Monoclonal Antibody Differentiation of *Mycoplasma mycoides* subsp. *mycoides* Small-Colony Strains Causing Contagious Bovine Pleuropneumonia from Less Important Large-Colony Strains

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Monoclonal antibody (MAb) PK-2 inhibited the in vitro growth of nine *Mycoplasma mycoides* subsp. *mycoides* small-colony strains. In contrast to the results with polyclonal antisera, growth inhibition by MAb PK-2 was specific for *M. mycoides* subsp. *mycoides* small-colony strains and constituted a reliable means of distinguishing them from other mycoplasmas.

*Mycoplasma mycoides* subsp. *mycoides* small-colony (MmmSC) strains cause contagious bovine pleuropneumonia (CBPP) in cattle and buffalo (8, 17), which is one of the most serious diseases of cattle in some parts of the world. The disease, once widespread, has been eradicated from many countries and is now mainly confined to the African continent, affecting most of the countries south of the Sahara and north of South Africa (14). However, in recent years there have been outbreaks of the disease in Spain, Portugal, and Italy (14). It is thought to exist in the Middle East and Asia (14).

Differentiation of MmmSC strains by serological and biochemical means has been difficult. This difficulty is caused by immunological cross-reactions and biochemical similarities of MmmSC strains with *M. mycoides* subsp. *mycoides* large-colony (MmmLC) strains from cattle (4, 5, 16), and even other animal *Mycoplasma* strains, including *M. mycoides* subsp. *capri*. Even growth inhibition with polyclonal antibodies (6) and indirect immunofluorescence (7), assays which are considered species specific and which are used to classify *Mycoplasma* isolates (9), cannot differentiate MmmSC strains from MmmLC strains. The situation has been made even more complex by the isolation of MmmSC variants from sheep and goats (1). There is no disease currently associated with MmmLC strains from cattle; however, MmmLC strains cause pleuropneumonia and associated arthritis in goats. MmmLC strains have a worldwide distribution and have been isolated in the United States, Europe, Australia, India, and Africa (5). It is therefore important to distinguish MmmSC strains from MmmLC strains, particularly in regions currently free of CBPP. This communication describes a growth-inhibiting monoclonal antibody (MAb) which makes the necessary differentiation between MmmSC and MmmLC strains from cattle and between MmmSC strains and several other *Mycoplasma* species.

**MAbs.** Production and testing of MAbs for growth-inhibiting activity to MmmSC strains and initial characterization of the epitope recognized by the MAbs have been described (12). All the MAbs were of the immunoglobulin M isotype and recognized a carbohydrate epitope. One MAb, PK-2, was selected for further evaluation of its growth-inhibiting effect on different *Mycoplasma* species and strains.

**Strains of Mycoplasma and their source.** The strains were from three sources: the National Veterinary Research Center, Muguga (NVRC-M), Kenya; the National Veterinary Research Centre, Kabete (NVRC-K), Kenya; and R. H. Leach, National Collection of Type Cultures (NCTC), Corrinde, England. The MmmSC strains (SC group) included T419 (NVRC-M), T1M44 (T1 vaccine strain) (NVRC-M), B467/92 Kabete (NVRC-K), Gladysdale (NCTC), Poumarat 4813 (NCTC), B613/87 (NCTC), B101/93 (NVRC-M), Oremut (NVRC-M), and U716 (NVRC-M). The MmmLC strains (LC group) included VR1/3172 LB (NCTC), 78/441 LC (NCTC), and Y goat M207/86 (NCTC). The MAb was also tested against other members of the *M. mycoides* cluster, which included the following strains: *M. mycoides* subsp. *capri* (capri group) Pendik (NCTC) and BOT (NCTC); *Mycoplasma capricolum* subsp. *capricolum* (capricolum group) MA4528/76 (NCTC), 74/3220 (NCTC), ZT 14 (NCTC), and 4528 (NCTC); bovine serogroup 7 (BS7 group) strains Poumarat BS7 (NCTC), L2917 BS7 (NCTC), and PG 50 (NVRC-K); and *M. capricolum* subsp. *capripneumoniae* (capripneumoniae group) G22 (NVRC-K), G94/83 (NVRC-K), and G280/80 (NVRC-K).

**In vitro growth inhibition.** Before use in growth inhibition assays, MAb PK-2 was isolated from hybridoma culture supernatants by gel filtration (19). The isolated PK-2 (2.0 mg/ml) was tested for growth inhibition of 24 mycoplasmas belonging to the *M. mycoides* cluster as described earlier (19). Briefly, 1 ml of log phase broth culture of test *Mycoplasma* organisms was spread evenly on Newing’s tryptose agar plates and allowed to dry for 10 min. Wells were punched into the agar, and 50 μl of MAb PK-2 was added and allowed to seep into the agar. Plates were incubated at 37°C for 3 to 5 days until the colonies were visible. The plates were examined with a low-power stereo microscope for zones of inhibition. MAb PK-2 caused growth inhibition of nine MmmSC strains (Table 1), and these strains were not inhibited by an unrelated immunoglobulin M control MAb (WM-25) which reacts with a carbohydrate epitope and inhibits the growth of *M. capricolum*.

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The mechanisms by which antibody inhibits the growth of *Mycoplasma* organisms are not clearly understood. It has been proposed that growth-inhibiting antibodies are directed against exposed surface membrane proteins (13). MmmSC cells are enclosed in a capsule of carbohydrate (11) composed of galactan (2, 3). It is not surprising, therefore, that attempts to make protein-reactive growth-inhibiting MAbs to MmmSC strains have not been successful. The first growth-inhibiting MAbs to MmmSC strains that were reported reacted with carbohydrate epitopes, as demonstrated by periodate sensitivity and proteinase K insensitivity of the recognized epitopes in Western blots (12). Recently, other growth inhibition of MmmSC strains by a pool of MAbs was reported, but the epitopes recognized were not described (18). The suggested involvement of galactan produced by MmmSC strains in the pathogenesis of CBPP (15) and the data demonstrating that growth of both MmmSC strains and *M. capricolum* subsp. *capri pneumoniae* can be inhibited by MAb-recognizing carbohydrate epitopes (12, 19, 20) suggest that immune responses to carbohydrate epitopes will protect against these pleurapneumonia-causing *Mycoplasma* organisms. In addition, growth-inhibiting antibody PK-2 provides a reagent to evaluate the relationship between in vitro growth inhibition and protective immune responses.

**Conclusions.** MAb PK-2 caused growth inhibition of nine MmmSC strains and did not inhibit either three MmmLC strains from cattle or 15 other strains belonging to the *M. mycoides* cluster. Use of MAb PK-2 in growth inhibition resolved the cross-reactions observed in this study, which were growth inhibitions of MmmSC strains with polyclonal antibodies to MmmLC strains. These data suggest that growth inhibition by MAb PK-2 could be used to identify MmmSC strains and differentiate them from MmmLC strains from cattle. Although molecular techniques can be used to make this differentiation (10), growth inhibition with MAb PK-2 is a more suitable test for most laboratories where CBPP is a concern.

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### REFERENCES


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### TABLE 1. Growth inhibition of MmmSC strains and other mycoplasmas with monoclonal and polyclonal antibodies to MmmSC strains

<table>
<thead>
<tr>
<th>Mycoplasma strain</th>
<th>Growth range (CFU/ml)</th>
<th>No. of isolates with growth inhibition/no. tested</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MAb PK-2 Control MAb</td>
<td>Polyclonal antiserum: MmmLC strains</td>
</tr>
<tr>
<td>MmmSC</td>
<td>4.5 x 10^3–2.5 x 10^5</td>
<td>9/9</td>
</tr>
<tr>
<td>MmmLC</td>
<td>1.5 x 10^3–2.5 x 10^3</td>
<td>0/3</td>
</tr>
<tr>
<td>Others ( ^* )</td>
<td>3.6 x 10^3–1.7 x 10^4</td>
<td>0/2</td>
</tr>
</tbody>
</table>

\( ^* \) The non-MmmSC and non-MmmLC strains were as follows: *M. capricolum* subsp. *capri pneumoniae*, *M. mycoides* subsp. *capri pneumoniae*, *M. capricolum* subsp. *capri*, *M. capricolum* subsp. *capripneumoniae* G22 (NVRC-K), G394/83 (NVRC-K), and G280/80 (NVRC-K). The growth of these mycoplasmas was inhibited by the homologous antiserum.

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*Notes:* CLIN. DIAGN. LAB. IMMUNOL.