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***Brief Communication***

# ISOLATION AND CHARACTERIZATION OF A NEW PORCINE MYCOPLASMA

In a study on the distribution of Mycoplasma sui- (hyo-) pneumoniae (M. suip.) among Danish swine it was found that most isolates possessing the characteristic colonial morphology of M. suip. would be inhibited significantly in the growth inhibition (g.i.) and metabolic inhibition (m.i.) tests by antiserum for a type strain* of this species. However, a few isolates were found to be completely unaffected by this antiserum. Five such strains have been recovered, viz. 4 from cases of catarrhal pneumonia in bacon pigs, 1 from the nasal cavity of a 40-kg pig. The pigs in question originated from 5 different herds. The recovery of 1 of the strains has been reported (*Friis* 1971b).

This paper deals with the identification of these M. suip.-antibody resistant strains (SAR-strains).

For cultivation, a medium here referred to as FF II and described by *Friis* (1971a) was used. All 5 SAR-strains were cloned twice on solid medium. Using rabbit hyperimmune serum against a selected SAR-strain, Ms42, g.i. and m.i. showed them to be closely related to one another.

Three SAR-strains, Ms30, Ms42, and Ms45, were compared to the above-mentioned type strain and 2 Danish isolates of M. suip.

The 6 strains were indistinguishable from each other in most tests. They were all slow-growing in FF II broth, in which a drop of the pH occurred (from 7.4 to 6.0—6.5). When they were inoculated onto solid medium small colonies would develop after 2 days. After 4 to 7 days a size of 0.5 to 1.0 mm would be reached. The colonies appeared uniform, though coarsely granular, and were devoid of a central nipple. There was no evidence of "film & spot".

By filtration experiments, using membraneous filters (Gelman), is was found that all 6 strains could easily pass 0.45 μm,

* Type NCTC 10110, Mycoplasma Reference Laboratory, Colindale, London.

but usually not 0.30 μm. By phase-contrast microscopy of broth cultures pleomorphism and mycelial growth were noticed. The organisms could be stained by the Giemsa method, but not by the usual bacteriological staining procedures.

None of the 6 strains would grow in serum-free medium FF II. In bacteriostatics-free medium FF II, none of the strains reverted to a parent bacterium after 3 passages in broth followed by cultivation on solid medium (L-phase variant test). All strains were resistant to 1.0 mg/ml of meticillin (a semi-synthetic penicillin) but sensitive to tetracycline, the minimal effective concentration being 0.1—0.01 μg/ml.

All 6 strains were found to metabolize glucose but neither arginine nor urea. They were negative in the tetrazolium reduction test (performed in broth with 0.005 % 2,3,5-triphenyl-2H-tetrazolium chloride) and also in the phosphatase activity test (performed in broth with 0.05 % phenolphthalein diphosphate added; alkalizing with NaOH after termination of growth).

The 3 SAR-strains were found dissimilar to the 3 M. suip. strains in 2 respects. During growth in broth the mycelial filaments of the SAR-strains show but little tendency to disintegrate, whereby very large aggregates are formed. The phenomenon was noticed by phase-contrast microscopy and after staining by the Giemsa method, but could be observed even with the naked eye, in that small flocky elements would appear in broth cultures after gentle shaking. Vigorous shaking forced the aggregates to disintegrate. This feature, however, seems to be lost after prolonged propagation in artificial media. The second point of difference is the fact that the SAR-strains will grow fairly well at 30°C, while strains of M. suip. will grow but poorly at this temperature.

The 8th passage ($10^{-13}$ of original tissue) of a strain recovered from a pneumonic lung and cloned once on solid medium was inoculated, by aerosol, on 4 9-week-old pneumonia-free pigs. No clinical signs of disease were noted. At necropsy 33 to 35 days p.i. no macroscopic changes were observed. Histologic examination of the nasal mucosa showed moderate infiltrations in the stratum proprium of lymphoid and histiocytic cells, and disintegration of the epithelial coat. Histologic examination of the lungs showed a distinct peribronchiolar and perivascular proliferation of lymphoid and histiocytic cells. From all 4 pigs, mycoplasmas serologically similar to Ms42 were recovered from

the nostrils on days 9 and 16, and from the nasal cavities and lungs at necropsy. From 2 of the pigs such mycoplasmas were also recovered from brain tissue. Cultures from other sites were negative. Two control animals kept in isolation showed no lesions at necropsy. They were found mycoplasma-free. Histologic examination was made only of the nasal mucosa, which was found normal.

The colonial morphology of the mycoplasmas described above is indistinguishable from that of M. suip. Nevertheless it is apparent that they differ from M. suip. not only in the serological features revealed by preliminary examination, but also in other respects. Especially their fairly good growth at 30°C. is remarkable and indicates that the upper respiratory tract is a natural habitat for these mycoplasmas. This was also confirmed by pig inoculation.

Serologic identification of this mycoplasma and its classification as a new species, Mycoplasma flocculare, is reported elsewhere (*Meyling & Friis* 1972).

*N. F. Friis*
The State Veterinary Serum Laboratory,
Copenhagen, Denmark.

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Reprints may be requested from: N. F. Friis, The State Veterinary Serum Laboratory, Bülowsvej 27, DK-1870 Copenhagen V, Denmark.