Synonymy of *Mycoplasma arginini* and *Mycoplasma leonis*

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Mycoplasma arginini Barile et al. 1968 and *Mycoplasma leonis* Heyward et al. 1969 are regarded as synonyms on the basis of comparisons of the original species descriptions and of the serological and biochemical characteristics of authentic strains, including the type strains, of each of the organisms. According to the rules of nomenclature, the correct name of the species formed by the union of these two taxa is *M. arginini* Barile et al., which name has priority over *M. leonis* Heyward et al.

In 1968, Barile and associates (1) proposed the name *Mycoplasma arginini* for a large group of mycoplasmas recovered from a variety of animal hosts. All of the strains examined were shown to hydrolyze arginine, and the extensive serological tests that were performed indicated that these organisms were serologically distinct from 27 other established *Mycoplasma* species. Shortly thereafter, Heyward and co-workers (5) reported on the characterization of several mycoplasmas of feline origin and proposed two new species, *Mycoplasma feliminutum* and *Mycoplasma leonis*, each represented by a single strain. One of the organisms, *M. leonis*, was recovered from the lungs and brain of a lion that had died of an encephalitis-like illness.

Recent serological analyses of the feline mycoplasmas revealed that the type strain of *M. leonis* was related to the type strain of *M. arginini*. A comparison of the biochemical and serological properties of the single *M. leonis* strain with those of several authentic *M. arginini* isolates was made in an attempt to confirm this relationship.

MATERIALS AND METHODS

Mycoplasma strains. The type strain of *M. arginini* is G230 (=ATCC 23838). [The original designation of the G230 strain as the "prototype" strain (1) may not meet the requirements of the Bacteriological Code, so we here designate the G230 strain as the type strain of *M. arginini*.] Several other *M. arginini* strains, identified by appropriate serological tests outlined below, were also utilized in this study. These included a tissue-culture isolate, designated BBL-88 (1), and a strain isolated by one of us (R.A.D.) from the lymph node of a sheep with pneumonia.

Strain LL (=ATCC 25528) is the type strain of *M. leonis* by monotypy. A culture of this strain was obtained from W. R. Dowdle.

Biochemical tests. Glucose fermentation tests were performed in a serum fraction broth (8) supplemented with 0.5% glucose and 0.002% phenol red and adjusted to pH 7.5. Tests for arginine hydrolysis were carried out in both a serum fraction and 20% horse serum broth (1) supplemented with 0.42% arginine and 0.002% phenol red and adjusted to pH 7.1. Control tubes consisting of medium and inoculum but without specific substrate were included in each series of tests.

Serological tests. Antisera for growth-inhibition tests were prepared in rabbits. Broth cultures of strains G-230 and LL were grown in serum fraction medium for 24 to 48 hr. The antigens were then washed three times in buffered saline (pH 7.5) and were administered intravenously to rabbits over a 2- to 3-week period. Undiluted antiserum was tested in the growth-inhibition procedure (2) against appropriate dilutions of mycoplasmas plated to serum fraction-agar. A portion of each antiserum was also conjugated with fluorescein isothiocyanate, and this antiserum was used in the plate-immunofluorescence procedure (4).

Polyacrylamide gel electrophoresis. Mycoplasmas grown in serum fraction broth were sedimented, the cell proteins were then solubilized in phenol-acetic acid-water (2:1:0.5 w/v/v), and the extracts were run in polyacrylamide gels containing 5 M urea and 35% acetic acid (7).

RESULTS AND DISCUSSION

Strains G-230 and LL were examined for their ability to ferment glucose and hydrolyze arginine. Repeated tests with both strains, even on different media, confirmed earlier findings

TABLE 1. Serological relationships among strains of *Mycoplasma arginini* and *M. leonis* strain LL as determined by growth-inhibition and immunofluorescence tests

Strain	Growth-inhibition adjacent to discs saturated with undiluted antiserum to (zones in mm)			Immunofluorescent reaction (reciprocal of serum conjugate titer) with antiserum to	
	<i>M. arginini</i> (G-230)	<i>M. leonis</i> (LL)		<i>M. arginini</i> (G-230)	<i>M. leonis</i> (LL)
		NIH	Dowdle ^a		
<i>M. arginini</i> (G-230)	6	1	4.5	320	40
(BBL-88)	7	4	6	160	20
(Sheep 8-058)	4	2	5	160	160
<i>M. leonis</i> (LL)	3	6	5	160	160

^a Antiserum obtained from W. R. Dowdle, Center for Disease Control, Atlanta, Ga.

(1, 5) that each organism hydrolyzed arginine but did not ferment glucose. Results of a serological study comparing the two strains by growth inhibition and agar-plate immunofluorescence are presented in Table 1. An antiserum prepared against the type strain of *M. arginini* (G-230) inhibited the growth of other *M. arginini* strains as well as of the type strain of *M. leonis*. In addition, fluorescein-conjugated antiserum prepared against strain G-230 stained colonies of other *M. arginini* strains and strain LL. Appropriate controls were included in each test to show that antisera of other *Mycoplasma* serotypes did not inhibit growth or stain colonies of these strains.

Reciprocal serological tests using *M. leonis* strain LL antiserum indicated a relationship between this strain and other *M. arginini* strains. Evidence of some serological heterogeneity among *M. arginini* strains and *M. leonis* strain LL was also apparent in the results of the growth-inhibition and fluorescent-antibody tests.

An analysis of the polyacrylamide gel electrophoretic patterns of strains G-230 and BBL-88 of *M. arginini* and of *M. leonis* strain LL indicated sufficient similarities in the cell-protein bands to consider these three strains to be closely related (Fig. 1).

M. leonis was initially thought to represent new feline mycoplasmas distinct from two earlier designated species, *M. felis* and *M. gateae*, isolated and described by Cole and associates (3). The serological properties of strain LL were compared to those of most of the established *Mycoplasma* species recognized at that time, and the strain appeared to belong to a distinct serotype (5). Although the

FIG. 1 Electrophoretic patterns of *Mycoplasma* cell proteins. A, *M. arginini* (G-230); B, *M. leonis* (LL); and C, *M. arginini* (BBL-88).

published description of *M. arginini* preceded that of *M. leonis*, the characterization of both strains occurred at almost the same time. Thus it is understandable that reciprocal serological tests on these strains were not performed at that time.

The serological study reported here among various authentic *M. arginini* strains, including the type strain, and strain LL, the type strain of *M. leonis*, indicates that the latter strain is related to *M. arginini*. This serological relationship has also been observed in other laboratories with both the growth-inhibition and indirect-hemagglutination tests (E. A. Freundt, personal communication). A review of the published biochemical characteristics of strain LL (5) indicates that they are compatible with those properties described for *M. arginini* (1). Similarities in the electrophoretic patterns of cell proteins of *M. arginini* strains and strain LL provide additional confirmation that the latter strain belongs to *M. arginini*.

From the data presented, it is clear that *M. arginini* and *M. leonis* are identical, and the names of these organisms are therefore to be regarded as subjective synonyms (i.e., names based on different types but regarded as synonyms). According to the rules of nomenclature, when two species are united, the older legitimate specific epithet is retained. Thus the correct name of the species formed by the union of *M. arginini* Barile et al. 1968 and *M. leonis* Heyward et al. 1969 is *M. arginini*.

M. arginini was originally isolated from sheep and goats and was shown to be a frequent tissue-culture contaminant (1). More recently this *Mycoplasma* species has been identified in the chamois and in several tissue sites in bovine

hosts (1a, 6). The initial recovery of strain LL from a lion and the present identification of this strain as *M. arginini* extends the known host range for this species.

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