NOTE

Elevation of *Mycoplasma agalactiae* subsp. *bovis* to Species Rank: *Mycoplasma bovis* (Hale et al.) comb. nov.

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Serological and deoxyribonucleic acid-deoxyribonucleic acid homology studies were performed with PG2 and Donetta, the type strains of *Mycoplasma agalactiae* subsp. *agalactiae* Wroblewski 1931 and *Mycoplasma agalactiae* subsp. *bovis* Hale et al. 1962, respectively. It is concluded that the two subspecies should rather be regarded as two different species. It is proposed, therefore, that *M. agalactiae* subsp. *bovis* be elevated to the rank of species. The species name is *M. bovis* (Hale et al.) comb. nov.

An organism causing bovine mastitis was isolated and tentatively classified as *Mycoplasma agalactiae* subsp. *bovis* by Hale et al. (10). The type strain of this proposed new taxon is Donetta (PG45) (6). Jain et al. (11) studied the serological relationships of several strains associated with mastitis, isolated at different laboratories in the United States. They concluded that some strains, including Donetta (synonymous with "Connecticut"), California 01, and Widanka, formed a homogenous and distinct group as determined by double immunodiffusion and growth inhibition tests. Jain et al. (11) did not find the name *M. agalactiae* subsp. *bovis* appropriate because of the lack of serological comparison with the type strain (PG2) of *M. agalactiae* Wroblewski 1931 (22) and therefore suggested the alternative name *M. bovimastitidis*. However, these authors did not present sufficient data to determine whether this group of mycoplasmas deserved the rank of a distinct species, rather than being classified as a subspecies of *M. agalactiae*. The necessity of carrying out comparative studies was emphasized by Leach (13) and has been discussed repeatedly by the Subcommittee on the Taxonomy of *Mycoplasmatales* (18, 20, 21).

Serological comparisons between *M. agalactiae* and the group in question were carried out by Al-Aubaidi and Fabricant using metabolism inhibition tests (1) and a direct immunofluorescence technique (2). They did not find any cross-reactions between *M. agalactiae* and their serotype *F* (synonymous with *M. agalactiae* subsp. *bovis*/*M. bovimastitidis*), which included the Donetta strain. Leach (14) obtained measurable metabolism inhibition titers when testing two

M. agalactiae strains with antisera against three strains, including strains Widanka and Donetta, of his serogroup 5. The titers varied and were usually less than 0.01 of those obtained with the homologous strain. Only one-way cross-reactions were observed. On the basis of these results, Leach (14) recommended that *M. agalactiae* subsp. *bovis* be conserved as the valid name for these organisms and that the name *M. bovimastitidis* be rejected. Statements in support of this view were given by Ernø et al. (8) and Ernø and Jurmanová (7).

The electrophoretic patterns of cell proteins of PG2 and Donetta were studied by Razin (15). Although the patterns "differed in several bands," it was concluded that the similarities observed were in favor of the inclusion of the two strains in one species. Ernø and Stipkovits (9), in an investigation of 17 type or reference strains of bovine and ovine sources, did not find that electrophoretic studies allowed definite conclusions regarding the taxonomic status of PG2 and Donetta.

In the present study, the type strains PG2 and Donetta were compared serologically by indirect epi-immunofluorescence, metabolism inhibition, and growth inhibition tests as described by Rosendal and Black (16), Ernø et al. (8) and Ernø and Jurmanová (7), respectively.

Furthermore, PG2 and Donetta were compared by deoxyribonucleic acid-deoxyribonucleic acid (DNA-DNA) hybridization experiments. The type strain (PG11) of *M. bovis* *genitalium* was included as a control because of its biochemical similarity to PG2 and Donetta. (The strains were obtained from D. G. ff. Edward, Public Health Laboratory, Dulwich

Hospital, East Dulwich Grove, London SE1W 8RH, England.) DNA was isolated as described previously (3). Labeled DNA was extracted from organisms grown in broth containing 0.5 to 1.0 μCi of [^3H]thymidine per ml. The DNA-DNA hybridization method was the membrane filter technique of Denhardt (5). Direct-annealing experiments, as well as competition experiments, were carried out with 10 μg of membrane-bound unlabeled DNA, 0.1 to 1.0 μg of ^3H -labeled DNA and, for competition, 150 μg of unlabeled DNA.

The results of the serological comparisons are presented in Table 1. It can be seen that strains PG2 and Donetta are clearly distinct by metabolism inhibition, growth inhibition, and indirect immunofluorescence tests.

Table 2 shows the results of the hybridization experiments with strains PG2, Donetta, and

TABLE 1. Antigenic relationship of the type strains PG2 and Donetta^a

Strain	Antiserum to:					
	PG2			Donetta		
	MI	GI (mm)	IMF	MI	GI (mm)	IMF
PG2	12,800	4	+	16	0	—
Donetta	16	0	—	8,000	5	+

^a MI, Metabolism inhibition; GI, growth inhibition; IMF, indirect immunofluorescence.

TABLE 2. DNA-DNA hybridization of *M. agalactiae* subsp. *agalactiae*, *M. agalactiae* subsp. *bovis*, and *M. bovigenitalium*^a

³ H-labeled DNA from:	Hybridization (%)		
	<i>M. agalactiae</i> subsp. <i>agalactiae</i> (PG2)	<i>M. agalactiae</i> subsp. <i>bovis</i> (Donetta)	<i>M. bovigenitalium</i> (PG11)
PG2	100 (3,543) ^b	38 (1,331)	14 (496)
Donetta	40 (3,088)	100 (7,785)	5 (394)

^a In the homologous reaction, 32 and 20%, respectively, of the labeled DNA were bound, whereas the unspecific binding to blank filters was 1.6 and 0.4%, respectively.

^b The numbers in parentheses show the actual measured counts per minute.

PG11. The homology values between PG2 and Donetta are 38 and 40%, respectively, whereas 14 and 5% homologies were found between these organisms and PG11. The results of the competition experiments between strains PG2 and Donetta are given in Table 3. According to these data, the homology values are 38 and 35%.

The Subcommittee (17–19) has supported the general view that the separation of a group of organisms into two or more species should be based on a variety of distinctive properties and that particular emphasis should be put on the results of growth and metabolism inhibition tests and gel electrophoresis and nucleic acid hybridization studies (18). In 1974, the Subcommittee (20) expressed the view that the data presented by Leach (14) in support of recognizing the Donetta-like strains as a subspecies of *M. agalactiae* were insufficient and, hence, would await the results of additional studies based on gel electrophoresis and nucleic acid hybridization techniques before it made recommendation on this issue. The Subcommittee later recommended (21) that the studies be extended to include California 01 as the reference strain of *M. bovimastitidis* (11). To involve in the studies a special reference strain for *M. bovimastitidis*, however, does seem to blur the issue, which is simply a question of classification and nomenclature of a certain group of mycoplasmas, as pointed out by Jain et al. (11). The serological identity of different strains within this group has never been disputed (1, 2, 14), notably not by Jain et al. (11), who, as mentioned previously, also compared strains Donetta and California 01.

The relative genetic homologies among organisms of different taxa have been studied most extensively for bacteria, in which the degree of relatedness at the subspecies level was proposed by Johnson (12) to be 60 to 70%. This proposal is in agreement with that of Brenner (4), who in most cases found a relatedness of 70% or more between strains of species of the family *Enterobacteriaceae*.

In summary, it appears that strains PG2 and Donetta have essentially the same biochemical

TABLE 3. Competition experiments between DNA from *M. agalactiae* subsp. *agalactiae* (PG2) and *M. agalactiae* subsp. *bovis* (Donetta)

Homologous system	Homologous competitor DNA	Heterologous competitor DNA	Homology ^a (%)
PG2 (14.4) ^b	PG2 (4.0)	Donetta (10.5)	38
Donetta (19.2)	Donetta (3.8)	PG2 (13.8)	35

^a Percent homology = [(percent binding in homologous system – percent binding in heterologous competitor system)/(percent binding in homologous system – percent binding in homologous competitor system)] $\times$ 100.

^b The values in parentheses are the percentages of the input radioactivity bound in the reactions.

and biological characters (9). They do have common antigens, as shown by double immunodiffusion (7), but cross-reactions have never been demonstrated by growth inhibition and immunofluorescence, and only weak or one-way cross-reactions have ever been found by metabolism inhibition. Considering the species concept of mycoplasmas in general and the homology levels cited above, it would not seem justifiable to classify the Donetta-like strains as a subspecies of *M. agalactiae* but rather as a separate species, with the type strain Donetta (PG45). The name for this new species is *M. bovis* (Hale et al.) comb. nov.

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REPRINT REQUESTS

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