acta Vet. Scand. 19:166–178.
3. Ausubel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A.
3. Askaa, G., H. Ernø, and M. O. Ojo. 1978. Bovine mycoplasmas: classification of
groups related to Mycoplasma mycoides. Acta Vet. Scand. 19:166–178.
4. Bairoch, A., P. Bucher, and K. Hofmann. 1990. Current protocols in molecular biology. Wiley
Interscience, New York, N.Y.
5. Bairoch, A., P. Bucher, and K. Hofmann. 1990. Current protocols in molecular biology. Wiley
Interscience, New York, N.Y.
6. Bairoch, A., P. Bucher, and K. Hofmann. 1990. Current protocols in molecular biology. Wiley
Interscience, New York, N.Y.
7. Bairoch, A., P. Bucher, and K. Hofmann. 1990. Current protocols in molecular biology. Wiley
Interscience, New York, N.Y.
8. Bairoch, A., P. Bucher, and K. Hofmann. 1990. Current protocols in molecular biology. Wiley
Interscience, New York, N.Y.
9. Christiansen, C., and H. Ernø. 1982. Classification of the F38 group of

caprine mycoplasma strains by DNA hybridization. J. Gen. Microbiol. 128:
2523–2526.
10. Corboz, L., H. Keller, A. Waldvogel, and U. Weideli. 1980. Spontaneous and
artificial cases of polyarthritides and synovitises in calves due to mycoplasma. II.
Bacteriological and patho-anatomical findings. Schweiz. Arch. Tierheilkd. 122:
479–481.
11. Costas, M., R. H. Leach, and D. L. Michelfelter. 1987. Numerical analysis of PAG
protein patterns and the taxonomic relationships within the ‘Mycoplasma
mycoides cluster’. J. Gen. Microbiol. 133:3319–3329.
12. DaMassa, A. J., S. P. Wakenell, and D. L. Brooks. 1992. Mycoplasmas of
goats and sheep. J. Vet. Diagn. Invest. 4:101–113.
13. Frey, J., X. Cheng, M. F. Monnerat, E. M. Abdo, M. Krawinkler, G. Bölcke,
and J. Nicolet. 1996. Genetic and serological analysis of the immunogenic
67-kDa lipoprotein of Mycoplasma sp. bovine group 7. Res. Microbiol. 149:
55–64.
14. Gourlay, R. N., and C. J. Howard. 1979. Bovine mycoplasmas, p. 49–102. In J.
G. Tully and R. F. Whitcomb (ed.), The mycoplasmas, vol. II. Academic
Press, Inc., New York, N.Y.
15. Harlow, E., and D. Lane. 1988. Antibodies: a laboratory manual. Cold Spring
Harbor Laboratory, Cold Spring Harbor, N.Y.
16. Hayashi, S., and H. C. Wu. 1980. Lipoproteins in bacteria. J. Bioenerg.
Biomembr. 22:451–471.
17. Kapoor, P. K., D. N. Garg, and S. K. Mahajan. 1989. Isolation of Mycoplasma
mycoides subsp. mycoides (LC variant, ‘goat’) from naturally aborted bovine
foetuses. Theriogenology 32:683–691.
18. Kumar, H., N. S. Parihar, K. Charan, and K. P. Singh. 1994. Pathology and
bronchoscopic studies in Mycoplasma mycoides subsp. capri infection in
goats. Indian J. Anim. Sci. 64:1005–1005.
19. Leach, R. H., M. Costas, and D. L. Michelfelter. 1989. Relationship between
Mycoplasma mycoides subsp. mycoides (‘large-colony’ strains) and M. mycoides
subsp. capri, as indicated by numerical analysis of one-dimensional
SDS-PAGE protein patterns. J. Gen. Microbiol. 135:2993–3000.
20. LppA IN MYCOPLASMA MYCIOIDES SUBSPECIES...
Mycoplasma mycoides subspecies mycoides from polyarthritis and mastitis of goats in Canada. Can. Vet. J. 24:54–56.

37. Smiley, B. K., and F. C. Minion. 1993. Enhanced readthrough of opal (UGA) stop codons and production of Mycoplasma pneumoniae P1 epitopes in Escherichia coli. Gene 134:33–40.

38. Taylor, T. K., J. B. Bashiruddin, and A. R. Gould. 1992. Relationships between members of the Mycoplasma mycoides cluster as shown by DNA probes and sequence analysis. Int. J. Syst. Bacteriol. 42:593–601.

39. ter Laak, E. A. 1992. Contagious bovine pleuropneumonia. A review. Vet. Q. 14:104–110.

40. Thiaucourt, F., and G. Bölske. 1996. Contagious caprine pleuropneumonia and other pulmonary mycoplasmoses of sheep and goats. Rev. Sci. Tech. 15:1397–1414.

40a. Thiaucourt, F. Unpublished observations.

41. Villalba, E. J., J. B. Poveda, A. Fernandez, J. L. Rodriguez, C. Gutierrez, and J. Gomez Villamandos. 1992. An outbreak caused by Mycoplasma mycoides species in goats in the Canary Islands. Vet. Rec. 130:330–331.

42. Yamao, F., A. Muto, Y. Kawauchi, M. Iwami, S. Iwagami, Y. Azumi, and S. Osawa. 1985. UGA is read as tryptophan in Mycoplasma capricolum. Proc. Natl. Acad. Sci. USA 82:2306–2309.