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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-phenylacridan (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-phenylacridan (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-

TABLE 1. *Minimal inhibitory concentration (MIC) of C-390 against a spectrum of microorganisms*[a]

Microorganism	No. of strains	MIC (μg/ml)
Acinetobacter spp.	2	0.024–3.1
Alcaligenes faecalis	1	6.2
Bacillus spp.	2	0.024–0.048
Brevibacterium ammoniagenes	2	0.012
Corynebacterium xerosis	1	0.38
Enterobacter spp.	8	6.2–25.0
Escherichia coli	5	0.19–3.1
Haemophilus vaginalis[b]	1	0.024
Klebsiella pneumoniae	2	6.2–12.5
Propionibacterium acnes	1	0.048
Proteus spp.	13	0.75–3.1
Pseudomonas aeruginosa	13	50–>100
Pseudomonas diminuta	1	0.75
Salmonella spp.	6	0.75–12.5
Serratia marcescens	2	3.1–6.2
Shigella spp.	2	0.38
Staphylococcus spp.	11	0.003–0.75
Streptococcus spp.	3	0.19–0.38

[a] Single determinations by a serial dilution method in brain heart infusion.

[b] Brain heart infusion supplemented with calf serum at 10% and agar at 0.1%.

tuted with water and autoclaved. Plates of PAF with C-390 and of CET, PIA, and TSA (20 ml per plate) were prepared. The addition of C-390 to PAF resulted in a light blue medium.

All test microorganisms were grown for 24 h at 35 ± 1°C in Trypticase soy broth, and serial dilutions of the cultures were prepared with tubes of sterile distilled water. Plates of the above media were inoculated in duplicate with 0.1-ml samples from either the 10^{-5} or 10^{-6} dilutions (depending on growth). All plates were incubated for 48 h at 35 ± 1°C before colonies were counted and average yields were calculated per plate.

RESULTS

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 2. *Recovery of 53 P. aeruginosa strains on PAF with C-390*

Eaton code	Avg no. of colonies per plate[a] on the following media[b]: PAF with C-390 at 30 μg/ml	PIA	CET	TSA
Ps-10	196	151	0	242
Ps-26[c]	23	1	0	37
Ps-44	191	108	7	295
Ps-61	341	279	1	275
Ps-82[d]	69	26	0	70
Ps-86	135	103	0	196
Ps-87	99	44	0	143
Ps-88	60	35	0	179
Ps-89[e]	186	49	0	197
Ps-90	42	33	0	120
Ps-91	7	2	0	34
Ps-92	205	120	27	183
Ps-93	313	184	0	269
Ps-96[f]	405	326	0	435
Ps-97	127	89	0	122
Ps-98	127	147	8	93
Ps-99	94	79	3	89
Ps-100	73	12	0	74
Ps-101	134	118	5	221
Ps-102	26	20	1	31
Ps-103	82	49	0	391
Ps-104	76	74	31	71
Ps-105	124	94	2	106
Ps-107	119	105	5	127
Ps-108	83	62	2	54
Ps-109	125	111	64	111
Ps-110	132	83	0	142
Ps-111	94	45	4	78
Ps-112	128	61	0	366
Ps-113	43	22	3	56
Ps-114	34	33	0	61
Ps-115	41	9	0	42
Ps-116	29	12	0	37
Ps-117	148	26	0	130
Ps-119	123	26	20	128
Ps-121	33	9	0	40
Ps-122	24	0	0	57
Ps-126	71	163	1	307
Ps-127	124	13	0	140
Ps-128	168	99	0	202
Ps-129	94	73	2	138
Ps-134	100	35	0	102
Ps-135	463	387	5	539
Ps-136	70	13	0	167
Ps-137	162	105	4	130
Ps-138	103	57	19	89
Ps-139	106	99	27	105
Ps-140	112	43	0	137
Ps-141	108	80	0	106
Ps-142	164	64	26	149
Ps-144	165	148	72	153
Ps-147	138	101	2	163
Ps-149[g]	152	170	121	181

[a] Calculated from results of test determinations performed in duplicate.

[b] PAF, PIA, and CET were selective media; TSA was the nonselective control medium.

[c] ATCC 9721.

[d] ATCC 15442.

[e] ATCC 13388.

[f] ATCC 27835.

[g] ATCC 10145.

TABLE 3. *Selectivity of PAF with C-390 against pseudomonads other than P. aeruginosa*

Microorganism	Eaton code	Avg no. of colonies per plate on the following media[a]:			
		PAF with C-390 at 30 μg/ml	PIA	CET	TSA
Pseudomonas alcaligenes	Ps-106	0	0	0	46
P. diminuta	Ps-94[b]	0	0	0	319
P. fluorescens	Ps-83[c, d]	0	74	0	98
	Ps-118[d]	0	0	0	78
	Ps-123[d]	0	66	0	73
	Ps-143[d]	0	52	2	79
P. putida	Ps-124[d]	0	0	0	128
P. maltophilia	Ps-120	0	0	0	145
	Ps-125	0	0	0	51
	Ps-132	0	1	0	328
	Ps-145	0	0	0	192
P. mendocina	Ps-130	0	0	0	112
P. putida-like	Ps-148	0	83	0	130
P. stutzeri	Ps-133	0	0	0	31
	Ps-146	0	0	0	300
P. syringae	Ps-131	0	0	0	420

[a] See footnotes *a* and *b* to Table 2.
[b] ATCC 19146.
[c] ATCC 17397.
[d] Incubation at 30°C.

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*

Microorganism	Eaton Code	Avg no. of colonies per plate on the following media[a]: PAF with C-390 at 30 μg/ml	PIA	CET	TSA
Enterobacter aerogenes	Ae-54	0	0	0	130
	Ae-63	0	0	0	108
	Ae-67[b]	0	0	0	379
E. cloacae	Ae-8	0	0	0	231
	Ae-62	0	0	0	189
	Ae-65	0	0	0	78
	Ae-66	0	0	0	175
Klebsiella pneumoniae	Kl-23	0	0	0	127
Salmonella cholerae-suis	SaC-184[c]	0	0	0	108
S. pullorum	SaD-91	0	0	0	160
S. schottmuelleri	SaB-205[d]	0	0	0	169
Serratia marcescens	Se-2	0	0	0	153
	Se-4[e]	0	2	0	319
	Se-6	0	11	0	176
	Se-9	0	27	0	326
	Se-13	0	6	0	283
P. aeruginosa (control)	Ps-26[f]	23	1	0	37
	Ps-136	70	13	0	167

[a] See footnotes *a* and *b* to Table 2.
[b] ATCC 13048.
[c] ATCC 10708.
[d] ATCC 10719.
[e] ATCC 8195.
[f] 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*.

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