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***Brief Communication***

# A SELECTIVE MEDIUM FOR MYCOPLASMA SUIPNEUMONIAE

The isolation of Mycoplasma suipneumoniae (M. suip.) has not hitherto been possible in broth culture if the material also harboured Mycoplasma hyorhinis (M. hyor.), since the latter organism would outgrow M. suip. The present paper reports an effort to solve this problem.

In a previous experiment (*Friis*, unpublished) it was observed that the antibiotic cycloserine* exerted a selective inhibitory effect on M. hyor., the minimal inhibitory concentration being 0.05 mg/ml for strains of M. hyor. in primary isolation trials in broth, as against 0.5 mg/ml for strains of M. suip. and Mycoplasma hyosynoviae (M.hyosyn.)**. Experiments have been carried out to combine the inhibitory effect of cycloserine on M. hyor. with that of a specific rabbit hyperimmune M. hyor.*** antiserum. It was found that a medium containing 5 % of this antiserum and 0.5 mg/ml of cycloserine (i.e. the minimal inhibitory concentration for M. suip.) prevented replication (as judged by colorshift of phenol red in broth) of M. hyor. in primary isolation trials without influencing the growth of M. suip. Presumably, the slight inhibitory effect of cycloserine on M. suip. was counterbalanced by a slight stimulative effect of the antiserum.

This selective medium was tried in cultivation experiments with material from field cases of porcine catarrhal pneumonia. Twenty pneumonic pig lungs, originating from 18 different farms, were collected at 2 slaughter-houses. The material was prepared and cultivated as previously described (*Friis* 1971 a), both in ordinary mycoplasma medium (*Friis* 1971 b), and in the same medium modified as described above in order to make it selective for M. suip. For general bacteriostatic purposes bacitracin and meticillin were used, both at the level of 0.15 mg/ml. A sample was picked from a single pneumonic area of each lung and cultivated in 10-fold dilutions of ground tissue suspensions, up

* Kindly supplied by AB Kabi, Stockholm, Sweden.

** Previously referred to as M. suidaniae (*Friis* 1970).

*** Type NCTC 10121, kindly delivered by the Mycoplasma Reference Laboratory, Colindale, London, England.

to $10^{-9}$. Subcultivations, in ordinary medium, were made in 10-fold dilutions up to $10^{-3}$, if colorshift occurred. In addition, cultivation for M. hyosyn. was performed as described by *Friis* (1970). The final titers of the primary cultivations were read after 3 weeks.

In 9 lungs neither M. hyor. nor M. hyosyn. was present. From 7 of these lungs M. suip. was isolated within 14 days, and the speed of growth as well as the final titers were similar in the 2 media. From the remaining 2 lungs M. suip. was recovered after 20 days of incubation, only in ordinary medium, and only from the dilution $10^{-1}$.

M. hyosyn. was present in 5 lungs (25 %). From 4 of these, M. suip. could be isolated. The growth of M. hyosyn. in ordinary medium is rather sparse and its viability quickly lost, so that M. suip. may eventually be recovered in pure culture even if M. hyosyn. is present to begin with.

In 6 lungs (30 %) M. hyor. was present. From these lungs M. suip. could be isolated by using selective medium (Table 1), but not in ordinary medium. It appears from the table that in some of the lower dilutions the selective medium yielded M. hyor. However, subcultivations in broth and on solid medium gave reason to believe that this was the result not of replication but of simple survival af M. hyor. Moreover, subcultivations on consecutive days from the same tube showed, in several trials, a gradual disappearance of M. hyor. parallel to the progressing of the yellow colorshift.

Isolates of M. suip. were identified by their speed of growth in broth, by their colony morphology on solid medium, and by metabolic inhibition tests (*Friis* 1971a) with antisera for M.suip.* and M. hyor. It should be mentioned that 1 isolate, recovered from a lung yielding neither M. hyor. nor M. hyosyn., and preliminary referred to M.suip. on the basis of growth characteristics (glucose-fermentation, cycloserine resistance, slow growth in broth and on solid medium, dependance on $CO_2$ for growth on solid medium, colony morphology on solid medium indistinquishable from that of M. suip.) was not inhibited by any of the 2 antisera used.

Isolates of M. hyor. and M. hyosyn. were identified by growth inhibition tests.

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* Type NCTC 10110, kindly delivered by the Mycoplasma Reference Laboratory, Colindale, London, England.

Table 1. Results obtained by cultivation in normal and selective medium in 10-fold dilutions of pneumonic tissue suspensions harbouring M. hyor.

| Lung no. | Endpoint of growth of M. hyor. in normal medium | Isolates obtained from dilutions in selective medium | | | | Endpoint of growth of M. suip. in selective medium |
|---|---|---|---|---|---|---|
| | | $10^{-1}$ | $10^{-2}$ | $10^{-3}$ | $10^{-4}$ | |
| My 768 | $10^{-2}$ | s | s | s | o | $10^{-3}$ |
| My 770 | $10^{-6}$ | h | s | s | s | $10^{-6}$ |
| My 810 | $10^{-5}$ | h | s | s | o | $10^{-3}$ |
| My 814 | $10^{-6}$ | s | s | s | s | $10^{-4}$ |
| My 819 | $10^{-5}$ | h | h | s | h | $10^{-5}$ |
| My 824 | $10^{-5}$ | h | s | s | s | $10^{-4}$ |

h = M. hyor. s = M. suip.

It should be noted that the same principle for suppression of M. hyor. has been used with success for primary isolation of M. hyosyn. from the nasal cavity of a pig.

For production of specific rabbit hyperimmune antiserum the following procedure has been followed: A broth culture of mycoplasmas is washed 4 times by centrifugation (approx. 20,000 × g for 45 min.) and resuspended in PBS so as to obtain a 20-fold concentration as compared with the original. This antigen is mixed with an equal volume of Freund's complete adjuvant and 2 ml applied s.c. to a rabbit on day zero. The procedure is repeated on day 60 using incomplete adjuvant. Additionally, 1 ml of pure antigen is given i.v. on days 0, 30, 45, 60, 70, 80, 82, 84, 86, 88, 90, and if necessary on days 100, 110, 120, 122, and 124. Hereafter the animals are bled and inoculated once a week for several months. The method is time-consuming, but has always given large quantities of potent antiserum, no matter the species of mycoplasma worked with.

With the technique described in the present paper Mycoplasma suipneumoniae has been recovered from 95 % of cases of porcine catarrhal pneumonia in slaughtered bacon pigs. This would seem to support the view that the main responsibility for chronic catarrhal pneumonia in pigs should be assigned to that organism.

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