11-1-1967

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Synthetic and Complex Media for the Rapid Detection of Fluorescence of Phytopathogenic Pseudomonads: Effect of the Carbon Source

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Received for publication 10 July 1967


<table>
<thead>
<tr>
<th>Species</th>
<th>NA&lt;sub&gt;GL&lt;/sub&gt;</th>
<th>NA&lt;sub&gt;AG&lt;/sub&gt;</th>
<th>NA&lt;sub&gt;M&lt;/sub&gt;</th>
<th>NA</th>
<th>N&lt;sub&gt;GL&lt;/sub&gt;</th>
<th>N&lt;sub&gt;G&lt;/sub&gt;</th>
<th>N&lt;sub&gt;M&lt;/sub&gt;</th>
<th>A&lt;sub&gt;G&lt;/sub&gt;</th>
<th>NBY&lt;sup&gt;e&lt;/sup&gt;</th>
<th>B&lt;sub&gt;GL&lt;/sub&gt;&lt;sup&gt;d&lt;/sup&gt;</th>
<th>B&lt;sub&gt;G&lt;/sub&gt;</th>
<th>B&lt;sub&gt;M&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. phaseolicola (22 strains)</td>
<td>++++++</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>+++++++</td>
<td>0</td>
<td>++++</td>
<td>++++</td>
<td>0-+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>P. syringae (5 strains)</td>
<td>+++++</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>+++++</td>
<td>0</td>
<td>++++</td>
<td>++++</td>
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<td>++</td>
<td></td>
</tr>
<tr>
<td>P. tabaci (1 strain)</td>
<td>+++++</td>
<td>0</td>
<td>0</td>
<td>NA</td>
<td>0</td>
<td>+</td>
<td>0</td>
<td>+</td>
<td>++</td>
<td>++++</td>
<td>0-+</td>
<td></td>
</tr>
<tr>
<td>P. fluorescens (3 strains)</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+++</td>
<td>0</td>
<td>++++</td>
<td>+++++</td>
<td>++++</td>
<td>++</td>
<td></td>
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<tr>
<td>P. aeruginosa (1 strain)</td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>NA</td>
<td>+</td>
<td>+++</td>
<td>0</td>
<td>++++</td>
<td>+++++</td>
<td>++++</td>
<td>++</td>
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</tbody>
</table>

<sup>a</sup> Average of two to four experiments. Growth was good to excellent on all media except N<sub>M</sub>, NA, and N<sub>AM</sub>.

<sup>b</sup> All species produced blue fluorescent pigment on NA, N, and A media, irrespective of the carbon source.

<sup>c</sup> All species produced blue-green or blue fluorescent pigment.

<sup>d</sup> Medium B of King et al.; blue-green or green fluorescent pigment was produced, irrespective of the carbon source.

<sup>e</sup> Only 14 strains tested.


<sup>1</sup> Published as paper no. 2133 Journal Series, University of Nebraska Agricultural Experiment Station, Lincoln.

All synthetic media contained, in grams per liter: MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2; KH<sub>2</sub>PO<sub>4</sub>, 3.0; Na<sub>2</sub>HPO<sub>4</sub>, 6.0, and glucose, glycerol, or maltose, 5.0. The sugars and glycerol were autoclaved separately as 10% (w/v or v/v) solutions, and added aseptically. Medium A had L-asparagine (Nutritional Biochemicals Corp., Cleveland, Ohio), 0.5 g per liter, as nitrogen source; medium NA had 0.5 g
of l-asparagine per liter plus 1 g of NH₄Cl per liter; and medium N had 1 g of NH₄Cl per liter. The subscripts, G, M, or Gly indicate the carbon source as glucose, maltose, or glycerol, respectively.

NBY medium contained, in grams per liter: Nutrient Broth (Difco), 8.0; yeast extract (Difco), 2.0; K₂HPO₄, 2.0; KH₂PO₄, 0.5; glucose, 5.0; and MgSO₄·7H₂O, 0.25. Glucose (10%, w/v) and MgSO₄ (1 M) were autoclaved separately and added aseptically.

Medium B was prepared from the constituents, with glucose, maltose, or glycerol as carbon source. Commercial medium B (Difco Pseudomonas Agar F) was also used ( = Bₓ).

Solid media contained agar (Difco) at 15 g per liter.

Both NBY and Medium B have a considerable amount of blue autofluorescence; media N, NA, and A do not.

Fluorescence under ultraviolet light was determined daily on cultures incubated at 24 to 26 C. For inocula, distilled water suspensions of cultures grown for 24 to 48 hr on NBY, Medium B (Difco), or NAₓ were used.

The results in Table 1 show that all tested phytopathogens previously classified as fluorescent, and Pseudomonas fluorescens and P. aeruginosa, showed intense fluorescence on synthetic agar media with glucose or glycerol as carbon source, and on complex agar media with glycerol. Maltose in synthetic and complex media was poorest for fluorescence of the phytopathogens, but not for P. fluorescens and P. aeruginosa in complex media. Development of fluorescence on synthetic media with galactose occurred, but was slower than with glycerol or glucose.

Fluorescence, if detectable, generally was seen by 24 hr.

Corynebacterium flaccumfaciens var. aurantiacum, 2-A, Escherichia coli B, K-12, Proteus vulgaris PV-1, P. solanacearum K30, and Xanthomonas phaseoli XP4, XP104 Smᵣ, and XP104W showed no fluorescence on any media. C. flaccumfaciens ATCC 6887, Agrobacterium tumefaciens AT-1, E. coli C, and X. phaseoli XP6022, XPS, and K-4 showed weak (+) fluorescence on Bₓ and Bₓ₁, after a week or longer.

Efforts to use liquid synthetic media for fluorescence studies led to variable results.

R. Y. Stanier et al. (J. Gen. Microbiol. 43:159, 1966) have shown that maltose is rarely metabolized by fluorescent pseudomonads. This may explain the equivalence of media differing only in maltose (NA and NAₓ). Fluorescence on Bₓ is probably not due to the presence of maltose.

This report shows that fluorescence of phytopathogenic pseudomonads can be detected rapidly and readily on certain complex and synthetic media, but not on the commercial medium for this purpose. Carbon sources affect fluorescence of phytopathogenic pseudomonads, but have little or no effect on fluorescence of P. fluorescens and P. aeruginosa.

This investigation was supported by grant GB5470 from the National Science Foundation.

It is a pleasure to acknowledge the technical assistance of Mary Lou Mathys.