PREPARATION OF MYCOPLASMA SYNOVIAE ANTIGEN FOR THE TUBE AGGLUTINATION TEST

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SUMMARY

A satisfactory tube antigen was produced from isolate WVU 1853 Mycoplasma synoviae grown in a modification of mycoplasma broth formulated by Frey. The tube antigen, employed in the tube test, showed good specificity when tested with 187 known M.-synoviae-positive serums, 60 known M.-gallisepticum-positive, and 30 normal. The serums were confirmed by HI test as being positive or negative to M. synoviae or M. gallisepticum.

INTRODUCTION

The literature has little information on the production of a Mycoplasma synoviae antigen for the tube agglutination test and its use for diagnosing infection.

Olsen et al. (5) produced an M. synoviae antigen for the serum plate and tube tests. They reported the results of M. synoviae and M. gallisepticum serum plate tests on antiserums from birds exposed to one of three agents: 1) WVU 1853, an ISD-PPLO (M. synoviae); 2) WVU 1791 M. gallisepticum; and 3) agent WVU 1675, an unidentified agent that produces arthritis. They reported that the tube test with the ISD-PPLO antigen was used on pooled samples from each of the above groups (1853, 1675 and 1791) and isolated controls. Pooled serums from birds exposed to 1853, an ISD-PPLO, were agglutinated in the 1:100 serum dilution, the highest dilution used in the tube test. No reactions occurred in the 1:12.5 serum dilutions of the pooled samples of serum from groups 1675, 1791, and isolated controls. They read the test after 48–96 hours of incubation at 37 C.
Roberts and Olesiuk (6) made *M. synoviae* antigens for the serum plate and tube tests. They reported that the tube antigen was not satisfactory, since 2 of 15 serum samples gave a partial agglutination reaction in the 1:12.5 and 1:25 serum dilution at 3 and 4 weeks postinoculation into foot pad with *M. synoviae*. The tubes were incubated at 37 C and read at 18-24 hours. Serum from 15 of 15 birds gave positive serum plate agglutination reactions with their *M. synoviae* serum plate antigen, at 2 weeks postinoculation with *M. synoviae*.

Hofstad (3) used a serum dilution of 1:12.5 to 1:200 for the *M. gallisepticum* tube test, and read the test as either positive or negative after 18-24 hours of incubation at 37 C.

Jungherr *et al.* (4) used a dilution of 1:25 to 1:2010 in the *M. gallisepticum* tube test, and read each tube as 1+ to 4+ agglutination after overnight incubation at 37 C.

For diagnosing *M. gallisepticum* with the tube test, Hall (2) used a 1:25 dilution and read the test after 18-24 hours of incubation at 37 C.

As a screening test for *M. gallisepticum* antibodies, the USDA (7) recommends a 1:12.5 dilution and reading the test after 18-24 hours of incubation at 37 C.

This study was done to prepare an *M. synoviae* antigen for the tube test and confirm its specificity.

**MATERIALS AND METHODS**

The culture medium used for producing the *M. synoviae* antigen was the same as previously described (8) and that formulated by Frey *et al.* (1) except that 1.5 g dextrose per liter was used instead of 10.0 g, and phenol red was not used in the medium for producing the antigen. Phenol red was used in the medium for serial passage of the culture. The medium was sterilized by filtration through a sterile 2,500-ml Hormann filter using a D-9 pad. The medium with phenol red was dispensed in 3.75-ml quantities in sterile 15-ml screw-cap vials for serial transfers; 56-ml quantities in sterile 125-ml Erlenmeyer screw-cap flasks for seeding; and 1,000-ml quantities without phenol red in sterile 2,000-ml Erlenmeyer screw-cap flasks for antigen production.

To obtain a rapid-growing culture, serial passages of *M. synoviae* were made by inoculating 0.25 ml of the culture into 3.75 ml of medium and incubating at 37 C. When the culture began to change from red to slight orange, a transfer was made. Four ml of the rapid-growing culture was transferred to 56 ml of medium.
Table 1. A comparison of results of the tube agglutination and hemagglutination-inhibition tests.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Infection agent</th>
<th>Method of infection</th>
<th>Tube agglutination test</th>
<th>Hemagglutination-inhibition test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>M. synoviae</td>
<td>Artificial</td>
<td>MS 57/57</td>
<td>MS 57/57</td>
</tr>
<tr>
<td>2A</td>
<td>M. synoviae</td>
<td>Artificial</td>
<td>31/31</td>
<td>31/31</td>
</tr>
<tr>
<td>3B</td>
<td>M. synoviae</td>
<td>Contact</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>4C</td>
<td>M. synoviae</td>
<td>Aerosol</td>
<td>21/21</td>
<td>21/21</td>
</tr>
<tr>
<td>5D</td>
<td>M. synoviae</td>
<td>Aerosol</td>
<td>13/13</td>
<td>13/13</td>
</tr>
<tr>
<td>6D</td>
<td>M. synoviae</td>
<td>Natural</td>
<td>1/24</td>
<td>0/24</td>
</tr>
<tr>
<td>7</td>
<td>M. gallisepticum</td>
<td>Artificial</td>
<td>0/25</td>
<td>0/25</td>
</tr>
<tr>
<td>8E</td>
<td>M. gallisepticum</td>
<td>Artificial</td>
<td>1/24</td>
<td>1/24</td>
</tr>
<tr>
<td>9F</td>
<td>M. gallisepticum</td>
<td>Natural</td>
<td>0/30</td>
<td>0/30</td>
</tr>
</tbody>
</table>

*Groups 1 and 2 were the same birds, with serum samples obtained 10 and 15 weeks post-inoculation with M. synoviae into foot pad or hock joint.

*Serum samples from birds that had IM or IV inoculations weekly or twice weekly for 6 weeks with M. synoviae egg yolk or standard antigen and bled from 1 week to 3 weeks post-injection.

*Serum samples obtained 15 weeks post-contact with M. synoviae-inoculated birds.

*Groups 5 and 6 were the same birds with serum samples obtained 5 and 8 weeks post-aerosol-inoculation with M. synoviae egg yolk.

*Serum samples obtained 11 weeks postinoculation with M. gallisepticum into air sacs.

*Only 14 of 24 serum samples were tested with the HA antigens, but the 24 were 4+ reactors to M.G. serum plate test, and all were negative to the MS serum plate antigen.

and incubated at 37 C. As soon as the color changed from red to slight orange, 60 ml of the culture was inoculated into 1,000 ml of medium and incubated at 37 C. The antigen was harvested when the pH of the culture was between 6.0 and 6.5. The culture was centrifuged at 5 C for 2 hours at 2,000 rpm in a Model PR-2, or at 5 C for 1 hour at 10,000 rpm in a Model HR-1, International Equipment Company. The sediment was resuspended in 0.25% phenolized phosphate-buffered saline (PPBS), pH 7.1-7.2, and ground in a glass tissue grinder. The phenolized phosphate-buffered saline is made by mixing 8.5 g sodium chloride (C.P.), 0.68 g potassium dihydrogen phosphate (C.P.), 2.5 g phenol (Crystal) (C.P.), distilled water to make 1,000 ml, and enough sodium hydroxide (C.P.) to bring the solution to pH 7.1-7.2. The antigen was washed 3 times in PPBS and was standardized by packed cell volume (PCV). The PCV should be 0.05 ± 0.01 ml when 5.0 ml of the standard antigen is placed in a Hopkins tube and centrifuged in a PR-2 International centrifuge at 2,000 rpm for 90 minutes. The yield of the standard antigen usually runs between 2.0 and 3.0% of the volume of culture medium. For the tube test, 1 ml of the standard antigen was mixed with 19 ml of PPBS. The tube agglutination test was carried out with .08, .04, .02, .01, and .005 ml of each individual serum to which was added 1 ml antigen, re-
Preparing mycoplasma antigen for tube agglutination

resulting in respective serum dilutions of 1:12.5, 1:25, 1:50, 1:100, and 1:200. Tubes of 12 × 75 mm were used. The tubes were incubated at 37 C, and the test was read after 24 hours of incubation. A positive agglutination test was evidenced by a clearing of the fluid in the upper portion of the tube and an accumulation of antigen-antibody aggregates of cells at the bottom of the tube. The test was considered negative if the liquid in the tube appeared cloudy or milky and there was no flocculation at the bottom of the tube. Each tube was recorded as either negative, 1+, 2+, 3+, or 4+ reactors. In this test, only 4+ reactors are reported as positive for that particular serum dilution.

The experimental M. synoviae tube antigen and Salsbury's M. gallisepticum tube antigen serial No. 867 were tested on serums from chickens that had been infected by: 1) injecting isolate WVU 1853 (Olson) or ISU 1331 (Yoder) M. synoviae egg-yolk material into the hock joint or foot pad; 2) aerosolization with M. synoviae egg yolks; 3) contact with birds infected with M. synoviae; 4) injecting M. gallisepticum into air sac, foot pad, or hock joint; 5) natural infection; and 6) normal birds. The serums were confirmed by HI test as being positive or negative to M. synoviae and M. gallisepticum.

RESULTS AND DISCUSSION

Table 1 shows the serological results of the M. synoviae and M. gallisepticum tube agglutination and hemagglutination-inhibition tests on the various groups of serums tested. Serums from birds exposed to M. synoviae agglutinated M. synoviae tube antigen, and serums from birds exposed to M. gallisepticum agglutinated Salsbury's M. gallisepticum tube antigen No. 857. There were some cross-agglutination reactions to both antigens, but only in the serum dilutions of 1:12.5 and 1:25.

Four of the 13 M. synoviae serum samples in Group 3 cross-reacted with M. gallisepticum tube antigen. This was probably due to the excessive number of injections of M. synoviae, the method of injection, and not waiting long enough after the last injections to collect the serums. Roberts and Olesiuk (6) reported that birds inoculated via foot pad with M. synoviae, E. rhusiopathiae bacterin, and avian mycoplasma serotype P would cause reactions to the M. gallisepticum serum plate test, and stated that this was due to a rheumatoid-factor activity in the serum. Antibodies to the serum plate test and the tube agglutination test are the same antibodies.
All of the M. synoviae serums in Group 1-7 gave a 4+ tube agglutination reaction at a dilution of 1:25 or above except that 1 serum in Group 7 reacted at a 1:12.5 dilution. All of the M. gallisepticum serums in Groups 8-10 gave a 4+ tube agglutination reaction at a dilution of 1:25 or above, except that 1 serum in Group 10 reacted at 1:12.5 dilution. If an agglutination at a dilution of 1:25 or above were considered positive, there would have been 4 M. synoviae serums of the 187 tested that cross-reacted with the M. gallisepticum tube antigen; one of the 60 M. gallisepticum serums would have cross-reacted with the M. synoviae tube antigen; and none of the normal serums would have reacted to either antigen. This is not shown in the table.

Serum samples that are hemolyzed, cloudy, or contaminated make accurate readings difficult when .08 ml of serum is used. When .08 ml of positive hemolyzed serum is used, one may not see the clearing of fluid in the tube and might report it as negative. If a negative serum sample is contaminated with organisms, one might read it as positive because the organisms settle at the bottom of the tube. A 1:25 dilution is much easier to read, and it takes half as much serum. The authors believe that diagnostic laboratories could use a 1:25 dilution as a screening test and check each serum against M. synoviae and M. gallisepticum tube antigens. If the flock were infected, there would probably be many reactors at this dilution.

REFERENCES

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