Selective Medium for Growth of Rhizobium

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A new medium has been developed for selectively isolating strains of Rhizobium from soil.

Though media exist for the selective isolation of agrobacteria (5), there is at present no satisfactory means for isolating rhizobia directly from soil (4). This causes considerable difficulty in soil competition studies (J. M. Ireland, M. Sc. Thesis, Univ. of Sydney, Sydney, NSW) and in assessing soil populations of Rhizobium. For these reasons and because I am currently interested in the isolation and testing of indigenous Indian Leguminosae and their rhizobia, a medium selective for strains of Rhizobium has been developed.

The medium, yeast mannitol antibiotic agar (YMAA), has the following composition (grams/liter): mannitol, 5.0; lactose, 5.0; K2HPO4, 0.5; NaCl, 0.2; CaCl2·2H2O, 0.2; MgSO4·7H2O, 0.1; FeCl3·6H2O, 0.1; yeast extract, 0.5; agar, 20.0; water, 1,000 liters. After autoclaving the above ingredients, cycloheximide (200 mg), pentachloronitrobenzene (100 mg), sodium benzyl penicillin (25 mg), chloromycetin (10 mg), sulfathiazole (25 mg), and neomycin (2.5 mg) are added. Congo red (2.5 ml of a 1% solution) may also be added if desired. The pH is then adjusted to 7.0 and the medium is poured.

Strains of fast-growing rhizobia incubated on YMAA for 3 to 5 days at 28 C produce smooth, white, glistening colonies 1 to 3 mm in diameter. Colonies produced by the slow-growing rhizobia are always less than 1 mm in diameter, but are also white, glistening, and raised. Of the 52 Rhizobium strains tested on this medium, all but three have grown well. By contrast, none of the limited number of Agrobacterium strains available grew satisfactorily on the medium.

When soil suspensions or nonsterile nodules are streaked directly onto petri plates containing YMAA, colony growth is usually evident in 2 to 3 days. More than 99% of the colonies obtained are rhizobial in appearance and properties, with readily differentiated pseudomonads as infrequent contaminants. Identification of isolated rhizobia

by the conventional plant test can pose a problem; however, when samples are taken from swards with one legume dominant, a high proportion of the isolates will infect that host. As a routine precaution, we apply the ketolactase test of Bernaerts and de Ley (3) to all organisms isolated by this method. So far, no strains of Agrobacterium have been encountered.

Direct qualitative recovery of some strains from soil is also possible with this medium, the results obtained being more than comparable with those obtained for Agrobacterium by Schroth et al (5; Table 1). Counts taken directly from soils growing leguminous species indicate rhizobial populations as high as 3.84 × 109/g.

Albizo and Surgalla (1, 2) used a selective medium containing antiserum to isolate and identify fraction 1-positive Pasteurella pestis. If specific antirhizobial serum is incorporated into the YMAA medium, it could also be used to selectively identify and quantify rhizobial strains from soil. This would be most valuable in studying strain persistence and competition. Provided the strains used in such studies are carefully selected, this method is simpler and more rapid than existing ways of assessing soil rhizobia. The medium also has considerable usefulness in the isolation of rhizobiphage and suitable host rhizobia directly from soil.

TABLE 1. Recovery of Rhizobium strains from soil

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. added to soil</th>
<th>No. recovered</th>
<th>Recovery %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizobium meliloti strain 216</td>
<td>8 × 10⁸/g</td>
<td>3 × 10⁸/g</td>
<td>37.5</td>
</tr>
<tr>
<td>R. leguminosarum strain P14</td>
<td>49 × 10⁹/g</td>
<td>36 × 10⁸/g</td>
<td>73.4</td>
</tr>
<tr>
<td>Rhizobium sp. Groundnut strain 1</td>
<td>16 × 10⁹/g</td>
<td>15 × 10⁸/g</td>
<td>93.7</td>
</tr>
<tr>
<td>Peuraria strain 1</td>
<td>12 × 10⁹/g</td>
<td>88 × 10⁸/g</td>
<td>73.3</td>
</tr>
</tbody>
</table>

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LITERATURE CITED