

**Brief Communication**

**SELECTIVE ISOLATION OF SLOWLY GROWING  
ACIDIFYING MYCOPLASMAS FROM SWINE AND CATTLE**

The primary isolation of the slowly growing acidifying mycoplasma species from swine, *Mycoplasma suis pneumoniae* and *Mycoplasma flocculare*, and from cattle, *Mycoplasma dispar*, may be impossible from tissue containing also more rapidly growing, acidifying mycoplasma species. Thus, *Mycoplasma suis pneumoniae* (*M. suis*.) and *Mycoplasma flocculare* (*M. flocc.*) will be outgrown by *Mycoplasma hyorhinis* (*M. hyor.*) and *Mycoplasma dispar* (*M. dispar*) by *Mycoplasma bovirhinis* (*M. bovir.*).

Based on the observation that growth of *M. hyor.* and *M. bovir.* was retarded in medium enriched with a yeast extract prepared by acid extraction, selective media for the primary isolation of the three slowly growing species have been developed.

The medium mentioned above was a modification of the medium described by Friis (1975) for cultivation of *M. suis*. Horse and swine serum (10 % of each) was used. The watery extract of Fleischmann's yeast was replaced by the same volume of an acid extract of fresh baker's yeast (De Danske Spritfabrikker A/S, Copenhagen) similarly as in the medium of Herderscheé and Hers (*Herderscheé* 1963): Yeast, 250 g, and distil. water preheated to 40 C, 500 ml, are mixed well and another 500 ml distil. water added. Mix and adjust pH to 4.6 with conc. HCl, heat to 80 C and keep that temperature for 20 min. Cool, centrifuge at  $2000 \times g$  for 20 min, pass through a coarse filter and through a membrane filter (Gelman, TCM 0.45  $\mu\text{m}$ ). Storage at  $-25$  C.

*Selective isolation of Mycoplasma suis pneumoniae and Mycoplasma flocculare*

Eight pneumonic tissue suspensions known to contain *M. hyor.* were cultivated (10-fold dilutions to  $10^{-5}$ ) in liquid medium enriched with watery extract of Fleischmann's yeast and in medium enriched with acid extract of fresh baker's yeast. As evaluated from the degree of colorshift of the phenol red indicator, the speed, but especially the intensity, of growth was less in the latter medium than in the former. On the other hand, 15

suspensions with *M. suip.* and 10 with *M. floc.* showed the same speed and intensity of growth in the two media.

It has earlier been published (Friis 1971, 1975) that cycloserine has a selective inhibitory effect on *M. hyor.* and that this effect can be enhanced by antiserum for *M. hyor.* The selective medium for *M. suip.* and *M. floc.* is therefore composed as follows: Medium enriched with acid extract of fresh baker's yeast, 100 ml; inactivated rabbit hyperimmune antiserum for *M. hyor.*, 5 ml; cycloserine 31.5 mg (0.3 mg/ml).

Ten-fold dilutions to  $10^{-4}$  of tissue suspension in liquid selective medium are incubated and subcultivated on occurrence of yellow colorshift of the phenol red indicator. Non-selective medium with watery extract of Fleischmann's yeast can be used for subcultivation. As the final disappearance of *M. hyor.* from the selective cultures sometimes appears to depend on the acidifying growth of *M. suip./M. floc.*, it may be necessary to repeat the subcultivation at intervals of a few days.

Of a total of 70 isolations of *M. suip.* from pneumonic swine lungs, 17 were accomplished in spite of the presence of *M. hyor.* by using the new selective medium. For *M. floc.* the corresponding figures are 24 and seven.

#### *Selective isolation of Mycoplasma dispar*

Similarly as was the case with swine mycoplasmas, *M. bovir.* showed retarded growth in medium enriched with acid extract of fresh baker's yeast as compared to medium enriched with watery extract of Fleischmann's yeast. With *M. dispar* no difference was observed between the growth in the two media.

Since cycloserine does not have a selective inhibitory effect on *M. bovir.* (Friis, unpublished), the selective medium for *M. dispar* is composed thus: Medium enriched with acid extract of fresh baker's yeast, 100 ml; inactivated rabbit hyperimmune antiserum for *M. bovir.*, 5 ml.

Ten-fold dilutions to  $10^{-4}$  of tissue suspension in liquid selective medium are incubated and subcultivated on occurrence of colorshift. Non-selective medium with watery extract of Fleischmann's yeast can be used for subcultivation.

Of a total of 22 isolations of *M. dispar* from pneumonic calf lungs, six were obtained from this selective medium in spite of the presence of *M. bovir.*

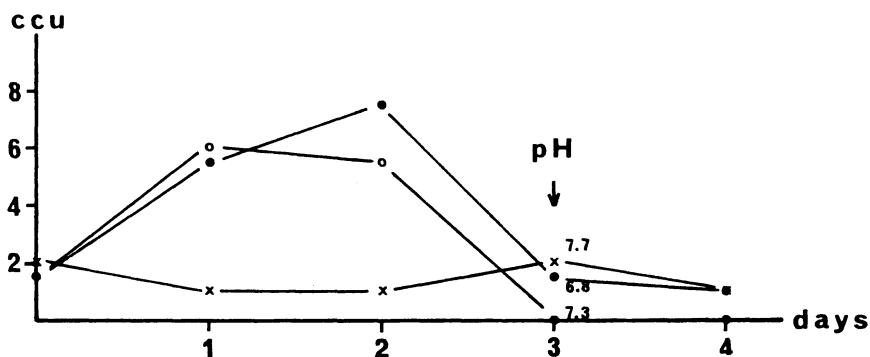


Figure 1. Influence of the source of yeast extract and of selective medium on the replication of *Mycoplasma bovirhinis*. (Cloned culture of strain Mk774 in third passage).

- Medium enriched with watery extract of Fleischmann's yeast.
  - Medium enriched with acid extract of fresh baker's yeast.
  - x—x Selective medium for *M. dispar*.
- ccu Log color changing units per 0.2 ml of culture.

In Fig. 1 is shown the growth of *M. bovir.* in liquid medium with watery extract of Fleischmann's yeast, in medium with acid extract of fresh baker's yeast, and in selective medium for *M. dispar*. A cloned second-passage culture of strain Mk774, diluted to  $10^{-4}$  in Hanks' balanced salt solution, was used for seeding. As appears, growth was poor in the selective medium and slightly reduced in medium with acid extract of fresh baker's yeast. These observations are consistent with the indicated pH estimations.

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#### REFERENCES

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