New Selective Agent for Isolation of *Pseudomonas aeruginosa*

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Results of minimal inhibitory concentration tests with a diversity of bacterial strains showed that 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridian (C-390) inhibited the growth of all microorganisms tested (other than *Pseudomonas aeruginosa*) at 25 μg/ml or less, whereas MICs obtained for *P. aeruginosa* ranged from 50 to >100 μg/ml. Therefore, C-390 was evaluated as a potential selective agent for *P. aeruginosa* in pseudomonas agar F. Recovery tests were conducted on this medium with 53 strains of *P. aeruginosa*, and the results were compared to those obtained in similar tests on commercially available selective media, i.e., pseudomonas isolation agar and Pseudosel agar. The results of these comparisons indicated that pseudomonas agar F with C-390 was significantly less inhibitory than Pseudosel agar and pseudomonas isolation agar and more selective than pseudomonas isolation agar. The incorporation of C-390 in pseudomonas agar F also provided a medium that was both selective and differential. Preliminary evidence also suggested that C-390 may be added to other basal media with comparable results.

A cetrimide agar formulation is currently recommended in the microbial limit test of the United States Pharmacopeia XX (7) as a selective medium for isolating *Pseudomonas aeruginosa*. However, this medium and pseudomonas isolation agar (PIA) are reported to be either inhibitory or lacking in selective specificity or both for *P. aeruginosa* (1, 4, 6). Consequently, a less inhibitory medium than either of these standard media was sought for isolating relatively low numbers of *P. aeruginosa*. Several compounds were selected for preliminary tests from computer-compiled lists of compounds with appropriate minimal inhibitory concentration data profiles. From results of these tests, 9-chloro-9-(4-diethylaminophenyl)-10-phenylacridian (C-390) was chosen for further evaluation of its antibacterial activity against a broad spectrum of microorganisms. The selective specificity of an agar medium containing C-390 was evaluated by a spread-plate colony count procedure with stock cultures of *P. aeruginosa*, other pseudomonads, and certain enteric microorganisms previously found to be least inhibited by C-390 in minimal inhibitory concentration tests. Results obtained after enumeration of the recovered colonies were compared to those obtained with a reference standard control medium.

**MATERIALS AND METHODS**

**Bacteria.** The microorganisms (76 in all) tested to determine the antibacterial activity (MICs) of C-390 were obtained from our culture collection (Table 1). The strains used in experiments to determine the selectivity and to challenge the selective specificity of the C-390 medium were also from the culture collection. The 53 strains of *P. aeruginosa* (Table 2) included 5 strains from the American Type Culture Collection, Rockville, Md., and 38 recent clinical isolates. Sixteen pseudomonads tested, other than *P. aeruginosa*, are listed in Table 3. For a list of the 16 enteric microorganisms tested, refer to Table 4.

**Chemical.** C-390 was originally provided by Morton Chemical Co., Chicago, Ill. Additional compound was also synthesized and provided by Norwich-Eaton Pharmaceuticals, Norwich, N.Y.

**Media.** Pseudomonas agar F (PAF), PIA, and brain heart infusion (BHI) were obtained from Difco Laboratories, Detroit, Mich. Pseudosel agar (CET), Trypticase soy agar (TSA) and Trypticase soy broth were obtained from BBL, Cockeysville, Md. All media were prepared according to the directions of the manufacturers.

**Detection of antibacterial activity.** A twofold serial dilution method (2) was used to determine the antibacterial activity of C-390 in BHI.

**Enumeration of test microorganisms.** The recovery of test microorganisms was determined by adding C-390 (30 mg) to sufficient dehydrated PAF required to prepare a liter. This mixture was reconsti-
Results of 76 single MIC tests (Table 1) showed that C-390 at 25 μg/ml or less inhibited the growth of all microorganisms tested except *P. aeruginosa*. The MICs of C-390 for *P. aeruginosa* ranged from 50 to 100 μg/ml.

Average yields of *P. aeruginosa* on PAF with C-390 are shown in Table 2. All but three of the strains (Ps-98, Ps-126, and Ps-149) yielded higher average colony counts on PAF with C-390 than on PIA. All strains yielded higher average counts on PAF with C-390 than on CET. However, in most instances average counts were higher on the nonselective control medium TSA than on any other medium tested.

Statistical analysis of the results indicated that PAF with C-390 was not significantly different (*P > 0.05*) in the number of *P. aeruginosa* recovered from the nonselective control. However, PAF with C-390 was significantly less inhibitory (*P < 0.001*) than either CET or PIA (3, 5).

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>No. of strains</th>
<th>MIC (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>2</td>
<td>0.024-3.1</td>
</tr>
<tr>
<td>Alcaligenes faecalis</td>
<td>1</td>
<td>6.2</td>
</tr>
<tr>
<td>Bacillus spp.</td>
<td>2</td>
<td>0.024-0.048</td>
</tr>
<tr>
<td>Brevisbacterium ammonigenes</td>
<td>2</td>
<td>0.012</td>
</tr>
<tr>
<td>Corynebacterium xerosis</td>
<td>1</td>
<td>0.38</td>
</tr>
<tr>
<td>Enterobacter spp.</td>
<td>6</td>
<td>6.2-25.6</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>5</td>
<td>0.19-3.1</td>
</tr>
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<td>Haemophilus vaginalis†</td>
<td>1</td>
<td>0.024</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>2</td>
<td>6.2-12.5</td>
</tr>
<tr>
<td>Propionibacterium acnes</td>
<td>1</td>
<td>0.048</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>13</td>
<td>0.75-3.1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>13</td>
<td>0.75-100</td>
</tr>
<tr>
<td>Pseudomonas diminuta</td>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>Salmonella spp.</td>
<td>6</td>
<td>0.75-12.5</td>
</tr>
<tr>
<td>Serratia marcescens</td>
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<td>3.1-6.2</td>
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<td>Shigella spp.</td>
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<td>Staphylococcus spp.</td>
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<td>0.003-0.75</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
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Table 1. Minimal inhibitory concentration (MIC) of C-390 against a spectrum of microorganisms*

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<tr>
<th>EATON code</th>
<th>PAF with C-390 at 30 μg/ml</th>
<th>PIA</th>
<th>CET</th>
<th>TSA</th>
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<td>151</td>
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<td>242</td>
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<td>Ps-26</td>
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<td>181</td>
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</table>
Recovery of other pseudomonads on this experimental medium and two commercially available selective media is shown in Table 3. Of the pseudomonads other than P. aeruginosa that were tested, none were recovered on PAF with C-390; three of four strains of Pseudomonas fluorescens and a Pseudomonas putida-like organism were recovered on PIA. One of four strains of Pseudomonas maltophilia grew on PIA, and one of four isolates of Pseudomonas fluorescens was recovered on CET. All the pseudomonads grew on TSA.

Results of experiments with enteric organisms on the same media are presented in Table 4. None of the test microorganisms grew on either PAF with C-390 or CET. Four of five strains of Serratia marcescens grew on PIA, but none of the other test microorganisms grew on this medium. Both P. aeruginosa controls were recovered on PAF with C-390 and on PIA, whereas neither of these microorganisms was recovered on CET. All test and control microorganisms grew on the nonselective control medium TSA.

**DISCUSSION**

C-390 has shown broad spectrum activity against all the microorganisms (other than P. aeruginosa) tested. These results suggested the utility of C-390 as a selective agent for the isolation of P. aeruginosa. Our conclusions were in accord with those in earlier reports regarding the inhibitory or nonspecific selectivity or both of cetrimide agar and PIA for the isolation of P. aeruginosa (1, 4, 6). Results of preliminary tests (data not shown) for the recovery of P. aeruginosa suggested that PAF with C-390 was superior to pseudomonas selective medium (Oxoid Canada Ltd., Ottawa, Ontario, Canada), PAF with Pseudo-Select reagent (Intechmark Corp., Palo Alto, Calif.) at 0.5% (vol/vol), and cetrimide agar (Difco). Results of limited testing of C-390 at 30 μg/ml in other basal media, including Sellers differential agar (Difco) with dextrose at 0.75%, Tech agar and Flo agar (BBL), have shown that all of these media appeared comparable (and in the case of Sellers, possibly superior) to PAF with C-390 in the recovery of P. aeruginosa. However, incorporation of C-390 at 30 μg/ml in TSA and nutrient agar (BBL) and in brain heart infusion agar, MacConkey agar, and Mueller-Hinton medium (Difco) resulted in selective media which appeared less satisfactory than PAF with C-390.

In addition, PAF with C-390 completely in-
### Table 4. Selectivity of PAF with C-390 against enteric microorganisms

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Eaton Code</th>
<th>Avg no. of colonies per plate on the following media*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PAF with C-390 at 30 µg/ml</td>
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<tr>
<td><em>Enterobacter aerogenes</em></td>
<td>Ae-54</td>
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<td>Ae-67b</td>
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<td>Ae-62</td>
<td>0</td>
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<tr>
<td></td>
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<td></td>
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<tr>
<td><em>Klebsiella pneumoniae</em></td>
<td>Kl-23</td>
<td>0</td>
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<tr>
<td><em>Salmonella cholerae-suis</em></td>
<td>SaC-184c</td>
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<td><em>S. pullorum</em></td>
<td>SaD-91</td>
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<td><em>S. schottmuelleri</em></td>
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<td><em>Serratia marcescens</em></td>
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<td></td>
<td>Se-4e</td>
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<td></td>
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<td>23</td>
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<td></td>
<td>Ps-136</td>
<td>70</td>
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</table>

* See footnotes a and b to Table 2.

** ATCC 13048.

*b ATCC 10708.

*b ATCC 10719.

* ATCC 8195.

* ATCC 9721.

* See footnotes a and b to Table 2.

** ATCC 13048.

*b ATCC 10708.

*b ATCC 10719.

* ATCC 8195.

*b ATCC 9721.

hibited the growth of the other pseudomonads and enteric microorganisms tested, whereas PIA and CET did not, thus indicating a potentially greater selective specificity of this experimental medium for *P. aeruginosa* than either PIA or CET. Further experimentation with additional strains would be required for confirmation. Exploratory studies (data not shown) with artificial mixtures containing *Enterobacter cloacae* and *P. aeruginosa* indicate that PAF with C-390 may be useful for isolating *P. aeruginosa* from natural samples of mixed microflora.

C-390 is water soluble, stable to autoclaving, and does not interfere with the oxidase test reaction or fluorescence of *P. aeruginosa*.

** ACKNOWLEDGMENTS**

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**LITERATURE CITED**


