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ELECTROPHORETIC ANALYSIS OF ISOENZYMES OF MYCOPLASMA SPECIES

By

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SALIH, M. M., H. ERNØ and V. SIMONSEN: *Electrophoretic analysis of isoenzymes of Mycoplasma species.* — Acta vet. scand. 1983, *24*, 14—33. — The purpose of this paper is to further characterize and determine relatedness among some Mycoplasma species or serogroups of significant practical veterinary interest. Twenty-four strains were examined for the presence of 35 enzymes by horizontal starch gel electrophoresis, revealing a total of 127 different electromorphs of 30 enzymes. Inter- as well as intraspecific differences were found demonstrating the application of isoenzyme studies in classification as well as epidemiology. It is concluded that the F38 group of *MacOwan* and group 7 of *Leach* constitute 2 new species. The elevation of the 2 subspecies of M. mycoides (mycoides and capri) to species level is favoured, but it is suggested that a decision be taken internationally, considering the practical consequences of a given nomenclature. Three alternative possibilities for classification are presented.

Regarding identification the results suggest that the presence or absence of maltase and ornithine transcarbamylase indicate whether an isolate is related to the agent of contagious bovine pleuropneumonia or merely a representative of either the caprine LC type of M. mycoides subsp. mycoides or the classical caprine subspecies, M. mycoides subsp. capri.

mycoplasmas; classification; isoenzymes; M. mycoides-related groups.

Serological, biochemical and genetic investigations of mycoplasmas have to be considered in order to make a meaningful and practical classification. Differences in the genetic constitution of mycoplasmas may be determined indirectly by polyacrylamide electrophoresis (*Razin* 1968, *Awaad et al.* 1978), two-dimensional electrophoresis (*Rodwell et al.* 1978, *Rodwell & Rodwell* 1978) and by immunoprecipitation test (*Archer* 1979) of cell proteins, or directly by nucleic acid hybridization techniques (*Askaa et al.* 1978, *Christiansen & Ernø* 1982). Recently studies of isoenzyme

analysis by thin layer starch, vertical starch and horizontal starch gel electrophoresis were found to be potentially useful for distinguishing strains of both genus Acholeplasma and Mycoplasma (*Lanham et al.* 1980, *O'Brien et al.* 1981, *Salih et al.* 1982).

The purpose of this study was to further characterize and determine relatedness among some species of genus Mycoplasma by using isoenzyme analysis. The following mycoplasmas were examined: M. bovigenitalium, M. capricolum, M. equigenitalium, M. ovipneumoniae, M. mycoides subsp. capri, M. mycoides subsp. mycoides, bovine serogroup 7 (*Leach* 1967), the F38 group (*Mac Owan & Minette* 1976), M. putrefaciens, a few field strains of each of M. mycoides subsp. capri, the F38 group, and the so-called small and large colony type of M. mycoides subsp. mycoides, including the ovine/caprine serogroup 8 (*Al-Aubaidi* 1972). More specifically the following questions of significant practical veterinary interest were considered in the light of isoenzyme analysis:

1) Is the F38 group so distinct that it should be considered a new species, a subspecies of M. mycoides or M. capricolum or rather grouped with bovine group 7?

2) Is bovine group 7 a separate species or a subspecies of M. mycoides?

3) Should the subspeciation of M. mycoides be retained or would it be reasonable to elevate M. mycoides subspp. capri and mycoides to separate species?

4) Do isoenzyme studies provide means for distinguishing the caprine small colony (SC) type of M. mycoides from the classical agent of contagious bovine pleuropneumonia (CBPP) as well as from the caprine large colony (LC) type of M. mycoides?

It is stressed, however, that final classification requires consideration of many other characteristics, as outlined in the proposed revision of the International Code of Nomenclature of Bacteria (*Lapage et al.* 1973).

MATERIAL AND METHODS

Mycoplasma strains

Twenty-four strains were included in the study. The type or reference strains of M. bovigenitalium (PG11), M. capricolum (Calif.kid), M. equigenitalium (T37), M. ovipneumoniae (Y-98), M. mycoides subsp. capri (PG3), M. mycoides subsp. mycoides

(PG1), M. putrefaciens (KS-1), bovine serogroup 7 (PG50), ovine/caprine serogroup 8 (Y-goat), and the caprine F38-like group (F38), were used along with caprine field strains of M. mycoides subsp. capri (*AMRC-C 513, C766 and C1408), bovine field strains of M. mycoides subsp. mycoides (C1369, C1582 and C1585), 5 caprine field strains of M. mycoides subsp. mycoides LC type (C1563, C1573, C1570, C1576, C1580) and 2 caprine field strains of the SC type (C1574, C1575), respectively, and one isolate belonging to the F38-like group (C1542). Table 1 lists the origin and serological identification of the strains.

These 24 strains were chosen, because they appear to represent relatively closely related species that have been extensively studied and compared by a number of different techniques (*Ernø & Jurmanova* 1973, *Ernø et al.* 1973, *Askaa et al.* 1978, *Cottew & Yeats* 1978, *Ernø et al.* 1978, *Cottew* 1979, *Gourlay & Howard* 1979, *Ernø & Salih* 1980). The F38-like strains isolated from goats in Kenya and Sudan are not only serologically and genetically related to bovine serogroup 7, but also related to other members of the serum digestion positive groups of mycoplasmas (*McOwan & Minette* 1976, *Ernø & Salih* 1980, *Christiansen & Ernø* 1982). The strains of our study were isolated from different geographic areas (Australia, England, India, Italy, Kenya, Nigeria, Somalia, Sudan, Turkey, and the U.S.A.).

Cultivation and harvest of organisms

Each strain was grown in 1—1.2 l of heart infusion broth (Difco) supplemented with yeast extract 10 % v/v, horse serum 5 %, glucose 5 % w/v, deoxyribonucleic acid (Sigma 0.2 % w/v solution), phenol red (0.06 % w/v solution), thallium acetate (1 % w/v solution), and penicillin (200,000 IU ml^{-1}); pH was adjusted to 7.8. When the pH had fallen to 7.2–7.3 – after 1—5 days of incubation — the titre of the culture was recorded, either by determination of the number of colony forming units (cfu/ml) on agar medium or by cell counting after staining with acridine orange (*Rosendal & Valdivieso-Garcia* 1981). The culture was harvested by centrifugation at 27,000 G for 30 min at 4°C, followed by washing twice in 0.25 mol/l NaCl buffered to pH 7.0 with Na_2HPO_4.

*The designation of the strains from the FAO/WHO Collaborating Centre for Animal Mycoplasmas, University of Aarhus, is for example AMRC-C 513, but is further on in this paper abbreviated to: C 513.

Preparation of culture lysates

Cell lysates were prepared as described by *Salih et al.* (1982).

Procedures for the isoenzyme electrophoresis

Horizontal starch gel electrophoresis of enzymes was carried out as described by *Hjorth* (1971). Thirty-five enzymes were selected for analysis of multiple enzyme expression. Thirty of these enzymes, and their references for buffer and staining procedures have been studied before in 3 Acholeplasma species (*Salih et al.* 1982). The 5 enzymes not included in the previous study were: 1) acid αglucosidase (Maltase, αGLU, EC 3.2.1.20); buffer: *Spencer et al.* (1964); stain: *Harris & Hopkinson* (1976). 2) Glucose dehydrogenase (GDH, EC. 1.1.1.47); buffer: *Spencer et al.*; stain: *Harris & Hopkinson.* 3) Glutamate pyruvate transferase (GPT, EC. 2.6.1.2); buffer: *Spencer et al.*; stain: *Harris & Hopkinson.* 4) Glycerate-2-dehydrogenase (G2DH, EC. 1.1.1.29); buffer: *Spencer et al., Carter* (1973) or *Shaw* (1968); stain *Siciliano & Shaw* (1967). 5) Sorbitol dehydrogenase (SORDH, EC. 1.1.1.14); the buffer described by *Carter, Spencer et al.* or *Shaw* and the stain as given by *Harris & Hopkinson.*

In each electrophoresis operation a control was included, consisting of incubated growth medium.

Estimation of dissimilarity coefficients

The theory for the method is that of *Brewer* (1970), elaborated by *Sneath & Sokal* (1973). We have used a simplified modification of this method, described in detail by *Wellendorf & Simonsen* (1978). For estimation of dissimilarity coefficients, all bands or electromorphs — a term used to describe enzymes with the same catalytic ability with identical or different charge — were taken into consideration. The number of identical electromorphs present in both strains were counted (a), identical electromorphs absent in both strains (b), electromorphs absent in one strain, but present in the other (c), and vice versa (d). The dissimilarity coefficient is then $(c+d)/(a+b+c+d)$. This value is then employed in an unweighted pair-group arithmetic clustering method for construction of dendrograms. The first dendrogram (Fig. 2) includes all reference or type strains of this study, while the other (Fig. 3) was made up of the reference or type strains of the left branch of the first dendrogram, supplemented with field strains of the same group.

RESULTS

Twenty-four strains were examined for the presence or absence of 35 enzymes. Table 2 presents the results relating to 10 of the enzymes found in 15 of the strains. These 15 strains were selected to illustrate the groups using the reference strains as well as one representative of the field strains of each category (Table 1).

Twenty enzymes were revealed in all strains: adenylate kinase (AK), aldolase (ALD), arginase (ARG), arginine deiminase (ARDI), carbamyl phosphokinase (CPK), galactose dehydrogenase (GADH), galactose 6 phosphate dehydrogenase (GA6PDH), glucose dehydrogenase (GDH), glucose 6 phosphate dehydrogenase (G6PDH), glucose phosphate isomerase (GPI), glutamate dehydrogenase (NAD), glutamate pyruvate transaminase (GPT), glycerate 2 dehydrogenase (G2DH), αglycerophosphate dehydrogenase (αGPDH), lactate dehydrogenase (LDH), mannose phosphate isomerase (MPI), phosphoglucomutase (PGM), 6 phosphogluconate dehydrogenase (6PGD), purine nucleoside phosphorylase (NP), and xanthine dehydrogenase (XDH).

Various electromorphs were observed of these 20 enzymes. Fig. 1 is depicting the zymograms of all strains, for 10 enzymes.

No distinct electromorphs were found in alcohol dehydrogenase (ADH), acid phosphatase (ACP), alkaline phosphatase (ALP), hexokinase (HK), and sorbitol dehydrogenase (SORDH).

The dissimilarity coefficients are estimated on the basis of the position of the 30 enzymes which were present in some or all 24 strains (Table 3).

The dendrograms are presented in Figs. 2 and 3. Strains F38 and C1542 have identical electrophoretic patterns, and in the cluster analysis they are consequently treated as one and the same strain.

In horse serum 4 enzymes were demonstrated: acid and alkaline phosphatase (ACP and ALP), esterase (EST), and glutamate oxaloacetate transaminase (GOT). When analyzing the zymograms, bands of these enzymes were regarded as mediaborne when they appeared in positions identical with those of horse serum, whereas bands of these enzymes with other positions were considered as being of mycoplasmal origin.

M. equigenitalium was peculiar in possessing 11 enzymes migrating towards the cathode, exemplified by PP and GPT (Fig. 1).

Table 1. Origin and serological identification of 24 strains of Mycoplasma.

Species or serogroup	Reference strain	Other strains		Origin
M. myc. subsp. mycoides (agent of CBPP)**	PG1			*Aarhus
		(Bovine)	AMRC-C 1369	Pathak
		—	— C 1582	Cottew
		—	— C 1585	Cottew
M. myc. subsp. mycoides (SC type of goats)		(Caprine)	— C 1574	Cottew
		—	— C 1575	Cottew
M. myc. subsp. mycoides (LC type of goats)	Y-goat			*Aarhus
			— C 1570	Mohan
			— C 1576	Cottew
			— C 1580	Cottew
M. myc. subsp. capri	PG3			*Aarhus
			— C 513	Cottew
			— C 766	Rottem
			— C 1408	Masiga
M. myc. subsp. capri or myc. LC type			— C 1563	Adler
			— C 1573	Sanguinetti
Bovine serogroup 7 of Leach	PG50			*Aarhus
M. capricolum	Calif.kid			*Aarhus
F38-like group	F38			MacOwan
			— C 1542	Harbi
M. ovipneumoniae	Y-98			*Aarhus
M. putrefaciens	KS-1			*Aarhus
M. bovigenitalium	PG11			*Aarhus
M. equigenitalium	T37			*Aarhus

*Aarhus: FAO/WHO Collaborating Centre for Animal Mycoplasmas.
**CBPP: contagious bovine pleuro-pneumonia.

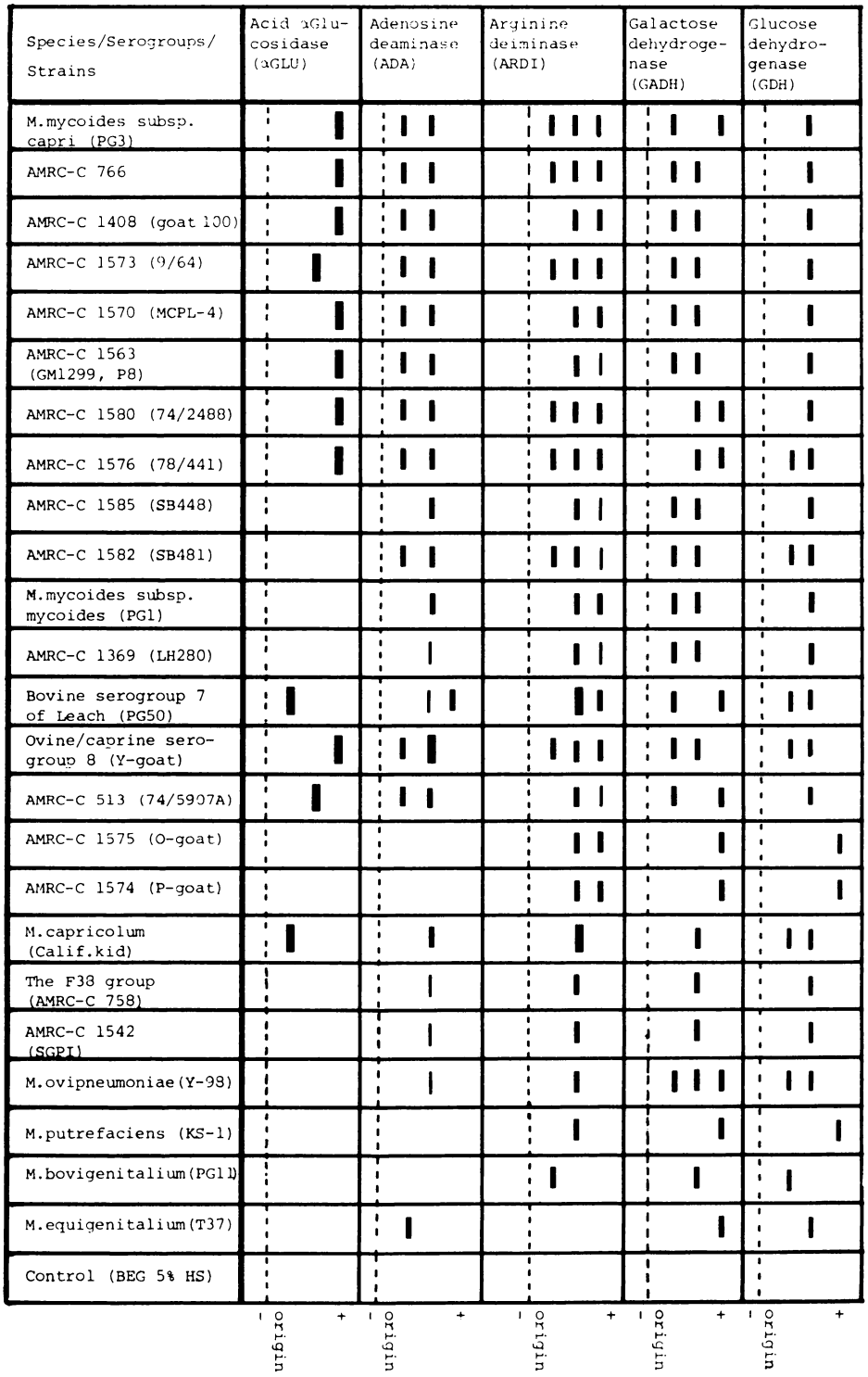

Figure 1. Diagrams of the zymograms for 10 enzymes in 24 strains of *Mycoplasma*.

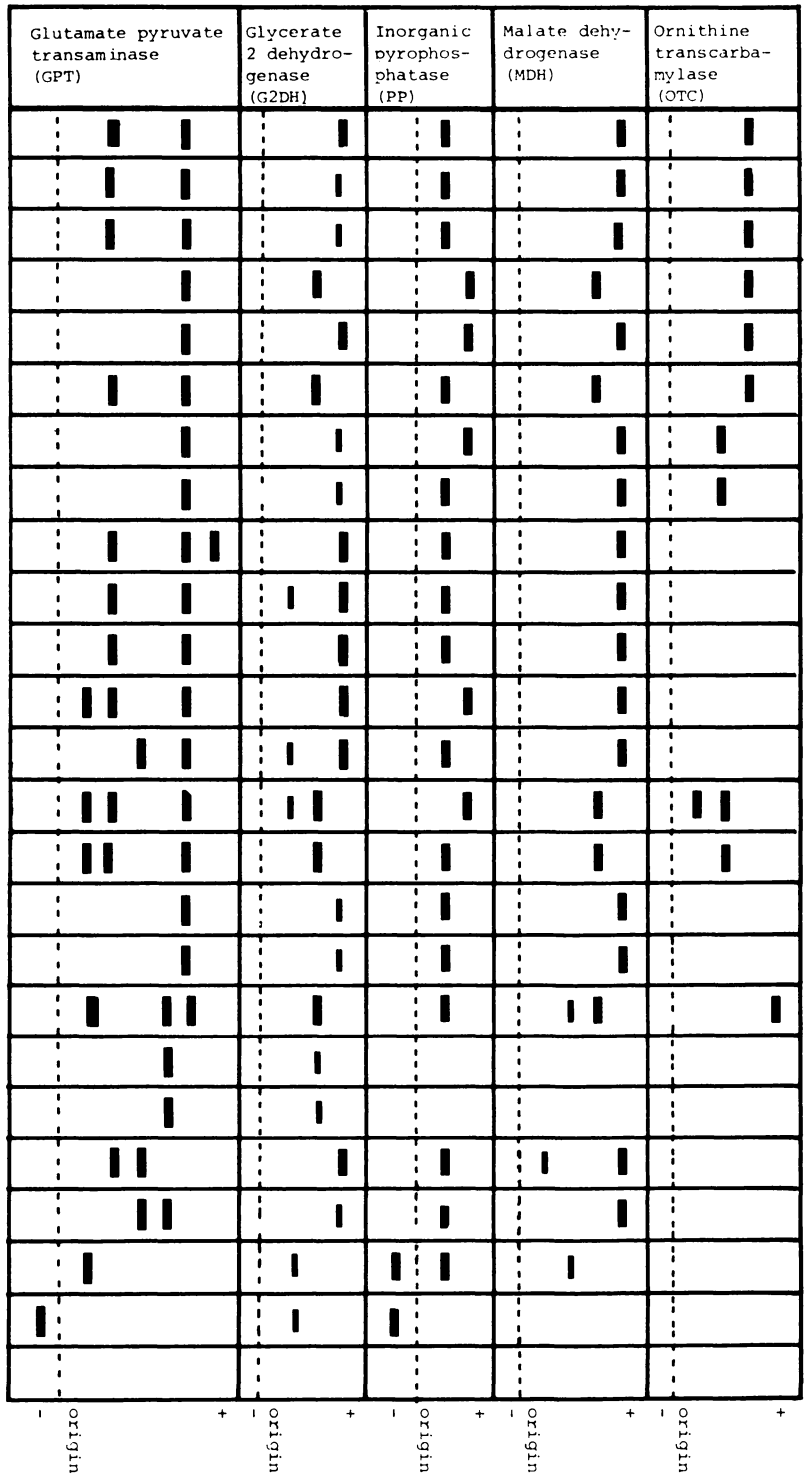
Glutamate pyruvate transaminase (GPT)
Glycerate 2 dehydrogenase (G2DH)
Inorganic pyrophosphatase (PP)
Malate dehydrogenase (MDH)
Ornithine transcarbamylase (OTC)
- origin +
- origin +
- origin +
- origin +
- origin +

Table 2. Position and bands due to migration rate in enzymes within the genus Mycoplasma.

Species/strains	ENZYME BANDS IDENTIFIED									
	* αGLU	ADA	EST	GLUD (NADP)	GOT	PP	MDH	OTC	PGK	SOD
	ABC	ABC	ABCDEFGHI	ABC	ABCDEFG	ABC	ABCD	ABCD	ABCDE	AB
M. mycoides subsp. capri (PG3)	100	110	100010000	100	0011000	010	1000	0100	01100	10
AMRC-C 1563 M. mycoides subsp. capri or mycoides LC type	100	110	100000101	100	1001000	010	0100	0100	11110	10
AMRC-C 1576 M. mycoides subsp. mycoides LC type	100	110	010110001	100	0101000	010	1000	0010	11110	10
M. mycoides subsp. mycoides (PG1)	000	010	000010000	100	0101000	010	1000	0000	01100	10
AMRC-C 1585 M. mycoides subsp. mycoides SC type	000	010	000000000	100	1001000	010	1000	0000	11100	10
Ovine/caprine serogroup 8 (Y-goat)	100	110	100110001	011	0111000	100	0100	0011	11100	10
AMRC-C 513 M. mycoides subsp. capri	010	110	000110001	010	0011000	010	0100	0010	11100	10
AMRC-C 1575 M. mycoides subsp. mycoides SC type	000	000	000000010	101	0101000	010	1000	0000	00100	01
Bovine serogroup 7 (Leach group 7) (PG50)	001	110	000111010	010	0011000	010	1000	0000	00100	11
M. capricolum (Calif.kid)	001	010	000110000	010	0000000	010	0110	1000	01100	10
F38-like group (F38)	000	010	000010000	001	0000000	000	0000	0000	00000	01
M. ovipneumoniae (Y-98)	000	010	000011000	100	0000100	010	1001	0000	00100	01
M. putrefaciens (KS-1)	000	000	000000000	010	0011000	010	1000	0000	01100	00
M. bovigenitalium (PG11)	000	000	000000001	001	0000101	011	0010	0000	00010	00
M. equigenitalium (T37)	000	001	000000100	000	0000010	001	0000	0000	00101	00

* αGLU = αGlucosidase, ADA = Adenosine deaminase, EST = Esterase, GLUD (NADP) = Glutamate dehydrogenase (NADP), GOT = Glutamate oxaloacetate transaminase, PP = Inorganic pyrophosphatase, MDH = Malate dehydrogenase, OTC = Ornithine transcarbamylase, PGK = Phosphoglycerate kinase, SOD = Superoxide dismutase.
1 = Band is present, 0 = the band is absent.
A,B,C, etc. indicate different band position on the gel ranking A with highest migration rate.

Table 3. Dissimilarity coefficient of 24 strains of genus Mycoplasma.

Species/ strains	PG3	C776	C1408	C1573	C1570	C1563	C1580	C1576	C1585	C1582	PG1	C1369	PG50	C513	Y-goat	C1575	C1574	Calif.kid	F38	C1542	Y-98	KS-1	PG11	T37
M. myc. subsp. capri (PG3)		0.07	0.11	0.25	0.20	0.20	0.23	0.20	0.26	0.24	0.24	0.24	0.29	0.27	0.31	0.29	0.28	0.38	0.44	0.44	0.33	0.41	0.49	0.48
AMRC-C 766			0.07	0.21	0.17	0.18	0.24	0.20	0.19	0.20	0.21	0.21	0.35	0.26	0.30	0.33	0.30	0.34	0.40	0.40	0.32	0.40	0.45	0.52
AMRC-C 1408				0.27	0.21	0.22	0.29	0.26	0.24	0.21	0.19	0.25	0.32	0.28	0.28	0.29	0.28	0.35	0.39	0.39	0.27	0.36	0.46	0.48
AMRC-C 1573					0.16	0.19	0.24	0.24	0.28	0.31	0.31	0.27	0.45	0.22	0.28	0.39	0.35	0.28	0.41	0.41	0.44	0.47	0.47	0.53
AMRC-C 1570						0.18	0.19	0.19	0.25	0.28	0.26	0.20	0.39	0.35	0.35	0.30	0.27	0.40	0.46	0.46	0.42	0.37	0.46	0.53
AMRC-C 1563							0.23	0.24	0.23	0.26	0.28	0.27	0.42	0.25	0.28	0.34	0.31	0.39	0.42	0.42	0.41	0.44	0.47	0.48
AMRC-C 1580								0.08	0.25	0.27	0.20	0.23	0.36	0.26	0.28	0.28	0.25	0.45	0.42	0.42	0.45	0.43	0.48	0.52
AMRC-C 1576									0.25	0.24	0.20	0.23	0.30	0.26	0.28	0.27	0.24	0.39	0.42	0.42	0.42	0.42	0.43	0.52
AMRC-C 1585										0.13	0.16	0.18	0.36	0.29	0.33	0.30	0.27	0.39	0.35	0.35	0.34	0.40	0.42	0.47
AMRC-C 1582											0.20	0.24	0.27	0.34	0.27	0.33	0.31	0.40	0.37	0.37	0.29	0.42	0.42	0.44
M. myc. subsp. myc. (PG1)												0.20	0.29	0.30	0.31	0.20	0.16	0.39	0.33	0.33	0.31	0.31	0.39	0.46
AMRC-C 1369													0.40	0.33	0.26	0.31	0.29	0.39	0.42	0.42	0.42	0.47	0.44	0.51
Bovine serogr. 7 (PG50)														0.40	0.36	0.31	0.31	0.35	0.34	0.34	0.32	0.32	0.46	0.39
ARMC-C 513															0.23	0.37	0.34	0.33	0.46	0.46	0.46	0.42	0.49	0.54
Ovine/caprine serogr. 8 (Y-goat)																0.42	0.42	0.35	0.43	0.43	0.46	0.53	0.50	0.53
ARMC-C 1575																	0.03	0.45	0.37	0.37	0.35	0.24	0.39	0.39
AMRC-C 1574																		0.43	0.35	0.35	0.35	0.23	0.39	0.39
M. capricolum (Calif.kid)																			0.30	0.30	0.38	0.44	0.42	0.45
F38 group (AMRC-C 758)																				0.00	0.28	0.33	0.35	0.29
AMRC-C 1542																					0.28	0.33	0.35	0.29
M. ovipneumoniae (Y-98)																						0.32	0.35	0.39
M. putrefaciens (KS-1)																							0.41	0.39
M. bovigenitalium (PG11)																								0.35
M. equigenitalium (T37)																								0.00

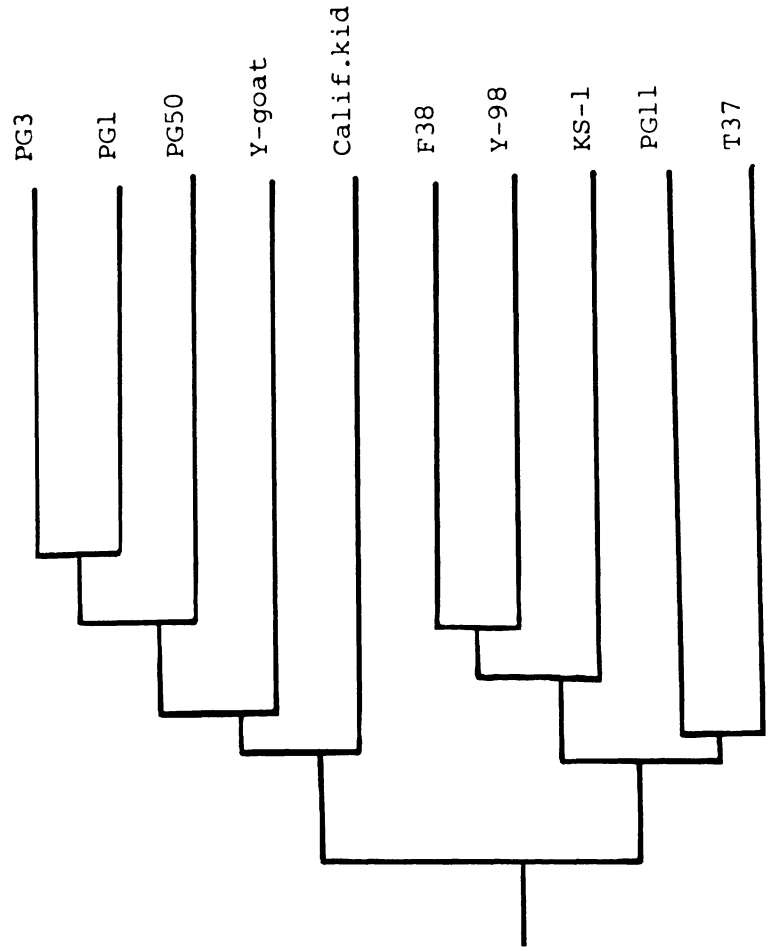

Figure 2. Dendrogram based on a group average cluster analysis of type — or reference strains representing 10 species or serogroups.

PG3	=	M. mycoides subsp. capri
PG1	=	M. mycoides subsp. mycoides
PG50	=	Bovine serogroup 7
Y-goat	=	Ovine/caprine serogroup 8 (M. mycoides subsp. mycoides LC type)
Calif.kid	=	M. capricolum
F38	=	F38-like group
Y-98	=	M. ovipneumoniae
KS-1	=	M. putrefaciens
PG11	=	M. bovigenitalium
T37	=	M. equigenitalium

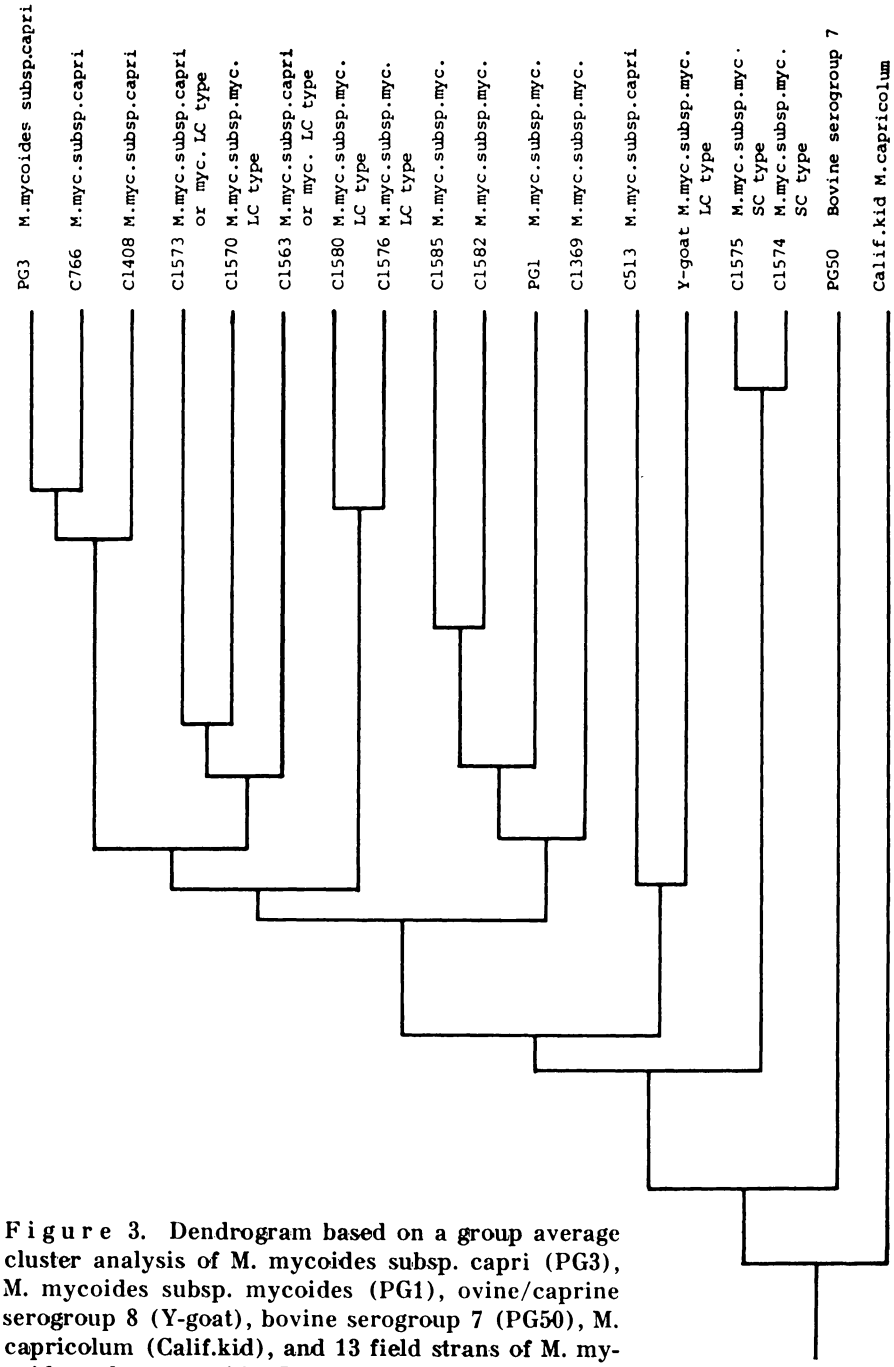

Figure 3. Dendrogram based on a group average cluster analysis of M. mycoides subsp. capri (PG3), M. mycoides subsp. mycoides (PG1), ovine/caprine serogroup 8 (Y-goat), bovine serogroup 7 (PG50), M. capricolum (Calif.kid), and 13 field strans of M. mycoides subsp. mycoides Large and Small colony types and M. mycoides subsp. capri (see Table 1).

DISCUSSION

To our knowledge the following (20) enzymes have not previously been reported in genus Mycoplasma: acid αglucosidase (αGLU), alcohol dehydrogenase (ADH), alkaline phosphatase (ALP), galactose dehydrogenase (GADH), galactose 6 phosphate dehydrogenase (GA6PDH), glucose dehydrogenase (GDH), glutamate dehydrogenase (NAD), glutamate dehydrogenase (NADP), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), glycerate 2 dehydrogenase (G2DH), αglycerophosphate dehydrogenase (αGPDH), hexokinase (HK), malate dehydrogenase (MDH), mannose phosphate isomerase (MPI), ornithine transcarbamylase (OTC), phosphoglucomutase (PGM), phosphoglycerate kinase (PGK), sorbitol dehydrogenase (SORDH), and xanthine dehydrogenase (XDH).

No greater discrepancies were found between our results and others. *Mitchell et al.* (1978), who examined M. mycoides subsp. mycoides, were unable to demonstrate the presence of ADA, contrary to our results. Concerning lactate dehydrogenase, we have confirmed the observations of *Neimark & Lemcke* (1972) and *Allsopp & Matthews* (1975), although we found not 1 electromorph, but 5.

The purpose of this study was, as already mentioned, to further characterize and possibly differentiate some reference and/or field strains of genus Mycoplasma. The results must be compared to the differentiation obtained by the serological methods most frequently used. It is obvious from the dissimilarity coefficients (Table 3) as well as the zymograms (Fig. 1) that the isoenzyme technique reveals inter- as well as intra-specific differences. Interestingly enough strains F38 and C1542 had identical isoenzyme pattern, which is of particular interest in regard to epidemiology. Strain F38 was isolated in Kenya (*MacOwan & Minette* 1976), while C1542 was recovered in Sudan (*Harbi et al.* 1981), also from a case of contagious caprine pleuropneumonia (CCPP).

It is well known, also in our laboratory, that serologically some field isolates seem equally related to the 2 subspecies of M. mycoides. The presence of maltase and ornithine transcarbamylase does indicate, however, that such strains are either M. mycoides subsp. capri or M. mycoides subsp. mycoides LC type from goats, and thus at least distinguishes between these 2 groups and the agent of CBPP (Table 4). Should a strain of M. mycoides,

Table 4. Alternative classifications of M. mycoides-related strains.

Present nomenclature	Alternative nomenclature I	Alternative nomenclature II	Alternative nomenclature III	* Presence of	
				αGlucosidase	Ornithine trans-carbamylase
M. mycoides subsp. mycoides (PG1)	M. mycoides	M. mycoides	M. mycoides biovar 1	0	0
M. mycoides subsp. mycoides (SC type from goats) (Strain O)	M. mycoides	M. mycoides	M. mycoides biovar 2	0	0
M. mycoides subsp. capri (PG3)	M. capri serovar 1	M. capri	M. mycoides biovar 3	+	+
M. mycoides subsp. mycoides (LC type from goats) (Y-goat)	M. capri serovar 2	New species	M. mycoides biovar 4	+	+
M. capricolum (Calif.kid)	M. capricolum	M. capricolum	M. capricolum	+	+
F38 group (F38)	New species to be named	New species to be named	New species to be named	0	0
Bovine group 7 (PG50)	New species to be named	New species to be named	New species to be named	+	0

* Neither αglucosidase nor ornithine transcarbamylase were demonstrated in: M. ovipneumoniae, M. putrefaciens, M. bovigenitalium, and M. equigenitalium; these species are therefore not incorporated in the Table.

isolated from a goat, possess neither maltase nor ornithine transcarbamylase, there is every reason to believe it is the agent of contagious *bovine* pleuropneumonia.

In the present study, it appears from Table 1 that the serologic examinations in 2 cases (C1563 and C1573) were inadequate in distinguishing between M. mycoides subsp. capri and M. mycoides subsp. mycoides, LC type. Neither has the presence or non-presence of maltase and ornithine transcarbamylase, as already mentioned, but the dissimilarity coefficients indicate a closer relation to the subsp. capri than to the LC type of M. mycoides subsp. mycoides, as exemplified with C1573, having dissimilarity coefficients of 0.25 and 0.28, respectively (Table 3).

The C513 strain was serologically classified as M. mycoides subsp. capri, but according to the dendrogram (Fig. 3) it should rather be M. mycoides subsp. mycoides, LC type.

It appears furthermore from Table 4 that the presence or absence of maltase and ornithine transcarbamylase is of help in distinguishing between M. capricolum, group 7 of *Leach* and the F38 group. M. capricolum possesses both maltase and ornithine transcarbamylase, the 2 strains of F38 possess neither of them, while group 7 is equipped with maltase only.

As to the question whether the F38 group should be considered a new species, a subspecies of either M. mycoides or M. capricolum, or possibly included with group 7 of *Leach*, we would, if the answer should be given by isoenzyme analysis alone, prefer a speciation of this group of mycoplasmas. Our conclusion is based on the fact that the lowest dissimilarity coefficient between strain F38 and strains of any other species or group included in this study, is 0.28, found between F38 and Y-98, the type strain of M. ovipneumoniae. Since the F38 group and M. ovipneumoniae are definitely different species by other parameters, isoenzyme analysis is supporting the establishment of the F38 group as a new species. The dissimilarity coefficients to M. capricolum, M. mycoides subspp. mycoides et capri, and group 7 of *Leach* are 0.30, 0.33, 0.44, and 0.34, respectively.

Following the same reasoning, group 7 should also be considered a new species, the lowest dissimilarity coefficient being 0.29, seen to M. mycoides subsp. mycoides (PG1).

The dissimilarity coefficient between the 2 subspecies of M. mycoides, i.e., capri and mycoides, was determined to be 0.24, a result lower than 0.28, and the above reasoning can therefore not directly be applied, as these 2 groups are not definitely different species by other taxonomical parameters as is the case with M. ovipneumoniae and the F38 group.

Classification is artificial anyway, however, and if 0.24 is chosen as the lower limit of dissimilarity coeffcients of 2 different species the 2 subspecies of M. mycoides may be elevated to species level. Following this point of view, 2 schemes of classification as shown in Table 4, may be suggested (alternatives I and II).

The question is now how much a classification solely based on isoenzyme studies is conflicting with other methods.

Rodwell (1980) stated in his Emmy Klieneberger-Nobel Award lecture that the 2-D electrophoresis method of comparing the patterns suggests that (1) the SC and LC strains of the subsp. mycoides and the subsp. capri strains are all fairly closely re-

lated; (2) the LC strains are more closely related to the subsp. capri strains than to the SC strains; (3) M. capricolum, group 7 of *Leach* and the F38 strain of *MacOwan* are more distantly related to M. mycoides. *Rodwell*, in a personal communication, states that the LC type of M. mycoides subsp. mycoides and M. mycoides subsp. capri are so closely related by 2-D electrophoresis that they may be regarded as serological variants.

Smith & Oliphant (1981 a) studied the immunogenicity of the SC and LC strains of M. mycoides subsp. mycoides in mice. The results suggested that although some protective antigens were shared between both groups of strains at least one of importance was present in the SC strains, but absent from the majority of LC strains, a qualitative and not merely a quantitative difference.

In an earlier study by *Smith & Oliphant* (1981 b), using the same method, no differences were demonstrated between caprine and bovine SC strains. The caprine LC strains were more related to M. mycoides subsp. capri than to the bovine subsp. of M. mycoides. It may be concluded that neither these studies nor those of *Rodwell* are in disagreement with our suggestions (Table 4).

The situation is different when studying the results of nucleic acid hybridization. *Christiansen & Ernø* (1982) found DNA from strain F38 to hybridize with DNA from M. capricolum (Calif.kid) to a degree of 80 %, justifying the classification — when based on hybridization alone — of the F38 group as a variant of M. capricolum. The classification of the 2 subspecies of M. mycoides might also be retained on the basis of the studies of *Christiansen & Ernø* using the proposal of *Johnson* (1973).

CONCLUSION

It is our opinion that the F38-like group of mycoplasmas as well as bovine group 7 should be elevated to species level. We also favour the elevation of the 2 subspecies of M. mycoides (mycoides and capri) to separate species. This has consequences regarding the classification of the SC and LC types of M. mycoides subsp. mycoides, isolated from goats. The SC types will be classified as M. mycoides, while we are uncertain about the position of the LC type. As appearing from Table 4, at least 2 possibilities exist. One is to regard this group of mycoplasmas as a serological variant of the PG3-like group, irrespective of the future taxonomical position of these mycoplasmas. Another possibility is to regard the LC type as a group sufficiently separate to be regarded as a new species.

From the Minutes of the Meeting of the *Subcommittee* on the Taxonomy of Mollicutes (1982) it appears that the *Subcommittee* endorsed the development of an informal biovar concept to accomodate the increasingly complex biological and pathological data from different isolates. Following this suggestion a third possibility appears, when a change of the present nomenclature is considered (Table 4, alternative III).

It is our hope that a final decision will soon be made as a result of discussions between taxonomists actively engaged in prevention of the important diseases related to the mycoplasmas we have considered in this work.

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SAMMENDRAG

Elektroforetisk analyse af isoenzymer hos mykoplasmer.

Formålet med dette arbejde er, ved hjælp af isoenzymstudier, yderligere at karakterisere og vurdere slægtsskabet af visse grupper eller arter af mykoplasmer, som har betydelig veterinær interesse i sygdomsmæssig henseende. Ved horisontal stivelseselektroforese blev 24 stammer undersøgt for tilstedeværelse af 35 enzymer. Der påvistes 127 elektromorfer blandt 30 enzymer. Inter- såvel som intraspecifikke forskelle demonstrerede anvendelsesmuligheder for isoenzymstudier i henseende til både klassifikation og epidemiologi.

Det konkluderes, at den caprine gruppe F38 (*MacOwan*) og bovin gruppe 7 (*Leach*) begge bør opfattes som nye, selvstændige arter. I henseende til den taksonomiske status af de 2 underarter af M. mycoides — subspecies mycoides og subspecies capri — vil forfatterne anbefale, at de klassificeres som selvstændige arter. Det foreslås dog, at beslutningen tages på internationalt niveau på grund af de praktiske konsekvenser af en given nomenklatur, netop ved denne gruppe af mykoplasmer. Der vises 3 forskellige muligheder for alternativ nomenklatur.

Med hensyn til rutinemæssig identifikation synes resultaterne at åbne mulighed for, at undersøgelse for maltase og ornithintranscarbamylase kan indicere, hvor vidt en given stamme er at betragte som tilhørende den gruppe, som er årsag til oksens ondartede lungesyge.

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