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Brief Communication

SEROLOGICAL IDENTIFICATION OF A NEW PORCINE MYCOPLASMA SPECIES, M. FLOCCULARE

In a preceding paper (*Friis* 1972), the so-called SAR (M. suipneumoniae antibody resistant) mycoplasma strains were described. As the reported findings indicated that these isolates belonged to a separate species, a representative cloned strain, Ms42, was selected for serological comparison with the known acid-producing mycoplasma species.

Serological techniques. The growth inhibition (GI) and metabolic inhibition (MI) tests were performed as described by *Friis* (1971). The indirect fluorescent antibody test (IFA) was conducted with air-dried broth-culture concentrates as antigens. The complement fixation test (CF) was conducted according to the LBCF procedure adapted to microtechnique (*U. S. Department of Health, Education and Wellfare* 1965). CF-antigens were produced from M. suipneumoniae, M. hyorhinis, and Ms42. Antigens for the agar gel diffusion test (AGT) (Ouchterlony) were prepared from organisms grown in standard medium enriched with horse serum. The wells punched in the agar were 2.5 mm in diameter and 3.0 mm apart.

Antisera for as well as cultures of the following acid-producing mycoplasma strains* were used for comparison with Ms42: M. agalactiae; M. agalactiae var. bovis (Donetta); M. anatis; M. bovigenitalium; M. bovine serogroups 6 (Squire), 7, K and L; M. bovirhinis; M. canis; M. dispar; M. edwardii; M. felis; M. fermentans; M. gallinarum; M. gallisepticum; A. laidlawii A and B; M. mycoides var. myc., PG 1 and Brack; M. mycoides var. capri; M. neurolyticum, Sabin A; M. pneumoniae; M. pulmonis; A. granularum, S 39; M. hyorhinis; M. hyosynoviae, M. 60; M. suipneumoniae.

Antisera for the porcine species used for GI and MI were produced in rabbits immunized with antigen grown in the standard medium. For sera used in CF and AGT rabbits had been immunized with antigen grown in rabbit meat infusion broth enriched with rabbit serum.

All the sera for porcine and non-porcine mycoplasma species failed to inhibit the growth of Ms42 on solid medium (GI) and to prevent the pH change produced by it in liquid medium. Conversely, an anti-Ms42 serum inhibited none of the type strains

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Table 1. Serological comparison of Ms42 with M. suipneumoniae and M. hyorhinis.

Antigen	Ms42 antiserum				
	GI	MI	CF	IFA	AGT*
Ms42	+	320	80	40	0
M. suipneumoniae (NCTC 10110)	0	<10	20	<10	0
M. hyorhinis (NCTC 10121)	0	<10	<10	<10	0

Table 1 (continued).

Antigen	M. suipneumoniae antiserum (NCTC 10110)				
	GI	MI	CF	IFA	AGT*
Ms42	0	<10	160	40	1
M. suipneumoniae (NCTC 10110)	+	160	1280	640	3
M. hyorhinis (NCTC 10121)	0	<10	40	20	0

Table 1 (continued).

Antigen	M. hyorhinis antiserum (NCTC 10121)				
	GI	MI	CF	IFA	AGT*
Ms42	0	<10	<40	<10	0
M. suipneumoniae (NCTC 10110)	0	<10	<40	20	0
M. hyorhinis (NCTC 10121)	+	5000	1280	640	2

* Figures indicate number of lines.

except, in the MI test, M. squire. However, this reaction was considered to be non-specific, since M. squire was also inhibited by antisera for M. suipneumoniae, M. hyorhinis, and M. granularum (to the same dilution as by its homologous serum). In the indirect FA-test the sera showed strong homologous staining reactions with titers ranging between 1:80 and 1:640, or above. None of the sera for non-porcine strains stained the Ms42 antigen in dilution 1:10. And an anti-Ms42 serum having a homologous staining titer of 1:1280 did not stain any of the type strains. All sera to non-porcine mycoplasmas were negative to Ms42 antigen

in the agar gel diffusion test. However, the homologous reactivity of the sera was not tested with this technique.

The results of the serological comparison between Ms42, M. suipneumoniae, and M. hyorhinis are given in Table 1. The GI and MI separate Ms42 completely from the 2 other organisms, while some low-order crossing was detected in AGT, CF, and IFA. In AGT, an M. suipneumoniae antiserum formed at least 3 strong lines with M. suipneumoniae antigen, and 1 distinct line with Ms42 antigen. This line was confluent with one of the lines formed with M. suipneumoniae antigen. Incorporation of 50 % complete standard medium in the agar did not affect the strength and position of the common line. The relationship of Ms42 to M. suipneumoniae revealed in the AGT manifested itself only one way; however, the available Ms42 antisera (free from antibodies to medium components) did not have a strength comparable to that of the M. suipneumoniae antiserum. The CF and the IFA also show that Ms42 and M. suipneumoniae are serologically different, although some crossing is seen.

It is generally accepted that the outcome of GI and MI tests determines the separation between species, whereas CF and AGT may reveal interspecies relationships; it is therefore concluded, on the basis of the data outlined above, that a new porcine species of mycoplasma has been discovered. With reference to the characteristic flocky elements visible in broth cultures, it is proposed that this new species be given the name M. flocculare (latin flocculus = small flock).

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