Mycoplasma canadense, a New Bovine Species

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Three mycoplasmas isolated from the male bovine genital tract and a mastitic udder exhibited serological properties which distinguished them from any of the known *Mycoplasma* species. Further, a variety of serological procedures failed to identify these organisms with any of the wide range of members of the order *Mycoplasmatales* tested. These strains are described as belonging to a new species, for which we propose the name *Mycoplasma canadense*. Strain 275C, designated as the type strain, has been deposited in the National Collection of Type Cultures, Great Britain, under the number 10152.

Three *Mycoplasma* strains, one of which has been reported previously (15), have been characterized but they could not be identified with any known bovine species. These strains and 27 others were found to constitute a separate serological group.

The purpose of this paper is to validly publish the name and to provide a description of the new species to which these strains have been allocated.

MATERIALS AND METHODS

Bacterial strains. The *Mycoplasmatales* strains used in this study were supplied by the National Collection of Type Cultures, Public Health Laboratories, London, England; by J. M. Al Aubaidi, as representative of species reported by him (1); and by the National Institutes of Health, Bethesda, Md. Strain 275C was recovered from the prepuce of a bull, strain 383C was from semen processed for artificial insemination, and strain 466 was from the mastitic udder of a cow. A list of the strains and species studied is presented in Table 1.

Staining procedures. Staining procedures were as described previously by Langford and Leach (9).

Media. Hayflick medium composed of PPLO broth (HB) or agar (HA) was modified from previously published formulations (9) to contain 0.024 calf thymus deoxyribonucleic acid (Sigma Chemical Company, no. D-1501) per 100 ml.

Metabolism tests. Metabolic activity was determined by the methods of Aluotto et al. (2) employing broth medium at pH 7.6 supplemented with 0.5% glucose, mannitol, mannose, maltose, lactose, salicin, sucrose, arabinose, dulcitol, and sorbitol, 0.5% arginine at pH 7.2, and urea at pH 7.0.

Reduction of methylene blue, tellurite, and tetrazolium was observed directly by the methods of Aluotto et al. (2). Phosphatase, oxidase, and catalase reactions were determined by the methods of Al-

uotto et al. (2). Determination of the reaction on egg yolk medium was as described by Fabricant and Freundt (6).

Growth requirements. Serum requirements were determined by the method previously reported by Langford and Leach (9) except that the inoculum in each case consisted of organisms washed in serum-free broth media, followed by a wash in 1 M phosphate-buffered saline (pH 7.3), and suspended in serum-free broth at one-half of the initial volume. Growth in the presence of 20% human, porcine, bovine, lapine, guinea pig, or ovine serum was determined with the same type of inoculum, substituting one of the above in HA and HB. Cholesterol requirements were determined as previously reported (9), but with arginine substituted for glucose.

Inhibition by digitonin and sodium polyethane sulfonate. Inhibition tests were performed as described by Rosendal (13).

Colonial hemadsorption test. The colonial hemadsorption test was performed by the method described by Del Giudice et al. (4).

Hemolysis tests. The test for hemolysis was similar to that described by Somerson et al. (16). Human, rabbit, guinea pig, sheep, horse, chicken, and bovine erythrocytes were used.

Filtration studies. A 24-h-old broth culture was titrated, and 2.5 ml was passed through membrane filters (Millipore Corp.) with the following nominal pore diameters: 800, 650, 450, and 220 nm. Serial 10-fold dilutions of the filtrates were made in arginine broth and incubated for 5 days at 37 C before reading.

Cloning. Cloning was done by filtering a culture through a 450-nm filter and plating the filtrate. Several single, well-isolated colonies were picked and transferred to individual broths by Pasteur pipette. The broths were incubated for 48 h and the procedure was repeated three more times.

Bacterial reversion. The initial broth containing the inhibitors penicillin and thallium acetate in which strain 275C grew was replated on HA devoid of inhibitors. Colonies with the morphology of classical mycoplasmas were picked to individual tubes

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TABLE 1. *Mycoplasmas and acholeplasmas and their antisera used in growth-inhibition and other serological tests*

Strains	Antisera provided by ^a
1. 275C	ADRI
2. 383C	ADRI
3. 466	H. L. Ruhnke
4. <i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (PG1)	RL
5. <i>M. bovis</i> (PG 11)	RL & ADRI
6. <i>Acholeplasma laidlawii</i> (PG 8)	RL & ADRI
7. <i>M. bovirhinis</i> (5M331)	RL & ADRI
8. <i>M. agalactiae</i> subsp. <i>bovis</i> (Donetta)	ADRI & RL
9. <i>A. modicum</i> (Squire)	RL & ADRI
10. <i>Mycoplasma</i> sp. (Group 7) (N29)	RL & ADRI
11. <i>M. alkalescens</i> (D12)	RL & ADRI
12. <i>M. dispar</i> (462/2)	RL & ADRI
13. <i>M. arginini</i> (G230)	RL & ADRI
14. <i>M. bovoculi</i> (M165/69)	RL & ADRI
15. <i>M. gateae</i> (12PA) ^a	ADRI
16. <i>M. gallinarum</i> (B142P) ^a	ADRI
17. <i>M. verecundum</i> (107)	ADRI
18. <i>M. conjunctivae</i> (M164/69)	ADRI
19. <i>M. gallisepticum</i> (PG 13)	ADRI
20. <i>M. hominis</i> (PG 21)	NIH
21. <i>M. salivarium</i> (PG 20)	NIH
22. <i>M. orale</i> (Ch 19299)	NIH
23. <i>M. agalactiae</i> subsp. <i>bovis</i> (PG 45)	NIH
24. <i>M. gallinarum</i> (PG 16)	NIH
25. <i>M. alkalescens</i> (DBS 803)	NIH
26. <i>M. iners</i> (PG 30)	NIH
27. <i>M. meleagridis</i> (17529)	NIH
28. <i>M. spumans</i> (PG 13)	NIH
29. <i>M. maculosum</i> (PG 15)	NIH
30. <i>M. arthritis</i> (PG 6)	NIH
31. <i>M. fermentans</i> (PG 18)	NIH
32. <i>M. pneumoniae</i>	NIH
33. <i>M. anatis</i> (1340)	NIH
34. <i>M. neurolyticum</i> Type A	NIH
35. <i>M. pulmonis</i> (GP 34)	NIH
36. <i>M. hyorhinis</i> (BTS 7)	NIH
37. <i>M. canis</i> (PG 14)	NIH
38. <i>A. laidlawii</i> (PG 9)	NIH
39. <i>A. granularum</i> (BTS-39)	NIH
40. <i>M. agalactiae</i>	Plum Island
41. <i>M. mycoides</i> subsp. <i>capri</i>	H. Erno
42. <i>M. synoviae</i>	Plum Island
43. <i>M. ovipneumoniae</i>	Cottew
44. <i>Mycoplasma</i> sp. (B144 P) ^b	ADRI
45. <i>A. axanthum</i> (H86N)	ADRI

^a Abbreviations: ADRI, Animal Diseases Research Institute (Western), Lethbridge, Alberta, Canada; RL, R. H. Leach (prepared at Wellcome Research Laboratories, Beckenham, Kent, England); NIH, National Institutes of Health, Bethesda, Md.

^b Provided by J. Al-Aubaidi.

and passed seven times through non-inhibitor broth and agar for a total of 15 passages.

OF test. Glucose agar was used according to the method of Aluotto et al. (2).

Temperature requirements. Vials containing 20 ml of Hayflick broth inoculated with 2×10^3 organisms were incubated at 22, 30, 35, 37.5, 40, and 42 C. Tenfold titrations in arginine broth were made after 6, 24, 48, 72, 96, and 120 h of incubation.

Film and spot production. Film and spot production was determined as previously described by Fab-

ricant and Freundt (6) and Langford and Leach (9).

G+C content of DNA. The guanine plus cytosine (G+C) content of deoxyribonucleic acid (DNA) was determined by the method of Marmur (10).

Antisera. The antisera used were in part those used in a previous characterization study (9). Sera against *Mycoplasma bovoculi*, *M. gateae*, *M. gallinarum*, *M. conjunctivae*, *M. gallisepticum*, *M. verecundum*, *Acholeplasma axanthum*, Al Aubaidi strain B144P, and strains 275C and 383C were prepared in rabbits in essentially the same manner. Antiserum against strain 466 was prepared by the method of Al Aubaidi and Fabricant (1). Sera against 21 other strains were supplied by the National Institutes of Health, Bethesda, Md. (Table 1).

Metabolic-inhibition tests. Metabolic-inhibition tests were carried out by the method of Taylor-Robinson et al. (18) or of Purcell et al. (11), depending on whether the strains catabolized glucose or hydrolyzed arginine.

Growth-inhibition test. Approximately 0.1 ml of a titrated broth culture containing 10^6 colony-forming-units (CFU) per ml was spread evenly over HA, 4 to 5 mm deep in a 55-mm plastic petri dish. The inoculated plates were air dried. Wells, 5 mm in diameter, were cut in the agar, and 0.05 ml of each of the test antisera was placed in separate wells. Zones of inhibition were measured from the edge of the well to the zone of growth.

Growth-precipitation tests. Growth-precipitation tests were carried out as described by Erno and Jurmanova (5), but the wells were partially filled with 0.1 ml of HA before the inoculum was added. The antisera-impregnated disks were added after 16 to 18 h of incubation.

Indirect immunofluorescence test. The indirect immunofluorescence test was carried out as described by Rosendal and Black (14).

Counterimmunoelectrophoresis tests. Counterimmunoelectrophoresis tests were carried out by the method of Cho and Langford (3).

Electron microscopy. The method of electron microscopy was similar to that reported previously by Langford and Leach (9). The pictures were taken with a Hitachi model HU-12A at an accelerating voltage of 75 kV. The sections were stained with lead acetate and uranyl acetate.

RESULTS

Morphological characteristics. The test strains (275C, 466, and 383C) had the typical colonial morphology of classical *Mycoplasma* strains (Fig. 1). Giemsa-stained impression films of colonies (Fig. 2) showed that the colonies were as previously described for other mycoplasmas (17).

Light microscopy of Giemsa-stained films prepared from broth cultures of three strains revealed minute coccoid and bipolar elements with large ring, signet-ring, and other pleomorphic forms. The organisms were gram negative, taking up the counterstain poorly. Electron microscopy of thin sections of strains 275C

FIG. 1. A 48-h-old colony of *Mycoplasma canadense* on Hayflick agar. Bar equals 100 μ m.

FIG. 2. Giemsa-stained, 24-h-old colony of *Mycoplasma canadense*. $\times 250$.

and 383C confirmed the absence of a cell wall and the presence of a triple-layered membrane approximately 12 nm thick (Fig. 3 and 4), as described for mycoplasmas (12). There was no evidence of morphological instability after 15

successive passages through media devoid of bacterial and mycological inhibitors.

Serum-cholesterol requirements. Serum (horse) was required for growth at the 2% level in both broth and agar; no growth was observed

FIG. 3. Electron micrograph of a thin-section preparation of strain 275C. $\times 200,000$. Bar equals 100 nm.

FIG. 4. Electron micrograph of a thin-section preparation of strain 383C. $\times 50,000$. Bar equals 100 nm.

in media containing less than 2% serum. Growth improved as the serum concentration was increased up to 10%. Growth occurred when rabbit, sheep, bovine, human, or guinea pig serum replaced the horse serum. Cholesterol was required for growth.

DNA base composition. The G+C content of the DNA of strains 275C and 383C averaged 29 mol%. These estimates lie within the range of 25 to 35 mol% as given by Hill (7) for mycoplasmas.

Metabolic reactions. Detailed testing by the methods of Aluotto et al. (2) indicated that strains 275C and 383C hydrolyzed arginine, did not reduce tetrazolium aerobically but did reduce it anaerobically, did not catabolize glucose or hydrolyze urea, and did not form film or spots. The results of other biochemical tests with these strains are given in Table 2.

Reactions with erythrocytes. Alpha hemolysis of the erythrocytes of sheep, cattle, horses, guinea pigs, chickens, rabbits, and humans (type A, Rh negative) was observed. Neither hemadsorption nor hemagglutination was observed.

Cultural properties. No growth was observed when cultures of the organism were incubated at 22 or 30 C. Growth occurred at 35,

37.5, 40, and 42 C. Titrations of broth cultures indicated that the optimum temperature is above 37.5 C and close to 40 C. Cells of strains 275C and 383C were destroyed by heating at 50 C for 15 min but not at 44 C for 24 h.

Filterability. The organism passed through 800-, 650-, 450-, and 220-nm filters with reduction of the initial broth titration (1.8×10^7) by 1, 3, 4, and 6 logs, respectively. Since the organism passed through a 220-nm filter, the test was repeated, and on this occasion the titer was reduced by 7 logs by the filter. Similar observations were made for strains 383C and 466.

Serology. Two-way growth inhibition tests (GIT) between antisera and cultures of the described bovine strains, numbers 1 to 18 in Table 1, failed to reveal cross-inhibition. No antiserum had less than a 2-mm zone of inhibition against the homologous antigen. Antisera against the remainder of the strains in Table 1 did not inhibit the growth of the test strains.

The serological results for strain 275C are supported by findings kindly made available by J. G. Tully, who found that this strain was not inhibited in the GIT by antisera against the strains listed in Table 1 or, in addition, those listed in Table 3, with the exception of the strains of *M. agalactiae*, *M. synoviae*, *M. ovipneumoniae*, and strain 144P. The same tests were repeated for strain 466 by J. G. Tully, and

TABLE 2. Summary of biochemical reactions and growth requirements of strains 275C and 383C

Test	Result
Carbohydrate breakdown	
Glucose	—
Mannose	—
Maltose	—
Mannitol	—
Arabinose	—
Lactose	—
Raffinose	—
Xylose	—
Sucrose	—
Salicin	—
Sorbitol	—
Arginine hydrolysis	+
Urea hydrolysis	—
Tetrazolium reduction	— ^a
Tellurite reduction	— ^a
Film and spot production	—
Gelatin hydrolysis	—
Serum-slope digestion	—
Phosphatase	+ ^b
Catalase	+
Oxidase	—
Methylene blue	Not reduced
SPS	Inhibits growth
Digitonin	Inhibits growth
Serum requirements	2%
Cholesterol	Required

^a Aerobic, negative; anaerobic, positive.

^b After 14 days of incubation.

TABLE 3. Strains on which the growth-inhibition test was performed at NIH^a

Species	Strain
<i>Mycoplasma buccale</i>	CH20247
<i>M. faucium</i>	DC-333
<i>M. lipophilum</i>	MaBy
<i>M. primum</i>	HRC 292
<i>Mycoplasma</i> sp.	(Monkey) HRC291
<i>Mycoplasma</i> sp.	(Goat) G145
<i>Mycoplasma</i> sp.	(Avian Group L) 694
<i>Mycoplasma</i> sp.	(Dog) MH5408
<i>M. hyosynoviae</i>	S16
<i>M. gateae</i>	Cat Mart
<i>Mycoplasma</i> sp.	Calif. calf
<i>M. putrefaciens</i>	KS1
<i>Mycoplasma</i> sp.	(Avian C) 33F
<i>Mycoplasma</i> sp.	(Avian D) 887
<i>Mycoplasma</i> sp.	(Avian F) 1197
<i>Mycoplasma</i> sp.	(Iowa 695 avian I complex)
<i>M. mycoides</i> subsp. <i>capri</i>	PG3
<i>M. capricolum</i>	Calif. kid
<i>M. caviae</i>	G122
<i>M. suis</i>	J
<i>M. suipneumoniae</i>	PG24
<i>M. edwardii</i>	H-831
<i>M. cynos</i>	H-542
<i>M. molaris</i>	Cat 27
<i>M. felis</i>	Cat Ben
<i>M. feliminutum</i>	H86N
<i>Acholeplasma axanthum</i>	19L
<i>A. oculi</i>	D-1 (swine)
<i>Acholeplasma</i> sp.	B-3046
<i>Acholeplasma</i> sp.	Maroc
<i>Spiroplasma citri</i>	

^a National Institutes of Health, Bethesda, Md.

the results were identical to those for strain 275C.

The antisera against strains of all of the recognized bovine species and strains 275C, 383C, and 466 gave satisfactory homologous titers in the metabolic-inhibition test (Table 4). Table 4 records the results of the heterologous tests between antigens 275C, 383C, and 466 and antisera against strains of the recognized bovine species and the 22 other *Mycoplasma* and *Acholeplasma* species listed in Table 1. The results of the converse test are given in Table 5.

Reciprocal growth-precipitation tests failed to reveal any cross-reaction between the test organisms and the glucose-catabolizing strains tested. There was either a one- or two-way cross between antigens 275C, 466, and 383C or their antisera and the arginine-hydrolyzing strains of *M. alkalescens*, *M. arginini*, and *M. gateae* and their antisera (Table 6).

The results of the counterimmunoelectrophoresis study were similar to those noted for the growth precipitation test except that there was a complete two-way cross between the arginine-hydrolyzing mycoplasmas (Table 6). The presence of a common antigen in arginine-hydrolyzing strains of bovine mycoplasmas has been reported previously (5). Indirect fluorescent antibody tests (FAT) were positive at a dilution of

TABLE 5. *Metabolic-inhibition tests with strains of recognized bovine species and other species of Mycoplasmatales against antiserum from strains 275C, 383C, and 466*

Mycoplasma strain tested	Reciprocal metabolic-inhibition titers against antisera to strain:		
	275C	383C	466
Test strains:			
275C	5,120	5,120	5,120
383C	2,560	5,120	5,120
466	2,560	5,120	5,120
Strains of recognized bovine species:			
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (PG1)	10	10	10
<i>M. bovigenitalium</i> (PG11)	10	10	10
<i>Acholeplasma laidlawii</i> (PG8)	10	10	10
<i>M. bovirhinis</i> (5M331)	10	10	10
<i>M. agalactiae</i> subsp. <i>bovis</i> (Donetta)	10	10	10
<i>A. modicum</i> (Squire)	10	10	10
<i>Mycoplasma</i> sp. (Group 7) (N29)	10	10	10
<i>M. alkalescens</i> (D12)	10	10	10
<i>M. dispar</i> (462/2)	10	10	10
<i>M. arginini</i> (G 230)	10	10	10
<i>M. bovoculi</i> (M 165/69)	10	10	10
<i>M. gateae</i> (12PA)	10	10	10
<i>M. gallinarum</i> (B142P)	10	10	10
<i>M. verecundum</i> (107)	10	10	10
Other species:			
<i>M. conjunctivae</i> (M 164/69)	10	10	10
<i>M. gallisepticum</i> (PG 13)	10	10	10

TABLE 4. *Metabolic-inhibition tests of strains 275C, 383C, and 466 by antisera to strains of recognized bovine species and other species of the order Mycoplasmatales*

Antiserum to:	Reciprocal antiserum titers in metabolic-inhibition test against strain:			
	Homologous	275C	383C	466
275C	>5,120	>5,120	>5,120	>5,120
383C	>5,120	1,280	>5,120	>5,120
466	>5,120	2,560	>5,120	>5,120
Recognized bovine species:				
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (PG1) ^a	320	10 ^b	10	10
<i>M. bovigenitalium</i> (PG11)	320	10	10	10
<i>Acholeplasma laidlawii</i> (PG8)	160	10	10	10
<i>M. bovirhinis</i> (5M331)	1,280	10	10	10
<i>M. agalactiae</i> subsp. <i>bovis</i> (Donetta)	160	10	10	10
<i>A. modicum</i> (Squire)	640	10	10	10
<i>Mycoplasma</i> sp. (Group 7) (N29)	1,280	10	10	10
<i>M. alkalescens</i> (D12)	2,560	10	10	10
<i>M. dispar</i> (462/2)	640	10	10	10
<i>M. arginini</i> (G230)	640	10	10	10
<i>M. bovoculi</i> (165/69)	1,280	10	10	10
<i>M. gateae</i> (12PA)	640	10	10	10
<i>M. gallinarum</i> (B142P)	2,560	10	10	10
<i>M. verecundum</i> (107)	640	10	10	10
<i>M. mycoides</i> subsp. <i>capri</i>	ND ^c	10	10	10
<i>M. synoviae</i>	ND	10	10	10
Other <i>Mycoplasma</i> species as listed in Table 1	>160	10	10	10

^a For sources of antisera used in these tests, see Table 1.

^b All results are ≤ 10 .

^c ND, Not done.

TABLE 6. Other serological tests between strains of bovine species of *Mycoplasmatales* and their antisera

Antigen	Homologous antisera				275C antiserum			383C antiserum			466 antiserum			
	GIT ^a	GPT ^b	CIEP ^c	FAT ^d	GIT	GPT	CIEP	GIT	GPT	CIEP	GIT	GPT	CIEP	FAT ^e
<i>Mycoplasma mycoides</i> subsp. <i>mycoides</i> (PG1)	+	+	+		-	-	-	-	-	-	-	-	-	ND ^f
<i>M. bovis</i> <i>genitalium</i> (PG11)	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>Acholeplasma laidlawii</i> (P68)	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>M. bovirhinis</i> (5M331)	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>M. agalactiae</i> subsp. <i>bovis</i> (Donetta)	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>A. modicum</i> (Squire)	+	ND	+	+	-	-	-	-	-	-	-	-	-	-
<i>Mycoplasma</i> sp. (Group 7) (N29)	+	+	+	+	-	-	-	-	-	-	-	-	-	-
<i>M. alkalescens</i> (D12)	+	+	+	+	-	+	+	-	+	+	-	+	+	-
<i>M. dispar</i> (462/2)	+	+	+		-	+	-	-	-	-	-	-	-	ND
<i>M. arginini</i> (G230)	+	+	+		-	+	+	-	+	+	-	+	+	-
<i>M. bovoculi</i> (M165/69)	+	+	+		-	-	-	-	-	-	-	-	-	ND
<i>M. gateae</i> (12PA)	+	+	+		-	+	+	-	+	+	-	+	+	ND
<i>M. gallinarum</i> (B142P)	+	+	+		-	-	-	-	-	-	-	-	-	ND
<i>M. verecundum</i> (107)	+	+	+		-	-	-	-	-	-	-	-	-	ND
275C	+	+	+	+	+	+	+	+	+	+	+	+	+	+
383C	+	+	+		+	+	+	+	+	+	+	+	+	+
466	+	+	+		+	+	+	+	+	+	+	+	+	+

^a GIT, Growth-inhibition test.^b GPT, Growth-precipitation test.^c CIEP, Counterimmunoelectrophoresis.^d FAT, Fluorescent-antibody test—direct.^e FAT, Fluorescent-antibody test—indirect.^f +, Positive.^g ND, Not done.

1/160 when antisera against strain 466 was reacted with strains 275C and 383C. There was no reaction with the eight other bovine species listed in Table 6. Direct FAT using the heterologous conjugated antisera listed in column 1, Table 6, did not react with the three test strains. Conjugated strain 275 antisera reacted with strains 383C and 466 but not against the heterologous bovine species listed in Table 6.

Growth- and metabolic-inhibition tests and growth-precipitation tests proved that at least 27 other arginine-hydrolyzing strains of *Mycoplasma* isolated at the Animal Diseases Research Institute (Western) from semen, preputial washings, and cervico-vaginal mucus are similar to the isolates studied. Previous preliminary studies (15) reported that a serologically identical organism had been recovered from the milk, joint, and umbilicus of a calf, the vaginal mucus of a cow (8), and from the semen and preputial washings of bulls in Ontario.

DISCUSSION

The observed morphological, physical, and cultural characteristics of the test strains are consistent with those regarded as defining membership in the order *Mycoplasmatales* (17). The serum and cholesterol requirements place these strains in the family *Mycoplasmataceae*. By the growth-inhibition, metabolic-inhibition,

growth-precipitation, and fluorescent-antibody tests, the strains were found to be antigenically homogeneous and serologically different from the 67 characterized and uncharacterized members of the order *Mycoplasmatales* listed in Tables 1 and 3. Therefore, we regard strains 275C, 383C, and 466 as belonging to a new species, for which we propose the name *Mycoplasma canadense* (can. a. den'se. M. L. adj. *canadensis* pertaining to Canada).

The following is a summary description of *M. canadense* sp. nov.

Morphology and staining characteristics. Minute coccoid and bipolar elements with ring and other pleomorphic forms are visible by staining. Gram-negative, but counter-stain weakly. The cells are bounded by a triple-layered cell membrane.

Size and filtration characteristics. Cells are approximately 250 to 600 nm in diameter, as determined by electron microscopy. Viable particles are filterable through a filter with a 450-nm pore diameter but are largely retained by a filter with a 220-nm pore diameter.

Oxygen requirements. Aerobic, microaerophilic, and anaerobic.

Agar colonies. Colonies on horse serum agar are 80 to 150 μ m in diameter and possess the typical "fried-egg" appearance with growth down into the agar.

Liquid cultures. Slightly turbid.

Film and spots. Film and spots are not formed on horse serum agar or egg yolk emulsion agar.

Biochemical reactions. Arginine, but not urea, is hydrolyzed. Glucose, mannose, mannitol, arabinose, lactose, raffinose, xylose, sucrose, salicin, and sorbitol are not utilized. Tetrazolium salts and tellurite were not reduced aerobically, but they were reduced anaerobically.

Temperature requirements. Optimum, 37 C to 40 C. No growth at 30 C or lower. Cells are not viable after 15 min at 56 C.

Hemadsorption and hemagglutination. Neither hemadsorption nor hemagglutination was observed with horse, sheep, human, rabbit, guinea pig, or chicken erythrocytes.

Hemolysis. Alpha hemolysis occurred with erythrocytes from horses, cows, sheep, humans (type A, Rh negative), rabbits, chickens, and guinea pigs.

Antigenic structure. The type strain was antigenically distinct from strains of the *Mycoplasma* species and subspecies with which it was compared by growth-inhibition and/or metabolic-inhibition tests. It was antigenically distinct from the glucose-catabolizing strains of bovine origin, but it shared antigens with all of the bovine arginine-hydrolyzing strains tested, with the exception of the strain of *M. gallinarum*, by the growth-precipitation and counterimmunoelectrophoresis tests.

Pathogenicity. Causes mastitis and possibly other conditions.

Source. The three strains reported were recovered from the reproductive tracts of cattle, from the mastitic udder of a cow, and from a joint and the umbilicus of a calf.

Habitat. Uncertain.

Type strain. Strain 275C. This strain has been deposited in the National Collection of Type Cultures, Central Public Health Laboratory, London, England, under the number 10152.

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REPRINT REQUESTS

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