Mycoplasma lipofaciens, a New Species of Avian Origin

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A mycoplasma designated strain R171^T (type strain) was isolated from the infraorbital sinus of an adult chicken. This organism was assigned to the class *Mollicutes* and the order *Mycoplasmatales* on the basis of its morphological, physical, and cultural characteristics. Its deoxyribonucleic acid base composition was 24.5 mol% guanine plus cytosine. Strain R171^T was sterol dependent, and since it did not produce helical forms or hydrolyze urea, it was assigned to the family *Mycoplasmataceae* and the genus *Mycoplasma*. Strain R171^T fermented glucose, hydrolyzed arginine, and produced films and spots. This strain was shown to be serologically distinct from 74 currently accepted mycoplasma species or serovars by growth inhibition, immunofluorescence, and immunodiffusion tests, which were supported in some cases by metabolism inhibition tests. Thus, strain R171^T appears to be a new and distinct mycoplasma species, for which we propose the name *Mycoplasma lipofaciens* (= NCTC 10191^T = ATCC 35015^T).

During a survey of mycoplasmosis in domestic poultry and wild birds, a number of isolates were recovered from the respiratory tracts of chickens; these isolates were apparently serologically distinct from previously recognized avian species (23). Particular interest was focussed on isolates which were biochemically similar to *Mycoplasma iowae* (previously avian serovar I) in having the ability to utilize both glucose and arginine but which showed no serological relationship to this group of mycoplasmas during preliminary tests.

In view of the importance of mycoplasma detection to the poultry industry and of the increasing evidence of the pathogenicity of *M. iowae* (8, 29, 32, 40, 41), we thought that it would be worthwhile to characterize these strains further. The aims of this paper are to establish that one of these isolates, strain R171^T (type strain), is a new *Mycoplasma* species and to provide a detailed description of the morphological, physical, cultural, biochemical, and serological properties of this organism in accordance with the recommendations of the International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of *Mollicutes* (22).

(Preliminary results of this investigation were presented at the 3rd Conference of the International Organization for Mycoplasmaology, Custer, S.D., in 1980.)

MATERIALS AND METHODS

***Mycoplasma* strains.** Most reference strains used in this study were obtained from the World Health Organization/Food and Agriculture Organization Collaborating Centre for Animal Mycoplasmas, Universi-

ty of Aarhus, Denmark; exceptions were *Mycoplasma sualvi*, *Mycoplasma faucium*, *Mycoplasma bovoculi*, and *Mycoplasma hominis* reference strains, which were obtained from the National Collection of Type Cultures, London, England. Strain R171^T was isolated from the infraorbital sinus of an adult chicken in 1975; the flock to which this chicken belonged was serologically positive for *Mycoplasma synoviae* but clinically normal.

Media. For isolation and laboratory examination, strains were normally propagated in a modified Hayflick (18) broth (MB) or modified Hayflick agar (MA) (6). These media, with certain modifications (see below), were also used for some biochemical and serological studies. To prepare strain R171^T antigen for rabbit inoculation, the serum in the growth medium was replaced with 1% (vol/vol) PPLO serum fraction (Difco Laboratories).

Some reference strains required special media for growth and for the serological tests; these media were U3 medium (20) for *M. sualvi* and FF74 medium (17) for *Mycoplasma dispar*, *Mycoplasma flocculare*, and *Mycoplasma hyopneumoniae*.

Cultivation and purification. *Mycoplasma* strain R171^T was routinely subcultured in MA or MB and incubated at 37°C in a candle jar to provide a carbon dioxide-rich atmosphere.

The isolate was purified by filter cloning. A broth culture was passed through a membrane filter (average pore diameter, 450 nm; Nuclepore, Wallabs Inc.), and dilutions of the resulting filtrate were inoculated onto solid medium (MA). After incubation, a single colony was transferred to a second MA plate, and the subsequent growth was inoculated into broth. The whole procedure was then repeated twice, and the resulting cloned culture was used in all tests. A second clone was derived similarly from the original isolate.

Morphological studies. The appearance of colonies on MA incubated in a candle jar or in an anaerobic jar was examined at magnifications of ×10 to ×40, and films of cell suspensions fixed in methanol and stained

with Giemsa stain were examined under oil immersion at a magnification of $\times 1,250$. Cells in broth culture were also examined by phase-contrast microscopy at a magnification of $\times 1,125$.

Electron microscope examinations were carried out with cells that were collected by centrifugation of broth cultures at $2,000 \times g$ for 5 min, embedded in agar (24), fixed in 5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.3), postfixed in 2% osmium tetroxide, dehydrated, and embedded in epoxy resin. Sections 60 to 90 nm thick were cut by using glass knives and a Reichert model OM U2 ultramicrotome and were examined with a Philips model EM 201 electron microscope at 80 kV.

Filtration studies. Viable counts (7) were determined with a 48-h broth culture of strain R171^T diluted 1:10 in phosphate-buffered saline (pH 7.3) containing 0.2% gelatin before and after passage of separate portions through membrane filters having average pore diameters of 450 and 220 nm.

DNA base composition. Deoxyribonucleic acid (DNA) was extracted from strain R171^T by a modification of the method of Kirby (26). Cells were collected by centrifugation at $7,500 \times g$ for 45 min at 4°C, lysed with a detergent mixture, and deproteinized by successive phenol treatments. Contaminating ribonucleic acid was removed by incubation with ribonuclease (50 µg/ml; Sigma Chemical Co.), and after further phenol treatments the DNA was precipitated with cold ethanol and dissolved in standard saline citrate diluted 1:10 (0.015 M NaCl, 0.0015 M sodium citrate, pH 7.0).

The guanine-plus-cytosine (G+C) content of DNA was determined by the buoyant density of the DNA in cesium chloride (35).

Reversion studies. Strain R171^T was subcultured 10 times on MA devoid of bacterial inhibitors. Blood agar plates were inoculated from MA plates after each passage, incubated for 7 days, and examined daily for growth of bacterial colonies. The mycoplasma colonies on MA were examined for changes in typical morphology and were examined by indirect immunofluorescence after each passage. Films of cell suspensions were made after each passage, stained by the Gram method and with Giemsa stain, and examined microscopically.

Strain R171^T was also subcultured 10 times in MB devoid of bacterial inhibitors. Blood agar plates were inoculated from each passage and examined for bacterial colonies, and smears from the broth were stained with Giemsa stain and examined microscopically.

Biochemical tests. (i) **Sensitivity to digitonin and sodium polyanethanol sulfonate.** The sensitivity of strain R171^T to 1.5% (wt/vol) digitonin and 5 and 20% (wt/vol) sodium polyanethanol sulfonate was determined as an indirect indication of the sterol requirement for growth of this strain (13, 14).

The sterol requirement for growth strain R171^T was assessed directly by the method of Edward (10).

(ii) **Utilization of glucose, arginine, and urea.** The methods of Aluotto et al. (1) were used to test for the utilization of glucose, arginine, and urea.

The tests were carried out in serum fraction broth containing PPLO broth (Difco) supplemented with 1% (vol/vol) serum fraction, 5% (vol/vol) aqueous boiled yeast extract (25%, wt/vol), 0.01% (wt/vol) nicotinamide adenine dinucleotide, 0.05% (wt/vol) thallium acetate, and 1,000 U of penicillin per ml. This medium

was used with the following five modifications: (i) without glucose and arginine supplements, pH 7.8; (ii) without glucose and arginine, pH 7.0; (iii) without arginine but with 1% (wt/vol) glucose, pH 7.8; (iv) without glucose but with 1% (wt/vol) arginine, pH 7.0; (v) without glucose and arginine but with 1% (wt/vol) urea, pH 7.0.

Glucose utilization was also assessed by measuring the depletion of available glucose in the broth medium used to grow strain R171^T by the glucose-oxidase method (11).

A second test was used to investigate the urease activity of the test strain (15, 36).

(iii) **Production of films and spots.** Egg yolk medium was used for the test for production of films and spots. This medium was prepared as MA was but with 10% (wt/vol) egg yolk emulsion (Oxoid), and the concentration of swine serum was reduced to 5% (vol/vol). A 24-h culture of strain R171^T in MB was inoculated onto egg yolk medium plates and incubated at 37°C for up to 12 days. The plates were examined daily for the production of films and spots.

(iv) **Optional tests.** The other biochemical tests used were tests for phosphatase activity, tetrazolium reduction, serum digestion (1), and arbutin and esculin hydrolysis (39).

In all biochemical tests known positive and negative *Mycoplasma* species were tested concurrently with strain R171^T as controls; uninoculated medium controls were also included where applicable.

Serological tests. Sera from rabbits hyperimmunized against 74 mycoplasma reference strains and some serovars were obtained from the World Health Organization/Food and Agriculture Organization Collaborating Centre for Animal Mycoplasmas, Aarhus, Denmark. Antiserum to *M. sualvi* was supplied by J. Tully, National Institutes of Health, Bethesda, Md.

Antiserum to *Mycoplasma* strain R171^T was prepared in rabbits by a modification of previously used method (28). Two New Zealand white rabbits were each given four intramuscular and four subcutaneous inoculations, each of which contained 2.5 mg of antigen protein in Freund complete adjuvant. An intravenous inoculation containing 5 mg of antigen protein without adjuvant was also given. Blood was collected after 3 weeks. The rabbits were given an intravenous booster of 5 mg of protein after 14 weeks, and blood was collected 10 days later.

GI tests. *Mycoplasma* strain R171^T was tested with the 74 reference antisera described above by the agar well modification of the growth inhibition (GI) test (5). Homologous controls were included for strain R171^T and all of the reference mycoplasmas.

In some GI tests (see Table 1) a modified medium was used; this medium contained reduced concentrations of swine serum (5%, vol/vol) and yeast extract (3%, vol/vol) and no nicotinamide adenine dinucleotide. These suboptimum growth conditions were needed to obtain good homologous controls for some strains and were used for all other GI tests with those strains. In all cases, GI plates were incubated for 2 days at 27°C and then transferred to 37°C. In reciprocal tests, the rabbit antiserum to strain R171^T was tested with all 74 *Mycoplasma* reference strains.

IFA tests. Indirect fluorescent antibody (IFA) tests were conducted using strain R171^T against all 74 reference antisera and all the reference strains against

rabbit antiserum to strain R171^T. Homologous controls were included for strain R171^T and all reference mycoplasmas, and all cultures were tested with normal rabbit serum as an extra control. The method used was that of Rosendal and Black (34), except that for IFA tests with *M. hyopneumoniae* colonies a colony imprint method (9) was used and for *M. dispar* and *M. flocculare* a modification (12) of the agar block method was adopted.

All IFA tests were repeated by using the second clone of strain R171^T with the reference antisera.

DID tests. Double immunodiffusion (DID) tests were performed by using a freeze-disrupted cell suspension (5 to 10 mg of protein per ml) of strain R171^T as the antigen (19). This antigen was tested with homologous serum and all 74 reference antisera.

MI tests. Where apparent cross-reactions were encountered, we attempted to clarify these cross-reactions by using the appropriate reference antiserum against strain R171^T in the metabolism inhibition (MI) test. Both the acid and alkaline color change methods were used (30, 38), and the cultures tested are indicated below (see Table 1).

RESULTS

Morphological studies. *Mycoplasma* strain R171^T grew well on MA both in a candle jar and anaerobically, producing typical "fried egg" colonies in 36 to 48 h at 37°C.

Giemsa-stained films revealed minute coccoid elements, and phase-contrast microscopy showed ring forms and short filaments typical of mycoplasma pleomorphism. Examination of thin sections by electron microscopy showed mainly spherical and elliptical particles (Fig. 1) having an average diameter of 520 nm, lacking a

FIG. 1. Thin sections of strain R171^T cells. Bar = 500 nm.

FIG. 2. Thin section of a strain R171^T cell. Bar = 100 nm.

cell wall, and bounded by a triple-layer membrane (Fig. 2).

Filtration studies. A culture of strain R171^T diluted 1:10 in phosphate-buffered saline supplemented with gelatin contained 6.2×10^6 colony-forming units per ml, and filtrates from the 450- and 220-nm filters contained 2.7×10^6 and 10 colony-forming units per ml, respectively.

DNA base composition. The G+C content of the DNA isolated from *Mycoplasma* strain R171^T was estimated to be 24.5 mol% by cesium chloride density gradient analysis.

Reversion studies. No bacteria-like colonies developed on the noninhibiting substrate or on blood agar subcultures from MA or MB during 10 serial passages, and microscopic examination did not reveal any evidence of reversion to bacterial forms.

Biochemical tests. *Mycoplasma* strain R171^T was sensitive to both 1.5% (wt/vol) digitonin and 5 and 20% (wt/vol) sodium polyanethol sulfonate, which produced inhibition zones 5, 3, and 5 mm, respectively.

The sterol requirement of this organism was confirmed by its positive growth response to increasing concentrations of sterol in the medium and its failure to grow on serum-free agar or serum-free agar supplemented with bovine serum albumin and palmitic acid.

The results of the other biochemical tests are summarized in the species description below.

Serological tests. Rabbit antiserum prepared against strain R171^T gave an inhibition zone of 5 to 6 mm and an IFA reciprocal titer of 1,280 with the homologous strain. Two strong precipitation lines and one weak line were produced in ho-

mologous DID tests, and homologous MI tests gave reciprocal titers of 80 and 160 with the glucose and arginine substrates, respectively.

The serological tests with 74 reference strains and their antisera did not give any positive results that were comparable to the results with the homologous controls (Table 1).

Inhibition zones were observed with strain R171^T in GI tests with antisera to 12 of the reference strains, but these zones were all 1 to 2 mm wide and very sharply defined, in contrast to the usual gradual diminution in colony number and size. Similar sharply defined zones were produced by some normal rabbit sera (Fig. 3).

Reciprocal GI tests with specific antisera to strain R171^T produced small inhibition zones with five *Mycoplasma* reference strains, but these strains were different from the 12 reference strains discussed above. *M. sualvi* was the only species to show a two-way reaction in GI tests with strain R171^T and gave an inhibition zone 6 mm wide with antiserum to strain R171^T. However, further GI tests with *M. sualvi* cultures revealed that a range of different rabbit antisera produced in our laboratory inhibited the organisms, resulting in zones 5 to 6 mm wide, whereas the homologous system produced a zone 10 mm wide.

Immunofluorescence tests revealed only a few weak reactions (Table 1), and none of these was confirmed by reciprocal tests. Fluorescence was graded arbitrarily from 0 to 3+, with 2+ being the minimum acceptable for homologous controls. None of the heterologous tests showed fluorescence greater than 1+. In some instances the same degree of fluorescence was observed with the normal rabbit serum control.

In DID tests antisera to seven *Mycoplasma* species gave rise to precipitation lines, and of these, five had one line that was common to one of the lines of strain R171^T (Table 1).

MI tests were carried out with antisera to 35 reference strains, and only one positive reaction was encountered. This was with ovine group 5 when the arginine substrate was used and was not confirmed by the tests with the glucose substrate.

DISCUSSION

The proposal for minimal standards for descriptions of new species in the class *Mollicutes* (22) defined the criteria that should be established before a new *Mycoplasma* species can be designated.

The observed morphological, physical, and cultural characteristics of *Mycoplasma* strain R171^T are consistent with membership in the class *Mollicutes*. The morphology and size of the particles are similar to those described by other workers (27, 31), and the smaller forms

present correspond to the forms frequently reported in such preparations (2, 27). The microscopic appearance of both stained films and phase-contrast preparations of the test strain showed the pleomorphism typical of mycoplasmas, and estimates of the G+C content of the DNA were consistent with those for other *Mycoplasma* species.

The sterol requirement of strain R171^T was confirmed directly by its growth response to cholesterol and its failure to grow on serum-free agar and indirectly by its sensitivity to digitonin and sodium polyanethol sulfonate, thus distinguishing strain R171^T from members of the family *Acholeplasmataceae*.

Cultural characteristics indicate that strain R171^T is a facultative anaerobe that lacks the ability to hydrolyze urea, and since no helical, motile filaments were observed, we suggest that this isolate should be assigned to the family *Mycoplasmataceae* and the genus *Mycoplasma*.

Species designation was based on serological and biochemical tests in which strain R171^T was compared with 74 reference strains. Strain R171^T did not appear to belong to any of the previously described *Mycoplasma* species examined, and the few cross-reactions observed were not comparable to homologous results and were not confirmed by reciprocal or alternative tests.

We thought that the positive results of GI tests with strain R171^T and some antisera may have been due to the sensitivity of this mycoplasma to a component of serum from certain rabbits. This feature has been reported previously in some porcine mycoplasmas (33) and some ureaplasmas (21).

The growth inhibition of *M. sualvi* by strain R171^T antiserum was considered to be nonspecific despite the large size of the zone. Similar zones were observed when strain R171^T was tested with rabbit antiserum prepared in our laboratory to other mycoplasmas; moreover, the reaction was not confirmed by the IFA, DID, or MI tests.

Strain R171^T is unusual in its ability to utilize both glucose and arginine, a property shared definitely by only six other previously described *Mycoplasma* species (*Mycoplasma alvi*, *Mycoplasma capricolum*, *Mycoplasma fermentans*, *M. iowae*, *Mycoplasma moatsii*, and *M. sualvi*) and possibly by two others (*Mycoplasma neurolyticum* and ovine group 6), whose reported ability to utilize arginine is variable (12, 15). Of these species, only *M. iowae* (formerly serovar I) is of avian origin, and at first we suspected that strain R171^T might be related to this species. A further similarity was the G+C content of the DNA, which was close to the value reported for *M. iowae* (25).

TABLE 1. Results of serological tests with *Mycoplasma* strain R171^{Ta}

Organism or serovar	Strain	GI test ^b		Indirect immunofluorescence ^c			No. of lines in DID test	MI test conducted
		Reference antiserum	Reference culture	Clone 1 reference antiserum	Clone 2 reference antiserum	Reference culture		
<i>M. lipofaciens</i>	R171 ^T	5-6		3+	3+		3	+
<i>M. alkalescens</i>	D12 ^T	1-2	0	0	0	0	0	+
<i>M. anatis</i>	1340 ^T	0	0	GI	0	0	0	+
<i>M. bovirhinis</i>	PG43 ^{Td}	2	0	0	0	0	0	+
<i>M. bovis</i>	Donetta ^{Td}	0	0.5-2	0	0	1+ ^e	1	+
<i>M. bovoculi</i>	M165/69 ^{Td}	?	?	0	0	1+ ^e	0	+
<i>M. buccale</i>	CH20247 ^{Td}	0	2	0	0	0	0	+
<i>M. citelli</i>	RG-2C ^T	0	0	GI	0	GI	0	+
<i>M. columbinasale</i>	694 ^T	0	0	0	0	0	1	+
<i>M. columbinum</i>	MMP1 ^T	2	0	0	1+	0	0	+
<i>M. columborale</i>	MMP4 ^T	2	0	0	0	0	0	+
<i>M. dispar</i>	462/2 ^T	2	0	1+	0	0	(1) ^f	+
<i>M. edwardii</i>	PG24 ^T	0	0	GI	0	0	0	+
<i>M. equirhinis</i>	M432/72 ^{Td}	0	0	0	1+	0	0	+
<i>M. faucium</i>	DC333 ^T	0	0	0	0	0	1	+
<i>M. feliminutum</i>	BEN ^{Td}	0	0	GI	0	0	0	+
<i>M. felis</i>	CO ^T	2	0	0	0	0	0	+
<i>M. flocculare</i>	Ms42 ^T	1	0	0	0	0	(2)	+
<i>M. gallinaceum</i>	DD ^{Td}	2	0	0	0	GI	1	+
<i>M. gallinarum</i>	PG16	0	0	1+	1+	0	0	+
<i>M. gateae</i>	CS ^T	2	0	0	0	0	0	+
<i>M. iners</i>	PG30 ^T	0	0	GI	0	0	0	+
<i>M. moatsii</i>	MK405 ^T	0	0	GI	0	0	0	+
<i>M. molare</i>	H542 ^T	0	2	0	0	GI ^e	0	+
<i>M. mycoides</i> subsp. <i>capri</i>	PG3	0	0	0	0	GI	0	
<i>M. neurolyticum</i>	A ^T	0	1-2	0	0	0	0	+
<i>M. orale</i>	CH19299 ^T	0	0	0	0	GI	0	
<i>M. pullorum</i>	CKK ^T	2	0	0	0	0	0	+
<i>M. salivarium</i>	PG20 ^{Td}	0	0	0	0	0	1	+
<i>M. spumans</i>	PG13 ^{Td}	0	0	1+	GI	0	0	+
<i>M. sualvi</i>	Mayfield B ^T	1	6	0	0	GI	0	+
<i>M. synoviae</i>	WVU1853 ^T	0	2	0	0	0	0	+
Avian serovar J	DJA	0	0	0	GI	0	0	+
Avian serovar R	DRA-0	0	0	GI	0	0	0	+
Bovine group 7	PG50 ^d	0	0	0	0	GI	0	+
Ovine group 5	Goat 145 ^d	0	0	0	0	1+	0	+
Ovine group 6	Goat 189 ^d	1	0	0	0	0	0	+
Ovine group 7	A1343 ^d	0	0	0	0	1+ ^e	0	+

^a Results of all tests were negative for comparisons with *M. agalactiae* PG2^T, *M. alvi* Ilsley^T, *M. arginini* G230^T, *M. arthritis* PG6^T, *M. bovirhinis* PG11^T, *M. canadense* 275C^T, *M. canis* PG14^T, *M. capricolum* California kid^T, *M. caviae* G122^T, *M. conjunctivae* HRC581^T, *M. cynos* H831^T, *M. equigenitalium* T37^T, *M. fermentans* PG18^T, *M. gallisepticum* PG31^T, *M. gallopavonis* WR1^T, *M. hominis* PG21^T, *M. hyopneumoniae* J^T, *M. hyorhinis* BTS-7^T, *M. hyosynoviae* S16^T, *M. iowae* 695^T, *M. lipophilum* MaBy^T, *M. maculosum* PG15^T, *M. meleagridis* 17529^T, *M. mycoides* subsp. *mycoides* PG1^T, *M. opalescens* MH5408^T, *M. ovipneumoniae* Y-98^T, *M. pneumoniae* FH^T, *M. primatum* HRC292^T, *M. pulmonis* PG34^T, *M. putrefaciens* KS-1^T, *M. subdolum* TB^T, *M. verecundum* 107^T, avian serovar K strain DK CPA, avian serovar N strain FMN, avian serovar Q strain L3-10B, bovine group L strain B144P, and ovine group 11 strain 2D. Modified MA was used in GI tests with *M. agalactiae* PG2^T, *M. arthritis* PG6^T, *M. canadense* 275C^T, *M. conjunctivae* HRC581^T, *M. fermentans* PG18^T, *M. hominis* PG21^T, *M. hyorhinis* BTS-7^T, *M. maculosum* PG15^T, *M. pneumoniae* FH^T, *M. pulmonis* PG34^T, *M. verecundum* 107^T, bovine group L strain B144P, and ovine group 11 strain 2D.

^b 0, Negative result; ?, result not determined because the homologous control was negative. The numbers indicate the sizes of the inhibition zones (in millimeters).

^c 0, Negative result; GI, colonies glowed green; 1+, weak fluorescence; 3+, strong fluorescence.

^d Modified MA was used in the GI test.

^e Culture gave the same reaction with normal rabbit serum.

^f The parentheses indicate that precipitation lines were not common with strain R171^T.

FIG. 3. GI test with a strain R171^T culture. Well 1, No serum; well 2, preinoculation rabbit serum; well 3, rabbit antiserum to strain R171^T.

Several workers have agreed that avian serovars I, J, K, N, Q, and R are biochemically similar and antigenically related and have demonstrated two-way cross-reactions in serological tests (3, 4, 16, 37). Despite the similarities described above, strain R171^T was biochemically different from this group in its ability to form films and spots, and there were no convincing serological reactions between it and any member of the group.

On the basis of these observations, we believe that this organism is a new *Mycoplasma* species, for which we propose the name *Mycoplasma lipofaciens* (li po fa' ci ens. Gr. n. *lipos* lipid; L. v. *facere* to make; L. part. adj. *lipofaciens* lipid making). Prevalence and pathogenicity studies are in progress.

***Mycoplasma lipofaciens* sp. nov.** Thin sections show mainly oval and elliptical particles with a triple-layer cell membrane and no cell wall.

Average diameter of cells is 520 nm. Cells pass through a filter with 450-nm pores but are retained by 220-nm pores.

Filaments and ring forms are produced.

Typical fried egg colonies are produced.

Cultures are resistant to penicillin and thallium acetate, and no reversion to cell wall forms occurs in the absence of these compounds.

Cultures show a positive growth response to cholesterol.

Glucose and arginine are utilized, but urea is not.

Films and spots are produced on routine and egg yolk agars.

Triphenyl tetrazolium chloride is reduced anaerobically but not aerobically.

Cultures produce no phosphatase activity, no

serum digestion, and no hydrolysis of esculin or arbutin.

The species is serologically distinct from other *Mycoplasma* species.

Isolated from the infraorbital sinus of an adult chicken.

Pathogenicity not known, but experimental infection with the type strain has caused some chicken and turkey embryo mortality.

The G+C content of the DNA is 24.5 mol%.

Type strain: strain R171 (= ATCC 35015 = NCTC 10191).

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