SHORT COMMUNICATION

Rapid Tissue Culture Method for Detection of *Mycoplasma hyorhinis*

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An easy, rapid tissue culture method for detection of *Mycoplasma hyorhinis* is described. The organism induces morphological changes in mink S + L – cells. This effect was not observed in eight other animal cell lines infected by *M. hyorhinis* and it did not occur in the mink cells infected with six other strains of mycoplasma. This cell system should be useful in research laboratories which do not have other standard techniques available for monitoring the presence of *M. hyorhinis*.

INTRODUCTION

Mycoplasmas are ubiquitous organisms which are often difficult to detect in tissue culture. They can remain latent during several cell passages without noticeable effects on the cells. Nevertheless, their presence can influence experimental procedures and growth of other organisms in tissue culture (McGarrity *et al.*, 1978, 1979; Tully & Whitcomb, 1979). Mycoplasmas are detected by a variety of procedures including growth on agar, fluorescent antibody (FA) staining, histological staining, electron microscopy, enzyme assays, DNA fluorochrome staining, and cytopathic effects (CPE) in certain cells (McGarrity *et al.*, 1978, 1979; Tully & Whitcomb, 1979). These techniques are usually performed by laboratories specializing in the identification of mycoplasmas and can take 2–4 weeks to give a final result. A very sensitive assay is growth of the organisms on agar, but strains of some mycoplasmas, particularly *Mycoplasma hyorhinis*, cannot grow on standard agar or in broth (Hopps *et al.*, 1973). These auxotrophic, fastidious organisms are identified in cell cultures by DNA fluorochrome staining or FA procedures (Barile & Grabowski, 1982; Chen, 1977). Recently, axenic cultivation of these *M. hyorhinis* strains has been achieved using cell lysate-supplemented medium (Del Giudice *et al.*, 1980).

While studying virus production in certain animal cell lines, we recognized the presence in one culture of a filterable agent which induced distinct changes in mink S + L – cells within 4–5 d. Instead of reaching a tight confluent monolayer with a cobblestone appearance, the mink S + L – cells had a fibroblastic morphology with some crossing over of the cells, and the culture medium became acidic. We analysed the supernatant for bacteria, viruses and mycoplasmas by standard techniques (Barile & Grabowski, 1982; Chen, 1977). By specific FA staining, we discovered that this CPE in the mink cells was due to *M. hyorhinis*. In this report, we present evidence that this bioassay is a new rapid *in vitro* test for *M. hyorhinis*.

METHODS

Cells and media. Several of the cell lines used for the experiments were obtained from Dr W. Nelson-Rees, Naval Biologic Laboratory, Oakland, California, U.S.A. These included SIRC (rabbit cornea), gazelle lung, horse dermis, mink lung (ATCC, CCL64), and bat lung cells. Duck embryo cells were established in culture in our laboratory from 18-d-old eggs. The normal rat kidney (NRK) line, NIH 3T3 mouse fibroblast, and human foreskin fibroblast cells are cultivated routinely in our laboratory. Cells were grown in Eagle's minimal essential medium, supplemented with 5% (v/v) heated (56 °C, 30 min) calf serum, 2 mM-glutamine, and the antibiotics

penicillin (100 units ml⁻¹) and streptomycin (100 µg ml⁻¹). The mink S+L- cell line (Peebles, 1975), originally obtained from P. Peebles, National Institutes of Health, Bethesda, Maryland, U.S.A., was maintained in RPMI 1640 medium supplemented with 5% (v/v) foetal calf serum and the above-mentioned antibiotics. All cell lines, prior to use, were free of mycoplasma infection as determined by the FA and DNA fluorochrome staining techniques (Barile & Grabowski, 1982; Chen, 1977) and the lack of colony growth on solid agar.

Mycoplasma strains and detection procedures. The GDL and BTS-7 reference strains of *M. hyorhinis* were obtained from Dr G. J. McGarrity, Institute for Medical Research, Camden, New Jersey, U.S.A. and the National Institute of Allergy and Infectious Disease (NIAID), Bethesda, Maryland, U.S.A., respectively. The DBS 1050 cultivar α strain of *M. hyorhinis* (Del Giudice *et al.*, 1980; Hopps *et al.*, 1973) was purchased from the American Type Tissue Culture Collection (ATCC 29052). Mycoplasmas were detected by the FA and DNA fluorochrome staining procedures (Barile & Grabowski, 1982; Chen, 1977). The *M. hyorhinis* antiserum for the FA studies was supplied by NIAID (serum M718-501-471). The fluorochrome stain was obtained from Riedel-De Haen Ag, Seelze-Hannover, F.R.G.

RESULTS

Based on the observation that morphological changes were induced in mink S+L- cells by the *M. hyorhinis* strain present in a cell line in our laboratory, we conducted several experiments to determine whether this type of CPE was specific for this strain of mycoplasma. Moreover, we investigated whether other cells were altered when infected with *M. hyorhinis*. We first inoculated mink S+L- cells with reference strains of *M. hyorhinis* 24 h after the cells had been plated at semiconfluency. The cultures were then maintained with fluid changes every 2-3 d. The results (Table 1) indicated that CPE in mink S+L- cells was only observed with the organisms identified specifically as *M. hyorhinis* by FA staining. All strains of *M. hyorhinis* produced the characteristic morphological change in mink S+L- cells, including the *M. hyorhinis* cultivar α strain, which requires a specific medium for axenic cultivation (Del Giudice *et al.*, 1980). Mycoplasma strains other than *M. hyorhinis* replicated in the mink S+L- cells as demonstrated by the DNA fluorochrome staining technique, but did not produce any CPE (Table 1). When serial dilutions of *M. hyorhinis* samples were tested, titres ranged from 10⁵ to 10⁷ CPE-inducing units per 0.4 ml. This effect in mink S+L- cells was first noted by the fourth or fifth day even in cultures which received the last positive end-dilution. The CPE was observed in cells receiving antibiotics but was more evident earlier in cultures not receiving medium containing these drugs. Cultures that did not show any CPE by the sixth day did not contain any detectable *M. hyorhinis* either by further passage of the supernatants or by testing the inoculated cells by the FA and DNA fluorochrome staining techniques. This sensitivity of mink S+L- cells for detection of *M. hyorhinis* was also observed with mink lung cells, but the CPE in these cells usually developed after 5-6 d; it consisted of degeneration of the cells rather than the

Table 1. *Detection of mycoplasmas*

Organisms were inoculated on to mink S+L- cells which were examined for cytopathic effects (CPE). Fluorescent antibody (FA) and DNA fluorochrome staining procedures were conducted on the cells as described in text. The antiserum of the FA test was specific for *M. hyorhinis*. The figures represent the number of times the organism was detected/number of assays conducted.

Strain	CPE in S + L - cells	FA staining	DNA fluorochrome staining
<i>M. hyorhinis</i>			
BTS-7 strain	6/6	6/6	6/6
GDL strain	8/8	8/8	8/8
DBS 1050 strain (cultivar α)	3/3	3/3	3/3
<i>M. orale</i>	0/3	0/3	3/3
<i>M. hominis</i>	0/2	0/2	2/2
<i>M. salivarium</i>	0/2	0/2	2/2
<i>M. fermentans</i>	0/2	0/2	2/2
<i>A. laidlawii</i>	0/3	0/3	3/3
<i>M. arginini</i>	0/3	0/3	3/3

alteration in the cell morphology observed with mink S+L- cells. The sensitivity for detection of *M. hyorhinis* in both mink cell lines was similar.

The eight other animal cell lines listed above were inoculated with *M. hyorhinis* but no CPE was noted. Nevertheless, the supernatants from these cultures, removed after 7 d, contained *M. hyorhinis* at titres of 10^7 – 10^8 CPE-inducing units ml⁻¹, as measured in mink S+L- cells. Thus, *M. hyorhinis* replicated to high levels in several animal cell lines, but CPE was only observed in mink cells. In an attempt to determine whether a product of the mink cells helped induce this effect, we transferred filtered spent medium from mink S+L- cells on to dog, SIRC and mouse cells inoculated with *M. hyorhinis* and added more of this conditioned medium at the fluid change on the third day. No CPE was observed.

We have also examined 30 cell cultures obtained from various laboratories at the University of California for mycoplasma infection. The mink S+L- assay as well as the FA and DNA fluorochrome staining techniques were used (Barile & Grabowski, 1982; Chen, 1977). Only the 10 samples in which *M. hyorhinis* was found to be present by the FA and DNA staining methods induced CPE in mink S+L- cells. With several samples, other mycoplasmas were detected by the DNA staining technique but no morphological changes were induced in the mink cells by the culture supernatants.

DISCUSSION

Mycoplasma hyorhinis is a porcine mycoplasma which inhabits the nasal cavity of young pigs (Whittlestone, 1979). It has been recognized in the laboratory because of its specific effectiveness in contaminating cell cultures throughout the world and is one of the major contaminants of uninoculated cell cultures; its presence has been recorded in as many as 19% of mycoplasmas isolated from cell cultures (Barile, 1973; McGarrity *et al.*, 1978; Tully & Whitcomb, 1979). The cultivar α variant of *M. hyorhinis* appears to be more common in cell cultures than the wild-type strain (Hopps *et al.*, 1973). Since it cannot be grown in standard broth or agar, it has generally been identified by other techniques such as DNA fluorochrome and FA staining. The use of lysate-supplemented medium has recently permitted its recognition by growth in agar (Del Giudice *et al.*, 1980).

The mink S+L- cell test described in this report offers the advantage of being both simple to perform and quick (CPE apparent within 4 d) to indicate the presence of *M. hyorhinis*, including the cultivar α strain, in a cell culture. Moreover, the altered morphology observed in the mink S+L- cell cultures is unusual since CPE associated with mycoplasmas generally results from cell degeneration (Barile, 1973; Whittlestone, 1979). Induction of these distinct morphologic changes in the mink cells appears to be specific for *M. hyorhinis*, although more mycoplasma strains must also be tested to substantiate this fact. This rapid tissue culture method for detecting *M. hyorhinis* should be useful as a screening test for cell culture laboratories which do not have access to standard assays for this mycoplasma.

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