Comparison of Two Plating Media for the Isolation of *Erysipelothrix rhusiopathiae* from Enrichment Broth Culture

RUBE HARRINGTON, JR., AND D. C. HULSE

Diagnostic Bacteriology Section, Animal Health Division, National Animal Disease Laboratory, Ames, Iowa 50010

Received for publication 5 April 1971

Sodium azide-crystal violet agar and a modified blood-azide (MBA)-agar were used to isolate *Erysipelothrix rhusiopathiae* from swine tissues. There was no significant difference in the number of isolations. However, 78% of the isolants from modified blood-azide medium required only 24 hr of incubation, whereas all of the isolants obtained on sodium azide-crystal violet medium required 48 hr.

Sodium azide-crystal violet (Packer's medium, SACV) medium is frequently used for the isolation of *Erysipelothrix rhusiopathiae* from specimens (1–3). The time required for growth on this medium (48 hr) is a disadvantage in cultural examination of swine tissue for *E. rhusiopathiae*. A selective plating medium allowing faster growth of *E. rhusiopathiae* is desirable in a diagnostic laboratory.

Modified blood-azide (MBA) has been used in this laboratory as part of a method for the isolation of *E. rhusiopathiae* from swine tissues. The medium was prepared by dissolving 40 g of Heart Infusion agar (Difco) and 0.4 g of sodium azide in 1,000 ml of distilled water. After sterilizing at 121 C for 15 min, the medium was cooled and 20 ml of defibrinated bovine blood and 50 ml of horse serum were added aseptically.

A study was conducted to compare the efficiency of SACV and MBA plating media for the isolation of *E. rhusiopathiae* from enrichment broth cultures.

The two media were compared during the course of bacteriological examinations of 638 swine tissues. The tissues were submitted to this laboratory by state and Animal Health Division veterinarians for differential diagnosis of hog cholera. Approximately 95% of the specimens were spleens; the remaining portion included kidney, liver, lung, and tonsil.

Approximately 1 g of swine tissue, consisting of small pieces, was placed in 10 ml of liquid selective medium (3, 4). After overnight incubation at 37 C, 5 ml of the liquid portion was removed, placed into a sterile test tube, and centrifuged for 20 min at approximately 1,400 × g. The supernatant liquid was discarded. Physiological saline solution (0.85%) was used to resuspend sediment to the density of McFarland standard no. 4. A loopful of the bacterial suspension was streaked on SACV and MBA agar plates. Colonies with morphological characteristics of *E. rhusiopathiae* appearing on SACV and MBA plates after 24 to 48 hr of incubation were selected and transferred to blood-agar slants. After overnight incubation at 37 C, a smear of the growth on the blood-agar slant was Gram stained and examined microscopically. Biochemical tests were conducted by methods previously described (4).

**Table 1. Comparison of modified blood-azide (MBA) and sodium azide-crystal violet (SACV) media for the isolation of *Erysipelothrix rhusiopathiae* from 638 swine tissues**

<table>
<thead>
<tr>
<th>Medium</th>
<th>No. positive at</th>
<th>No. negative at</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 hr</td>
<td>48 hr</td>
</tr>
<tr>
<td>MBA</td>
<td>126</td>
<td>161</td>
</tr>
<tr>
<td>SACV</td>
<td>0</td>
<td>160</td>
</tr>
</tbody>
</table>

The results of comparisons between the two plating media are presented in Table 1. Of the 638 tissues examined, 161 isolations were made from MBA medium and 160 were made from SACV medium. Statistical analysis of this data indicated that there was no significant difference between the two media for the isolation of *E. rhusiopathiae* from broth cultures (P < 0.01). However, 78% of the isolants from MBA medium were recovered after 24 hr of incubation, whereas 48 hr of incubation was required for all isolants on SACV medium. The more rapid isolation of
E. rhusiopathiae on MBA medium makes possible the reporting of cultural results within 96 hr.

We thank Mariam Fetters for technical assistance and C. K. Graham for statistical analysis.

LITERATURE CITED