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Original article

Mycoplasma mycoides subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* LC can be grouped into a single subspecies

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Abstract – *Mycoplasma mycoides* subsp. *capri* and *Mycoplasma mycoides* subsp. *mycoides* LC can be combined into one taxon on the basis of several contributions on both DNA sequence and protein analyses reported in the literature. Moreover, for the differentiation and identification of mycoplasmas of the "mycoides cluster", we investigated the *rpoB* gene, encoding the β-subunit of the RNA polymerase. A segment of 527 bp of the *rpoB* gene was amplified from 31 strains of ruminant mycoplasmas by PCR. The nucleotide sequences were determined and aligned, and accurate genetic relationships were calculated. Cluster analysis of *rpoB* DNA allowed species differentiation within the "mycoides cluster" and confirmed that *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC cannot be distinguished from each other. "*Mycoplasma mycoides* subsp. *capri*" is proposed as a common name for both subspecies

***Mycoplasma mycoides* subsp. *capri* / *Mycoplasma mycoides* subsp. *mycoides* LC / ruminant mycoplasmoses / taxon / serovar**

1. INTRODUCTION

Mycoplasma mycoides subsp. *capri* and *M. mycoides* subsp. *mycoides* large-colony type (LC) belong to the "mycoides cluster", a group of six closely related mycoplasmas [6]. *M. mycoides* subsp. *capri* causes mastitis, arthritis, pulmonary diseases and septicaemia specifically in goats [6, 14, 17, 22, 31, 34, 37]. *M. mycoides* subsp. *mycoides* LC is reported to cause a pattern of diseases similar to those induced by *M. mycoides* subsp. *capri* in goats, including mastitis, keratoconjunctivitis, polyarthritis, pneumonia and septicaemia [1, 6, 7, 9, 26, 28, 29]. It has also been isolated, rarely, from cattle [11, 23], sheep [20] and Vaal rhebok [19].

From an epidemiological point of view, differential identification of the six members of the "mycoides cluster" is of major importance, 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 "mycoides cluster" [2, 5, 8, 10, 18, 25, 33, 36].

M. mycoides subsp. *capri* and *M. mycoides* subsp. *mycoides* LC are antigenically very similar as assessed by

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numerical analysis of one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) protein patterns [4, 16], by two-dimensional PAGE protein patterns [21, 27], as well as by serological analysis [18]. A DNA probe based on a randomly selected genomic fragment was developed for the differentiation of the different members of the "mycoides cluster". This DNA probe grouped *M. mycoides* subsp. *capri* strains together with *M. mycoides* subsp. *mycoides* LC and could not allow differentiation between these two mycoplasmas, but it distinguished them from the other members of the "mycoides cluster" [32]. DNA-DNA hybridization studies revealed variable values for DNA homology between *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC (75 to 94%) depending on experimental conditions [18]. Several phylogenetic studies were performed in the view to distinguish these two strains. Sequence analysis of 16S rRNA genes revealed 99.9% similarity between *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC [24]. Since phylogenetic analysis based upon the 16S rRNA gene sequences alone provided only a limited understanding of species relationships and evolutionary history, and 16S rRNA operons show high intraspecific variation, sequences of protein-encoding genes have been suggested and used. Hence, sequence analysis of the *lppA* gene [18] and of a putative membrane protein gene [35] were assessed. These methods also clustered together both *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC. Very recently, tRNA gene PCR fingerprinting showed a very close relationship between these two mycoplasmas as well [30].

To give additional insight into the genetic identification of *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC, we decided to consider another genetic marker that has been used successfully in our laboratory for the elaboration of relationships in other bacteria [13]. This marker is the *rpoB* gene coding for the DNA-directed β-subunit of the RNA polymerase and has a higher discriminatory power than 16S rRNA gene sequences.

2. MATERIALS AND METHODS

2.1. Strains, growth conditions and DNA extraction

Strains of *M. mycoides* subsp. *mycoides* LC ($n = 8$) and *M. mycoides* subsp. *capri* ($n = 6$) and of the other *Mycoplasma* species ($n = 17$) are listed in Table I. The cells were grown in a standard mycoplasma medium (Axcell Biotechnologies, St. Genis l'Argentière, France) for 3 days at 37 °C to a density of 10^8–10^9 cells/mL. Growth and handling of live *M. mycoides* subsp. *mycoides* SC were performed in a biological safety laboratory fulfilling the BL3 containment safety standards. Lysis of mycoplasmas with GES buffer (5 M guanidium thiocyanate, 100 mM EDTA, 0.5% *N*-lauroylsarcosine) and extraction of genomic DNA were performed as previously described [3].

2.2. PCR and sequencing strategies

Polymerase chain reaction (PCR) was performed with a DNA thermal cycler Gene Amp PCR System 9600 (Applied Biosystems, Foster City, CA, USA) in a 30-μL reaction mixture (1 × reaction buffer B (supplied with FIREPol® DNA polymerase), 2.5 mM $MgCl_2$, 250 μM of each dNTP) that contained approximately 50 ng of genomic template DNA, 2.5 U of FIREPol® DNA polymerase (Solis BioDyne, Tartu, Estonia), and 400 nM of oligonucleotide primers CAMPrpoB-L4 (5'-CCAATTTATGGATCAAAT-3') and Rpob-R (5'-GTTGCATGTTNGNAC-CCAT-3'). The mixtures were subjected

Table I. *Mycoplasma* strains used.

Mycoplasma species	Strain	Origin	Isolated	Host	Accession number[a]
M. mycoides subsp.	Y-goat	Australia	1956	Goat/type strain	AM087655
mycoides LC	152/93	Grand Canary	1993	Goat	AM087656
	LC8065	France		Goat	AM087657
	D2482/91	Switzerland	1991	Goat	AM087658
	950010	France	1995	Goat	AM261514
	D2083/91	Switzerland	1991	Goat	AM261515
	CP271	Portugal	1991	Goat	AM261516
	D2503	Switzerland		Goat	AM261517
M. mycoides subsp.	PG3		1950	Goat/type strain	AM087659
capri	N108	Nigeria	1977	Goat	AM087660
	WK354/80[b]	Switzerland	1980	Goat	AM087661
	213	India	1984	Goat	AM261518
	9139-11/91	Turkey	1991		AM261519
	capri L	France	1975	Goat	AM261520
M. mycoides subsp.	PG1		1931	Cattle/type strain	ND[c]
mycoides SC	Afadé	Cameroon	1968	Cattle/lung	AM180630
	L2	Italy	1993	Cattle/lung	AM180631
	T1/44	Tanzania	1952	Cattle/vaccine strain	AM180632
Mycoplasma sp.	PG50	Australia	1963	Cattle/reference strain	AM087662
bovine group 7	PAD3186	India	1993	Goat/milk	AM180633
	FRD424	India	1993	Goat/milk	AM180634
	Calf 1	Nigeria		Cattle/blood	AM180635
	D318b	Germany		Cattle/semen	AM180636
Mycoplasma sp.	B144P	USA	1956	Cattle/joint	AM087663
serogroup L					
M. capricolum subsp.	California kid	USA	1955	Goat/type strain	AM087664
capricolum	173/87	Greece		Sheep	AM180637
	6443.90	France	1990	Goat	AM261521
M. capricolum subsp.	F38	Kenya	1976	Goat/type strain	AM087665
capripneumoniae	9081-487p	Oman	1990	Goat	AM180638
	Gabès	Tunisia	1981	Goat	AM180639
M. putrefaciens	KS1	USA		Goat/type strain	AM087666

[a] Deposited EMBL/GenBank accession numbers for the nucleotide sequences of the fragments from the *rpoB* genes.

[b] Taxonomic identification unclear; originally characterized as *Mycoplasma* sp. bovine group 7 [18].

[c] Sequence of *rpoB* not determined in this work since it is already available from the PG1 genome project [38].

to 3-min denaturation at 94 °C followed by 35 cycles of amplification with the parameters: 30 s at 94 °C, 30 s at 50 °C and 1 min extension at 72 °C, and a final extension step at 72 °C for 7 min. Amplicons were purified with the High pure PCR product purification kit (Roche Diagnostics, Rotkreuz, Switzerland).

DNA sequencing of the purified amplicons was performed with a DNA Sequenator AB 3100 genetic analyzer and the *Taq* dye deoxy terminator cycle sequencing kit (Applied Biosystems) using either primer CAMPrpoB-L4 or primer Rpob-R as previously described [13]. The assembling of DNA sequences and alignments of sequenced segments were done using the program Sequencher 4.6 (GeneCodes, Ann Arbor, MI, USA). Phylogenetic analysis was done using the program BioNumerics 4.0 (Applied Maths, Kortrijk, Belgium).

2.3. Nucleotide sequence accession numbers

The deposited EMBL/GenBank accession numbers for the nucleotide sequences of the fragments from the *rpoB* genes from the *Mycoplasma* strains used in this study are listed in Table I.

3. RESULTS

3.1. *rpoB* sequences

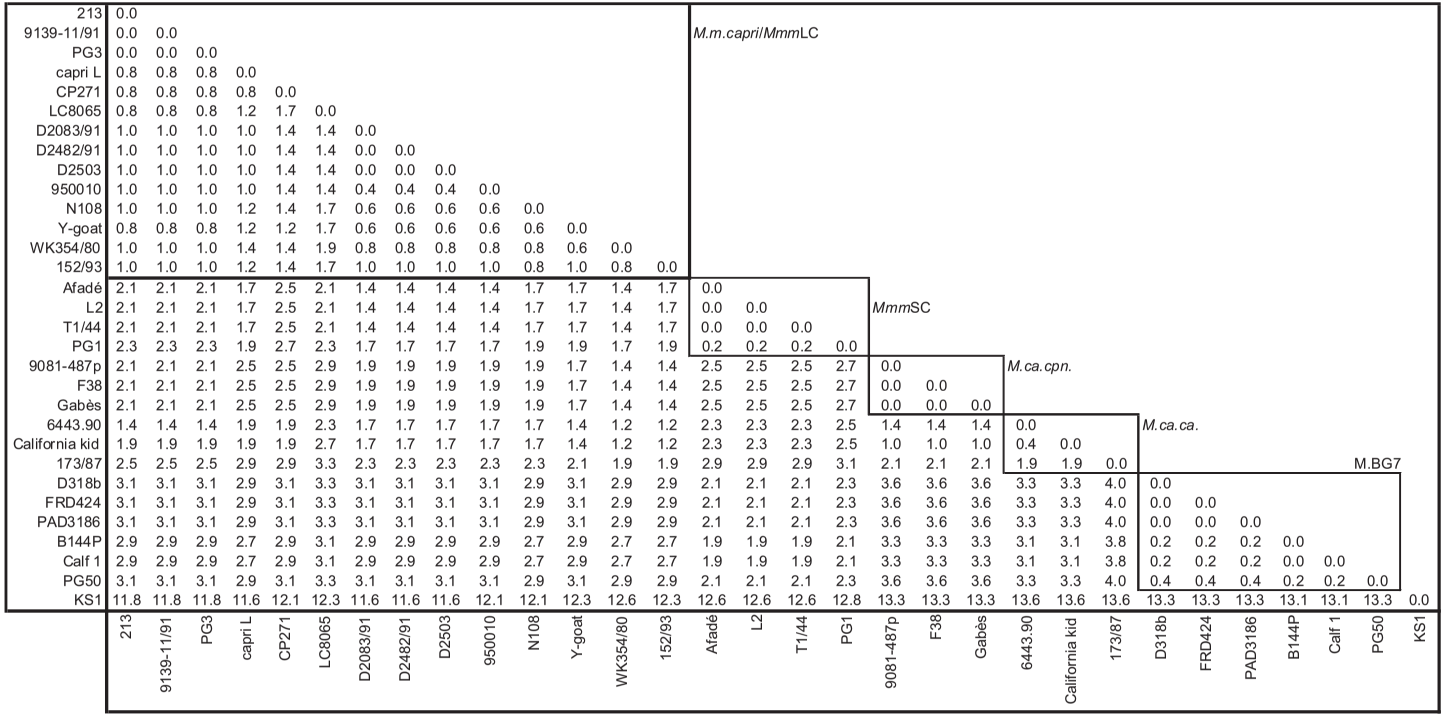
Amplicons of 527 bp from the *rpoB* genes of the 31 investigated *Mycoplasma* strains (Tab. I) were obtained. The nucleotide sequences (490 bp) of amplified DNA were determined and compared. The G+C contents of these sequenced DNA were 29.2 to 32.4%, reflecting the G+C-poor genome characteristic of mycoplasmas. No insertions or deletions were observed. The determined nucleotide sequences were compared pairwise for identity; the results showed that the 31 *Mycoplasma* strains were closely related to each other. In general, 0 to 13.6% divergence (i.e., 86.4 to 100% identity) was observed among the mycoplasmas tested (Fig. 1). Members of a same subspecies had almost identical sequences with only up to 1.9% divergence. The sequence divergences ranged from 0.0 to 1.9% also among strains of *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC. The maximal divergence within the "mycoides cluster" was of 4.0%, while the *M. putrefaciens* strain KS1 was easily differentiated from strains of the "mycoides cluster" (11.6 to 13.6% divergence).

3.2. Cluster analysis tree

Genetic relationships were calculated using the UPGMA clustering method (Fig. 2). All mycoplasmas of the "mycoides cluster" were relatively united. However, clustering of the mycoplasmas according to their species of affiliation was evident. Cluster analysis confirmed the inability to distinguish between *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC as the analyzed strains grouped together. *M. putrefaciens* strain KS1 was separated distantly from the mycoplasmas of the "mycoides cluster".

3.3. Amino acid sequences

The deduced amino acid sequences of amplified *rpoB* DNA comprised 163 amino acid residues (N_{556} to L_{718}) of the β-subunit of the RNA polymerase of 1291 amino acids from *M. mycoides* subsp. *mycoides* SC strain PG1 (Fig. 3). By considering the sequences of the 31 strains, we found a total of 81 codon variations in the 490 nucleotides analyzed. Most of these variations (n = 55 or 67.9% of the total) were characterized by a nucleotide change in the last position of the codons, with an amino acid change ratio of 5/55. Fourteen variations (17.3%) occurred at position 1



	213	9139-11/91	PG3	capri L	CP271	LC8065	D2083/91	D2482/91	D2503	950010	N108	Y-goat	WK354/80	152/93	Afadé	L2	T1/44	PG1	9081-487p	F38	Gabès	6443.90	California kid	173/87	D318b	FRD424	PAD3186	B144P	Calf 1	PG50	KS1
213	0.0																														
9139-11/91	0.0	0.0																													
PG3	0.0	0.0	0.0																												
capri L	0.8	0.8	0.8	0.0																											
CP271	0.8	0.8	0.8	0.8	0.0																										
LC8065	0.8	0.8	0.8	1.2	1.7	0.0																									
D2083/91	1.0	1.0	1.0	1.0	1.4	1.4	0.0																								
D2482/91	1.0	1.0	1.0	1.0	1.4	1.4	0.0	0.0																							
D2503	1.0	1.0	1.0	1.0	1.4	1.4	0.0	0.0	0.0																						
950010	1.0	1.0	1.0	1.0	1.4	1.4	0.4	0.4	0.4	0.0																					
N108	1.0	1.0	1.0	1.2	1.4	1.7	0.6	0.6	0.6	0.6	0.0																				
Y-goat	0.8	0.8	0.8	1.2	1.2	1.7	0.6	0.6	0.6	0.6	0.6	0.0																			
WK354/80	1.0	1.0	1.0	1.4	1.4	1.9	0.8	0.8	0.8	0.8	0.8	0.6	0.0																		
152/93	1.0	1.0	1.0	1.2	1.4	1.7	1.0	1.0	1.0	1.0	0.8	1.0	0.8	0.0																	
Afadé	2.1	2.1	2.1	1.7	2.5	2.1	1.4	1.4	1.4	1.4	1.7	1.7	1.4	1.7	0.0																
L2	2.1	2.1	2.1	1.7	2.5	2.1	1.4	1.4	1.4	1.4	1.7	1.7	1.4	1.7	0.0	0.0															
T1/44	2.1	2.1	2.1	1.7	2.5	2.1	1.4	1.4	1.4	1.4	1.7	1.7	1.4	1.7	0.0	0.0	0.0														
PG1	2.3	2.3	2.3	1.9	2.7	2.3	1.7	1.7	1.7	1.7	1.9	1.9	1.7	1.9	0.2	0.2	0.2	0.0													
9081-487p	2.1	2.1	2.1	2.5	2.5	2.9	1.9	1.9	1.9	1.9	1.9	1.7	1.4	1.4	2.5	2.5	2.5	2.7	0.0												
F38	2.1	2.1	2.1	2.5	2.5	2.9	1.9	1.9	1.9	1.9	1.9	1.7	1.4	1.4	2.5	2.5	2.5	2.7	0.0	0.0											
Gabès	2.1	2.1	2.1	2.5	2.5	2.9	1.9	1.9	1.9	1.9	1.9	1.7	1.4	1.4	2.5	2.5	2.5	2.7	0.0	0.0	0.0										
6443.90	1.4	1.4	1.4	1.9	1.9	2.3	1.7	1.7	1.7	1.7	1.7	1.4	1.2	1.2	2.3	2.3	2.3	2.5	1.4	1.4	1.4	0.0									
California kid	1.9	1.9	1.9	1.9	1.9	2.7	1.7	1.7	1.7	1.7	1.7	1.4	1.2	1.2	2.3	2.3	2.3	2.5	1.0	1.0	1.0	0.4	0.0								
173/87	2.5	2.5	2.5	2.9	2.9	3.3	2.3	2.3	2.3	2.3	2.3	2.1	1.9	1.9	2.9	2.9	2.9	3.1	2.1	2.1	2.1	1.9	1.9	0.0							
D318b	3.1	3.1	3.1	2.9	3.1	3.3	3.1	3.1	3.1	3.1	2.9	3.1	2.9	2.9	2.1	2.1	2.1	2.3	3.6	3.6	3.6	3.3	3.3	4.0	0.0						
FRD424	3.1	3.1	3.1	2.9	3.1	3.3	3.1	3.1	3.1	3.1	2.9	3.1	2.9	2.9	2.1	2.1	2.1	2.3	3.6	3.6	3.6	3.3	3.3	4.0	0.0	0.0					
PAD3186	3.1	3.1	3.1	2.9	3.1	3.3	3.1	3.1	3.1	3.1	2.9	3.1	2.9	2.9	2.1	2.1	2.1	2.3	3.6	3.6	3.6	3.3	3.3	4.0	0.0	0.0	0.0				
B144P	2.9	2.9	2.9	2.7	2.9	3.1	2.9	2.9	2.9	2.9	2.7	2.9	2.7	2.7	1.9	1.9	1.9	2.1	3.3	3.3	3.3	3.1	3.1	3.8	0.2	0.2	0.2	0.0			
Calf 1	2.9	2.9	2.9	2.7	2.9	3.1	2.9	2.9	2.9	2.9	2.7	2.9	2.7	2.7	1.9	1.9	1.9	2.1	3.3	3.3	3.3	3.1	3.1	3.8	0.2	0.2	0.2	0.0	0.0		
PG50	3.1	3.1	3.1	2.9	3.1	3.3	3.1	3.1	3.1	3.1	2.9	3.1	2.9	2.9	2.1	2.1	2.1	2.3	3.6	3.6	3.6	3.3	3.3	4.0	0.4	0.4	0.4	0.2	0.2	0.0	
KS1	11.8	11.8	11.8	11.6	12.1	12.3	11.6	11.6	11.6	12.1	12.1	12.3	12.6	12.3	12.6	12.6	12.6	12.8	13.3	13.3	13.3	13.6	13.6	13.6	13.3	13.3	13.3	13.1	13.1	13.3	0.0

Figure 1. Sequence pair distances of the 31 *Mycoplasma* strains analyzed based on their *rpoB* genes. Distances were determined by using the Jukes-Cantor algorithm. The strain names are given and the boxes represent the different species. *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC strains are grouped in a single box (thick-lined). Abbreviations: M.BG7: *Mycoplasma* sp. bovine group 7; *Mmm*SC: *M. mycoides* subsp. *mycoides* SC; *Mmm*LC: *M. mycoides* subsp. *mycoides* LC; *M.m.capri*: *M. mycoides* subsp. *capri*; *M.ca.ca.*: *M. capricolum* subsp. *capricolum*; *M.ca.cpn.*: *M. capricolum* subsp. *capripneumoniae*.

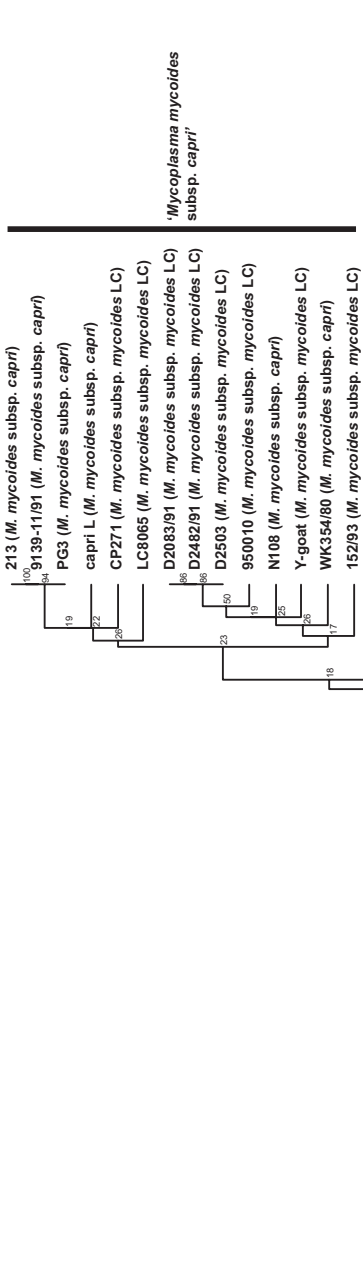


Figure 2. Representation of the genetic relationship of the *rpoB* sequences of all *Mycoplasma* strains analyzed. Distance matrix was calculated by the Jukes-Cantor algorithm and the cluster analysis tree was built by the UPGMA method. Bootstrap values of 500 replications are indicated at branches. The strain and species names are given. *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC strains are written in bold. The scale bar represents the percentage of sequence divergence.

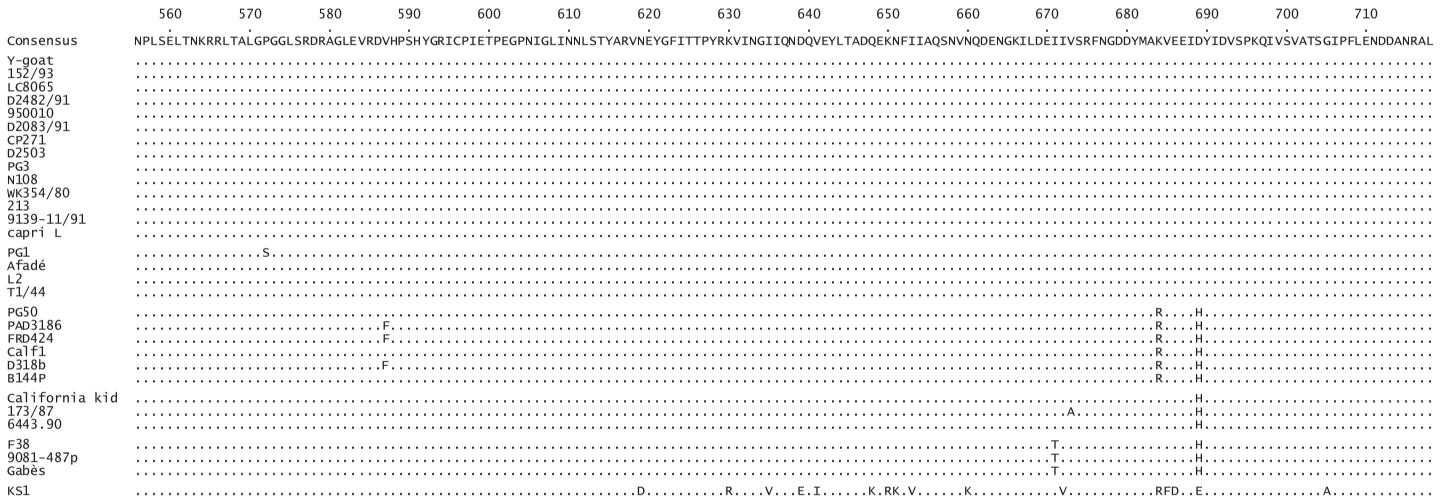


Figure 3. Multiple alignment of the deduced amino acid (N_{556} to L_{718} (*M. mycoides* subsp. *mycoides* SC strain PG1 numbering)) sequence of *rpoB*. The identical sequences to consensus are indicated as dots.

of the codons, with an elevated amino acid change ratio of 10/14. The 5 variations (6.2%) at position 2 of the codons were all producing an amino acid change. Interestingly, we found 5 variations (6.2%) at both positions 1+3 of the codons but only one of them allowed an amino acid change. Moreover, we found 1 variation (1.2%) at both positions 2+3 of the codons, which produced an amino acid change. Finally, there was 1 variation (1.2%) at all three positions of the codon, but it did not lead to an amino acid change. Hence, of the 81 codon variations, 22 (27.2%) were missense variations (Tab. II) and 59 (72.8%) were silent.

All strains of *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri* presented the same amino acid sequence in the region comprised between amino acid residues N_{556} and L_{718} of the DNA-directed RNA polymerase beta chain (Fig. 3). Thus, all 22 missense variations referred with the *rpoB* sequences from strains of *M. putrefaciens*, *M. capricolum*, *M. mycoides* subsp. *mycoides* SC, *Mycoplasma* sp. bovine group 7 or *Mycoplasma* sp. serogroup L in comparison to the *rpoB* sequences from strains of *M. mycoides* subsp. *mycoides* LC and subsp. *capri* (Tab. II). Among these 22 missense variations, 15 (68.2%) referred to the *M. putrefaciens* strain KS1 only, one to all *M. capricolum* subsp. *capripneumoniae* strains only (variation at position 2 of codon 671), and one to all *Mycoplasma* sp. bovine group 7 strains plus *Mycoplasma* sp. serogroup L strain B144P only (variation at positions 1+3 of codon 689). There was another missense variation involving codon 689 (variation at position 1 only), which was observed in all strains of the species *M. capricolum* (i.e., both subsp. *capricolum* and subsp. *capripneumoniae*). Interestingly, also one of the 15 missense variations referred to KS1 involved codon 689 (variation at position 3 only). We found a missense variation also involving codon 684 (variation at position 2) in all *Mycoplasma* sp. bovine group 7 strains plus B144P and the *M. putrefaciens* strain KS1. The remaining 3 missense variations were not related to species differentiation but rather to intraspecies variability (Fig. 3, Tab. II).

In the majority of the cases (16/22), the missense variation did not lead to any change of the amino acid charge (Tab. II). In contrast, in six cases, the missense variation led to a charge change of the encoded amino acid. Four of them involved a change from neutral to charged residues (all in *M. putrefaciens* strain KS1) and two led to a change from a negatively charged residue (Asp) to a positively charged residue (His). Interestingly, both cases referred to amino acid 689, whereby H_{689} was found in all strains of *M. capricolum* (due to their variation at codon position 1) and in all strains of *Mycoplasma* sp. bovine group 7 plus B144P (due to their variation at codon positions 1+3), whereas all other strains had D_{689} (excluding KS1 that had E_{689} due to its variation at codon position 3).

3.4. Silent variations

As already mentioned above, 59 of the 81 codon variations were silent. They were evenly distributed on the 490 nucleotides analyzed. Most of them (n = 50 or 84.7% of the total silent variations) were characterized by a nucleotide change at position 3 of the codons. Of the 59 silent variations, 26 (or 44.1%) referred to *M. putrefaciens* strain KS1 only (20 of which involving a change at codon position 3; the exceptional silent variation involving changes at all three positions of S_{704} with TCA instead of an AGT codon also referred to strain KS1 only).

Other particular silent variations were found by amino acids N_{556} (codon position 3 differentiating *M. mycoides* subsp. *mycoides* SC, *Mycoplasma* sp. bovine

Table II. Missense variations of *rpoB* in mycoplasmas.

Amino acid position[a]	Nucleotide change(s)[b]	Amino acid change	Charge change[c]	Mycoplasma(s) involved
572	CCT→TCT	Pro→Ser	0 → 0	PG1
587	GTT→TTT	Val→Phe	0 → 0	3 strains of *Mycoplasma* sp. bovine group 7
619	AAT→GAT	Asn→Asp	0 → –	KS1
630	AAA→AGA	Lys→Arg	+ → +	KS1
635	ATT→GTT	Ile→Val	0 → 0	KS1
639	GAT→GAA	Asp→Glu	– → –	KS1
641	GTT→ATT	Val→Ile	0 → 0	KS1
648	CAA→AAA	Gln→Lys	0 → +	KS1
650	AAA→AGA	Lys→Arg	+ → +	KS1
651	AAC→AAA	Asn→Lys	0 → +	KS1
653	ATT→GTT	Ile→Val	0 → 0	KS1
660	AAT→AAA	Asn→Lys	0 → +	KS1
671	ATT→ACT	Ile→Thr	0 → 0	all *M. capricolum* subsp. *capripneumoniae*
672	ATA→GTA	Ile→Val	0 → 0	KS1
673	GTT→GCT	Val→Ala	0 → 0	173/87
684	AAA→AGA	Lys→Arg	+ → +	all *Mycoplasma* sp. bovine group 7 + KS1
685	GTT→TTT	Val→Phe	0 → 0	KS1
686	GAA→GAT	Glu→Asp	+ → +	KS1
689	GAT→CAT	Asp→His	– → +	all *M. capricolum* (both subspp.)
689	GAT→CAC	Asp→His	– → +	all *Mycoplasma* sp. bovine group 7
689	GAT→GAA	Asp→Glu	– → –	KS1
705	GGT→GCA	Gly→Ala	0 →0	KS1

[a] The deduced amino acid sequences of amplified *rpoB* comprised amino acid residues N_{556} to L_{718} (*M. mycoides* subsp. *mycoides* SC numbering).

[b] Base changes are underlined. The first codon refers to *rpoB* in *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC, and the second codon to the mycoplasma(s) with the missense variation.

[c] 0, neutral residue; –, charged minus; +, charged plus.

group 7 plus *Mycoplasma* sp. serogroup L strain B144P from all other species), L_{608} and N_{633} (both codon positions 3 differentiating *Mycoplasma* sp. bovine group 7 plus B144P from all other species), I_{635} (codon position 3 differentiating *M. capricolum* subsp. *capripneumoniae* from all other species), and I_{688} (codon position 3 differentiating *M. mycoides* subsp. *mycoides* SC from all other species). The remaining 28 silent variations showed a more or less random characteristic and

allowed no particular clustering of the species but rather indicated intraspecies variability (not shown).

4. DISCUSSION

On the basis of several published works, the genetic and antigenic differences found between *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC seem to represent different serovars rather than different subspecies [4, 15, 16, 18, 21, 24, 27, 30, 32, 35]. This inability to distinguish these two organisms has been debated by the International Committee on Systematics of Prokaryotes – Subcommittee on the Taxonomy of Mollicutes, and it was suggested that an additional comparative analysis was required to definitively put an end to the inquiry about the relationship between these two mycoplasmas.

Recently, the usefulness of *rpoB* for phylogenetic study of *Mycoplasma* species was reported by the group of Kook [12], however, no mycoplasmas of the "mycoides cluster" were analyzed. Kook et al. have analyzed a region corresponding to that encoding amino acids 491–588 of the β-subunit of the RNA polymerase from *M. mycoides* subsp. *mycoides* SC strain PG1, which harbored insertion-deletion regions that led to size variations between species. On the contrary, we decided to analyze the region spanning amino acid residues 556–718 since it was free of insertion-deletion regions and it would also be an appropriate marker for mycoplasma differentiation due to the fact that sequence variations in this region occurred randomly, as it was shown for species of the family Pasteurellaceae [13].

The mycoplasma strains used in this study constituted representative populations for each species of the "mycoides cluster" comprising various years of isolation, various countries of origin and different pathological backgrounds. The level of divergence of *rpoB* among the mycoplasmas of the same species was usually from 0 to 1.4% (i.e., at least 98.6% identity was generally exhibited). However, one strain among the *M. mycoides* subsp. *mycoides* LC isolates (LC8065) showed 1.7% divergence to its type strain (Y-goat), with the result that the latter was more similar to the type strain of *M. capricolum* subsp. *capricolum* (California kid) than to LC8065 itself. Also, one strain among the *M. capricolum* subsp. *capricolum* isolates (173/87) showed a high divergence (1.9%) to its reference strain, with the result that California kid was more similar to some strains of the species *M. mycoides* than to 173/87. The reason for these results is the random characteristic of sequence variation in this *rpoB* region, which implies the possibility of a more elevated variation frequency. It has to be noted, however, that all of these variations involving strains LC8065 or 173/87 were silent, with the only exception of the $GTT_{Val} \rightarrow GCT_{Ala}$ transition of amino acid 673 by strain 173/87, which, however, did not alter the aliphatic hydrophobic neutral feature of the residue.

Analysis of the missense variations at codons 671, 684 and especially 689 of *rpoB* not only grouped together all strains of *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC, but also included all strains of *M. mycoides* subsp. *mycoides* SC, differentiating these three mycoplasmas from the other three members of the "mycoides cluster" (i.e., *Mycoplasma* sp. bovine group 7, *M. capricolum* subsp. *capricolum* and *M. capricolum* subsp. *capripneumoniae*), thus indicating that the species designation *M. mycoides* remains appropriate for both *M. mycoides* subsp. *mycoides* LC and *M. mycoides* subsp. *capri*.

In conclusion, all currently available results and our present work suggest that *M. mycoides* subsp. *capri* and *M. mycoides* subsp. *mycoides* LC can be grouped into a

single subspecies and therefore be named collectively "*Mycoplasma mycoides* subsp. *capri*". Moreover, substitution of the subspecies designation "*mycoides* LC" with "*capri*" would also provide clear name distinction between *M. mycoides* subsp. *mycoides* LC, which causes several diseases in small ruminants, and *M. mycoides* subsp. *mycoides* SC, the highly pathogenic etiological agent of contagious bovine pleuropneumonia (CBPP).

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