Lack of Genetic Relatedness Between *Mycoplasma alvi* and *Mycoplasma sualvi*

GUNNA CHRISTIANSEN,¹ CLAUS CHRISTIANSEN,² AND EYVIND A. FREUNDT^{1*}

Institute of Medical Microbiology, University of Aarhus, DK-8000 Aarhus C,¹ and The Genetechnology Group, The Technical University of Denmark, DK-2800 Lyngby,² Denmark

In hybridization experiments performed with deoxyribonucleic acids extracted from the type strains of *Mycoplasma alvi* and *Mycoplasma sualvi*, no hybridization was demonstrated. A representative strain (strain 1161-III) of a supposedly new species that exhibited some resemblance to *M. alvi* especially showed levels of deoxyribonucleic acid-deoxyribonucleic acid relatedness of less than 4% with both species.

Mycoplasma alvi Gourlay, Wyld and Leach 1977 (3) and *Mycoplasma sualvi* Gourlay, Wyld and Leach 1978 (4) share a number of prominent characteristics. The cells of both species are predominantly flask or club shaped with more or less well defined polar structures that probably serve as attachment organelles. In *M. alvi* each organelle contains a rod-shaped central core, which has not been demonstrated in *M. sualvi*. Although *M. alvi* and *M. sualvi* are facultative anaerobes, the growth of both organisms is better under anaerobic conditions. The biochemical properties of these two species, including utilization of glucose and arginine, are likewise identical. *M. alvi* is a common inhabitant of bovine alimentary and urogenital tracts, whereas *M. sualvi* occupies a similar ecological niche in pigs. Serologically, the two species are distinct. The guanine-plus-cytosine contents of the deoxyribonucleic acids (DNAs) of *M. alvi* and *M. sualvi* are 26.4 and 23.7 mol% (buoyant density method), respectively, and thus demonstrate a significant difference between the two species (3, 4).

The purpose of this study was to examine the degree of genetic relatedness between *M. alvi* and *M. sualvi* by using DNA-DNA hybridization. Our study was further prompted by the observation of a serological one-way cross-reaction between *M. alvi* and the representative strain (strain 1161-III) of a supposedly new species isolated from cell cultures. Strain 1161-III resembles *M. alvi* and *M. sualvi* in its ability to degrade both glucose and arginine and in possessing a polar terminal structure containing a central dense core (M. Salih, O. Vinther, and E. A. Freundt, unpublished observations). To determine the significance of the phenotypic similarities between *M. alvi* or *M. sualvi* and strain 1161-III, we decided to include this strain in the hybridization experiments.

The strains examined in this study are listed in Table 1. *Anaeroplasm* sp. strain 161, *Mycoplasma hominis* PG21^T (T = type strain), and *Mycoplasma mycoides* subsp. *mycoides* PG1^T were included for comparison. Culture material of *Anaeroplasm* sp. strain 161 was provided by I. M. Robinson, National Animal Disease Center, Ames, Iowa, and the other strains were type or reference strains kept at the Food and Agriculture Organization/World Health Organization Collaborating Centre for Animal Mycoplasmas, University of Aarhus, Aarhus, Denmark.

DNA was extracted as previously described (2). The guanine-plus-cytosine content of the DNA of *Mycoplasma* sp. strain 1161-III was determined by the method of Schild

TABLE 1. Hybrid yields (percentages of homologous reactions) with DNA-DNA hybrids of *Mycoplasma* and *Anaeroplasm* species

[³ H]DNA in solution	DNA on filter ^a		
	<i>M. alvi</i> Iisley ^T	<i>M. sualvi</i> Mayfield ^T	<i>Mycoplasma</i> sp. strain 1161-III
<i>M. alvi</i> Iisley ^T	100	0	2.4
<i>M. sualvi</i> Mayfield ^T	0	100	0
<i>Mycoplasma</i> sp. strain 1161-III	3.3	0.2	100
<i>M. hominis</i> PG21 ^T	1.2	1.4	0
<i>M. mycoides</i> subsp. <i>mycoides</i> PG1 ^T	0.3	0.3	0
<i>Anaeroplasm</i> sp. strain 161	0	0.3	0

^a Results are expressed as percentages of homologous reactions. Actual counts in homologous reactions were about 5,000 cpm; blank reactions were 150 cpm.

kraut et al. (5). DNA extracted from *Escherichia coli* was used as a reference (buoyant density, 1.7100 g/cm³). DNA-DNA hybridization experiments were performed as described previously by a membrane filter method (1, 2).

The buoyant density of the DNA of *Mycoplasma* sp. strain 1161-III was 1.6837 g/cm³ (standard deviation, 0.0001; *n* = 2); this gave a calculated value for the guanine-plus-cytosine content of 24.2 ± 0.1 mol%.

The results of the DNA-DNA hybridization experiments are shown in Table 1, which shows that no or only very insignificant hybridization occurred among the test strains.

In conclusion, our data confirm the mutual taxonomic distinctness of *M. alvi* and *M. sualvi*. Moreover, our data show that *Mycoplasma* sp. strain 1161-III is genetically unrelated to either of these species.

LITERATURE CITED

- Christiansen, C., G. Askaa, E. A. Freundt, and R. F. Whitcomb. 1979. Nucleic acid hybridization experiments with *Spiroplasma citri* and the cornstunt and suckling mouse cataract spiroplasmas. *Curr. Microbiol.* 2:323-326.
- Christiansen, C., F. T. Black, and E. A. Freundt. 1981. Hybridization experiments with deoxyribonucleic acid from *Ureaplasma urealyticum* serovars I to VIII. *Int. J. Syst. Bacteriol.* 31:259-262.
- Gourlay, R. N., S. G. Wyld, and R. H. Leach. 1977. *Mycoplasma alvi*, a new species from bovine intestinal and urogenital tracts. *Int. J. Syst. Bacteriol.* 27:86-96.
- Gourlay, R. N., S. G. Wyld, and R. H. Leach. 1978. *Mycoplasma sualvi*, a new species from the intestinal and urogenital tracts of pigs. *Int. J. Syst. Bacteriol.* 28:289-292.
- Schildkraut, C. L., J. Marmur, and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. *J. Mol. Biol.* 4:430-443.

* Corresponding author.