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Evaluation of Pseudosel Agar as an Aid in the Identification of *Pseudomonas aeruginosa*

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Growth of *Pseudomonas aeruginosa* and thirty-five other species of gram-negative bacilli was observed on 0.03% cetrimide in heart infusion agar medium and Pseudosel agar (BBL). The 0.03% cetrimide agar was more selective for growth of *P. aeruginosa* than was Pseudosel agar; however, certain bacteria other than *P. aeruginosa* also grew on the former medium. Although Pseudosel agar was not a highly selective medium for *P. aeruginosa*, it was preferable to technicolor agar for detection of the pyocyanin and pyorubin pigments produced by *P. aeruginosa*.

Cetrimide agar and Pseudosel agar (BBL) were devised to serve as selective media for the isolation and identification of *Pseudomonas aeruginosa* while supposedly inhibiting the growth of other microorganisms.

The use of 0.1% cetrimide agar as a selective medium for *P. aeruginosa* was first described by Lowbury (9), and it was later modified to 0.3% cetrimide by Lowbury and Collins (10). Brown and Lowbury (3) employed 0.03% cetrimide in King's medium B, and they reported that certain organisms other than *P. aeruginosa* also grew on this medium. Although 0.03% cetrimide in King's medium B was recommended by these authors, 0.09% cetrimide in heart infusion agar is used in the Center for Disease Control formulation for cetrimide agar (1, 12). A commercially available, cetrimide-containing agar, Pseudosel agar, contains 0.03% cetrimide and is recommended as a selective medium for the isolation and identification of *P. aeruginosa* (2). Furthermore, Pseudosel agar reportedly stimulates the production of pyocyanin and pyorubin pigments which aid in the identification of *P. aeruginosa*.

For the past 3 years in this laboratory, 0.09% cetrimide agar in heart infusion agar base and Pseudosel agar have been used to test for growth, or no growth, as a possible criterion in the identification of *P. aeruginosa* and other microorganisms. It was noted early that certain strains other than *P. aeruginosa* grew on 0.09% cetrimide agar and on Pseudosel agar but that 0.09% cetrimide agar was more selective than Pseudosel agar. However, it was also noted that Pseudosel agar was an excellent medium to stimulate the production of pyocyanin and pyorubin pigments elaborated by *P. aeruginosa*, and that many strains of *P. aeruginosa* that produced these pigments on Pseudosel agar failed to produce them on technicolor agar (King's medium A) (6).

This study was initiated to compare the selectivity of 0.09% cetrimide in heart infusion agar base with that of Pseudosel agar for growth of *P. aeruginosa* and other microorganisms. Secondly, Pseudosel agar and technicolor agar were compared to detect the production of pyocyanin and pyorubin pigments by strains of *P. aeruginosa*.

MATERIALS AND METHODS

The formulas for media used in this study were as follows. Cetrimide agar contained heart infusion agar (Difco), 40 g; cetrimide (22.5% solution), 4 ml; distilled water, 1,000 ml. Pseudosel agar was 45.3 g of the commercially prepared powder plus 1,000 ml of distilled water. Technicolor agar contained peptone (Difco), 20 g; agar (Difco), 15 g; glycerol (chemically pure), 10 g; $MgCl_2$ (anhydrous), 1.4 g; and K_2SO_4 (anhydrous), 10 g.

Cetrimide agar, Pseudosel agar, and technicolor agar slants were inoculated with one drop of a 24-hr heart infusion broth (Difco) culture. All slants were incubated at 35 C. If growth was poor, duplicate slants were incubated in a candle jar as determined by the growth requirements of the organism. Confluent growth was recorded as +; one to several scattered colonies were recorded as ±; and no growth was recorded as negative.

Strains examined in this study were original isolates from clinical specimens rather than cultures subjected to repeated transfers.

Biochemical tests routinely performed in this laboratory for the identification of gram-negative bacilli include 2% tryptone broth for indole production; 2% peptone broth (Difco) with 0.2% potassium nitrate for nitrate reductase; triple sugar iron agar (Difco) slant for fermentation and H_2S production; 1.2% gelatin (Difco) in heart infusion agar for gelatinase; litmus milk; esculin medium (14); Christensen's urea agar (Difco) for urease; catalase (1); oxidase; Moeller base for lysine and ornithine decarboxylases and arginine dihydrolase (11); and Simmon's citrate agar (Difco). The ability of each strain to grow on MacConkey agar (Difco) and on SS agar (Difco) was tested. Growth at 5, 22, 37, and 42 C was determined on tryptone-glucose-yeast-agar.

One of three carbohydrate bases, semisolid oxidative fermentative base (12), phenol red broth base (Difco), or fermentation base with Andrade's indicator (12), was used to study acid production from 11 carbohydrates. Choice of the base depended upon the organism tested; more than one base was used if required to ascertain a typical sacharrolytic pattern of a particular organism. Each carbohydrate was incorporated into a base in a final concentration of 1%. Carbohydrates included glucose, xylose, mannitol, lactose, sucrose, maltose, glycerol, arabinose, fructose, sorbitol, and trehalose.

Motility was determined in motility medium (Difco). Flagellar stains were performed on motile strains when needed for identification by the method of Leifson (7).

The nomenclature and biochemical schema for identification of strains used in this study were: *P. putida*, *P. acidovorans*, *P. cepacia*, and *P. pseudoalcaligenes* (15); all *Moraxella* species, *Aeromonas hydrophila*, and group Vd (1); *Xanthomonas* species (13); *Vibrio extorquens* (16); Herbicola-lathyri group (4); and *P. putrefaciens* (17). All other species were identified by the schema described by King (5).

RESULTS

Three hundred and four strains of *P. aeruginosa*, 262 strains of *Pseudomonas* species, and 250 strains of other gram-negative bacilli isolated from clinical specimens were chosen at random and inoculated to the two cetrimide-containing media.

Of the *P. aeruginosa* strains (Table 1), 99.3% grew on cetrimide agar as compared to 99.7% (303/304) that grew on Pseudosel agar. Seventy-six per cent (22/29) of *Pseudomonas fluorescens* and 91% (70/77) of *P. putida* grew on cetrimide agar, but all strains of these two species grew on Pseudosel agar. Only 54% (7/13) of *P. cepacia* grew on cetrimide agar, but all strains grew on Pseudosel agar. Both strains of *P. pseudoalcaligenes* grew on cetrimide agar and Pseudosel agar. One of the four strains of *P. acidovorans* grew on cetrimide agar, whereas two of these four strains grew on Pseudosel agar. All strains of *P. maltophilia* and *P. stutzeri* failed to grow on cetrimide agar, but 76% (93/123) of the former species and 83% (5/6) of the latter species grew on Pseudosel agar. All strains of *P. diminuta*, *P. dentrificans*, and *P. putrefaciens* failed to grow on both cetrimide agar and Pseudosel agar.

Sixty-seven per cent (6/9) of group III strains grew on cetrimide agar, although 89% (8/9) grew on Pseudosel agar; no strains of group IV grew on cetrimide agar, whereas 5 of 15 strains grew on Pseudosel agar; only one strain of group V grew on cetrimide agar, but 3 of these 13 strains grew on Pseudosel agar.

All strains of *Herellea vaginicola* and *Mima polymorpha* failed to grow on cetrimide agar, although 58% (27/47) of the *H. vaginicola* and 12% (4/34) of the *M. polymorpha* strains grew on Pseudosel agar.

All strains of *Pasteurella multocida*, *P. ureae*, *A. hydrophila*, *Flavobacterium* species, *F. meningosepticum*, *Xanthomonas* species, *Actinobacillus actinomycetemcomitans*, Herbicola-lathyri group, *V. extorquens*, *Mima polymorpha* var. *oxidans*, *Moraxella nonliquefaciens*, *M. kingii*, *M. phenylpyrouvica*, *M. osloensis*, and *Moraxella* species failed to grow on cetrimide agar (Table 1). However, 100% (15/15) of the *A. hydrophila*, 53% (15/28) of the *Flavobacterium* species, 87.5% (7/8) of the *F. meningosepticum*, and 66.6% (4/6) of the Herbicola-lathyri group strains grew on Pseudosel agar.

During this study, it was noted that pigmentation was often produced on Pseudosel agar when no pigment was produced on technicolor agar. Therefore, a comparison of pyocyanin and pyorubin production by strains of *P. aeruginosa* was made on technicolor agar and Pseudosel agar slants (Table 2). Sixty-three per cent (190/304) of *P. aeruginosa* produced pyocyanin, pyorubin, or both pigments on technicolor agar, but 81% (246/304) produced one or both pigments on Pseudosel agar. Therefore, Pseudosel agar was a more satisfactory medium to detect the production of pyocyanin, pyorubin, or both, than technicolor agar. Strains (513) of gram-negative bacilli other than *P. aeruginosa*, listed in Table 1, failed to produce pyocyanin or pyorubin.

DISCUSSION

Our results demonstrated that 0.09% cetrimide agar was a more selective medium for the growth of the gram-negative bacteria tested in this study than Pseudosel agar. However, cetrimide agar was of diagnostic use only in narrowing the species of bacteria that grew on this medium. Glucose oxidizers that grew

TABLE 1. *Growth on cetrimide agar and Pseudosel agar*

Organism	Cetrimide agar			Pseudosel agar		
	+[a]	±[b]	−[c]	+	±	−
Pseudomonas aeruginosa	301/304[d]	1/304	2/304	303/304	0/304	1/304
P. fluorescens	16/29	6/29	7/29	27/29	2/29	0/29
P. putida	61/77	9/77	7/77	75/77	2/77	0/77
P. cepacia	7/13	0/13	6/13	13/13	0/13	0/13
P. pseudoalcaligenes	1/2	1/2	0/2	2/2	0/2	0/2
P. acidovorans	0/4	1/4	3/4	1/4	1/4	2/4
P. maltophilia	0/123	0/123	123/123	44/123	49/123	30/123
P. stutzeri	0/6	0/6	6/6	1/6	4/6	1/6
P. diminuta	0/2	0/2	2/2	0/2	0/2	2/2
P. denitrificans	0/2	0/2	2/2	0/2	0/2	2/2
P. putrefaciens	0/4	0/4	4/4	0/4	0/4	4/4
Group III	5/9	1/9	3/9	8/9	0/9	1/9
Group IVc	0/5	0/5	5/5	0/5	1/5	4/5
Group IVd	0/4	0/4	4/4	0/4	2/4	2/4
Group IVe	0/1	0/1	1/1	0/1	1/1	0/1
Group IVf	0/5	0/5	5/5	0/5	1/5	4/5
Group Va	0/2	0/2	2/2	0/2	1/2	1/2
Group Vd	1/11	0/11	10/11	1/11	1/11	9/11
Herellea vaginicola	0/47	0/47	47/47	15/47	12/47	20/47
Mima polymorpha	0/34	0/34	34/34	0/34	4/34	30/34
Pasteurella multocida	0/4	0/4	4/4	0/4	0/4	4/4
P. ureae	0/2	0/2	2/2	0/2	0/2	2/2
Aeromonas hydrophila	0/15	0/15	15/15	12/15	3/15	0/15
Flavobacterium sp.	0/28	0/28	28/28	14/28	1/28	13/28
F. meningosepticum	0/8	0/8	8/8	6/8	1/8	1/8
Xanthomonas sp.	0/16	0/16	16/16	0/16	0/16	16/16
Actinobacillus actinomycetemcomitans	0/16	0/16	16/16	0/16	0/16	16/16
Herbicola-lathyri group	0/6	0/6	6/6	2/6	2/6	2/6
Vibrio extorquens	0/1	0/1	1/1	0/1	0/1	1/1
Mima polymorpha var. *oxidans*	0/6	0/6	6/6	0/6	0/6	6/6
Moraxella non-liquefaciens	0/4	0/4	4/4	0/4	0/4	4/4
M. kingii	0/1	0/1	1/1	0/1	0/1	1/1
M. phenylpyrouvica	0/2	0/2	2/2	0/2	0/2	2/2
M. osloensis	0/18	0/18	18/18	0/18	0/18	18/18
Moraxella sp.	0/4	0/4	4/4	0/4	0/4	4/4

[a] Varied from confluent growth to thin film.
[b] One to several scattered colonies.
[c] No growth.
[d] Number positive or negative/number examined.

on cetrimide agar included the three species of fluorescent pseudomonads, *P. aeruginosa*, *P. fluorescens*, and *P. putida*, as well as half of the *P. cepacia* strains and one strain of group Vd. The species of glucose nonoxidizers that grew on cetrimide agar included certain strains of *P. pseudoalcaligenes*, *P. acidovorans*, and group III.

That Pseudosel agar was less selective than cetrimide agar could be expected since Pseudosel agar contains only 0.03% cetrimide as opposed to a concentration of 0.09% cetrimide in cetrimide agar. The three species of fluorescent pseudomonads, as well as *P. cepacia* and *P. pseudoalcaligenes*, grew on Pseudosel agar; certain strains of *P. acidovorans* and group III and rare strains of group Vd also grew on Pseudosel agar.

Pseudomonas species that were completely inhibited on cetrimide agar but contained at least some strains that grew well on Pseudosel agar included *P. maltophilia* and *P. stutzeri*.

TABLE 2. *Pyocyanin and pyorubin production on technicolor agar and Pseudosel agar*

Organism	Technicolor agar		Pseudosel agar (BBL)	
	No. of strains positive	No. of strains negative	No. of strains positive	No. of strains negative
Pseudomonas aeruginosa				
Pyocyanin only	152 (50)[a]	152 (50)	122 (40)	182 (60)
Pyorubin only	17 (6)	287 (94)	35 (12)	269 (88)
Pyocyanin; pyorubin	21 (7)	283 (93)	89 (29)	215 (71)
Other gram-negative bacilli				
Pyocyanin and/or pyorubin	0 (0)	512 (100)	0 (0)	512 (100)

[a] Values in parentheses are expressed as percentages.

Three species of pseudomonads that failed to grow on both media were *P. diminuta*, *P. denitrificans*, and *P. putrefaciens*.

Certain strains of other bacteria which failed to grow on cetrimide agar grew on Pseudosel agar. Brown and Lowbury (3) reported that certain strains of *Proteus mirabilis*, *Providence*, and *Comamonas* grew on their selective medium that contained 0.03% cetrimide. Because of the diversity of bacterial species that grew on Pseudosel agar in this study, we cannot recommend it as a selective medium specifically for the isolation of *P. aeruginosa*.

However, 18% of the *P. aeruginosa* strains that produced pyocyanin, pyorubin, or both, on Pseudosel agar failed to produce either pigment on technicolor agar. Only 1.6% of the *P. aeruginosa* strains that produced one or both pigments on technicolor agar failed to produce either pigment on Pseudosel agar. Therefore, Pseudosel agar was the medium of choice for detection of pigment production by *P. aeruginosa*.

Sixty-three per cent of our *P. aeruginosa* strains produced pyocyanin, pyorubin, or both, on technicolor agar, whereas King, Ward, and Raney (6) reported that almost 100% of the *P. aeruginosa* strains that they examined produced one or both pigments on technicolor agar. Stanier, Palleroni, and Doudoroff (15) reported that 86% of their strains produced pyocyanin on King's medium A. There are two possible reasons why fewer strains in this study produced pigments than the strains reported by King et al. (5) and Stanier et al. (15). More strains were examined in our study than in either of the other studies, and, perhaps more important, all of our strains were primary isolates, whereas King's and Stanier's strains had been subjected to an unknown, and in some instances an innumerable, number of transfers. It has been our experience that pigmentation is more readily produced by certain subcultures than by the original isolates. Since many clinical laboratories perform a test only once and since early recognition of *P. aeruginosa* can be accomplished by detection of pyocyanin or pyorubin, or both, the use of Pseudosel agar for pigment production by *P. aeruginosa* is recommended.

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