Incidence and Identification of *Pseudomonas fluorescens* and *Pseudomonas putida* in the Clinical Laboratory

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Strains of *Pseudomonas* producing fluorescin but no pyocyanin or pyorubrin were studied by biochemical and antibiotic sensitivity testing. A rapid nitrate test was found to be useful in distinguishing *P. aeruginosa* (positive) from *P. fluorescens* and *P. putida* (both negative). A shortened gelatin test differentiated *P. fluorescens* (positive) from *P. putida* (negative). *P. fluorescens* and *P. putida* were very sensitive to low levels of kanamycin and resistant to carbenicillin, a pattern just the opposite of that obtained with *P. aeruginosa*.

The identification of nonfermentative gram-negative bacilli continues to pose a challenge in the diagnostic laboratory. Within the genus *Pseudomonas*, *P. aeruginosa* is the most commonly encountered species and is usually easily identified since most isolates produce the pigments pyocyanin and fluorescin. However, some *P. aeruginosa* strains do not produce detectable pyocyanin and can be confused with the other fluorescin-producing *Pseudomonas* species, *P. fluorescens* and *P. putida*. This paper reports the results of a study carried out to determine the incidence of the latter two species and to determine what diagnostic tests are most easily and rapidly used to differentiate them from apyocyaninogenic strains of *P. aeruginosa*.

MATERIALS AND METHODS

Reference strains used were *P. fluorescens* (ATCC 13525) and *P. putida* (ATCC 12633). Clinical isolates studied included 216 nonfermentative gram-negative rods which produced fluorescin but no pyocyanin or pyorubrin.

The organisms were tested for growth at 4, 35, and 42 C on heart infusion agar; incubation was extended for 10 days. Those organisms showing growth at 4 or 42 C were subcultured two more times and incubated at the appropriate temperature for 10 more days each time. Tech agar (BBL) was used for pyocyanin and pyorubrin production, and medium B of King et al. (4) was used to detect fluorescin. Other tests included oxidase, 10% lactose, oxidation of dextrose and maltose, nitrate reduction, gelatin liquefaction, pigment on heart infusion tyrosine agar, and growth on cetrimide; media used for these were according to

King (3). Shortened tests for nitrate reduction and gelatin liquefaction were performed in addition to the standard tests. For the shortened nitrate test, a heavy loopful of overnight growth from Triple Sugar Iron Agar (TSI) was emulsified in 0.5 ml of nitrate broth. After 2 hours of incubation in a heating block at 37 C, one drop of 0.8% sulfanilic acid in 0.2 N acetic acid and one drop of 0.5% alphanaphthylamine in 0.2 N acetic acid were added. A red color indicated reduction of nitrate. A pinch of zinc dust was added to all negatives to detect reduction beyond nitrite. For the shortened gelatin test, a heavy loopful of overnight growth from TSI was emulsified in 0.5 ml of saline, and a small strip of exposed, undeveloped X-ray paper was added. The tubes were incubated overnight in a heating block at 37 C. Gelatin liquefaction was indicated by removal of the emulsion from the paper, leaving a transparent blue strip. Flagellar stains were done using the method of Leifson (5). Antibiotic sensitivity studies were performed by the disc diffusion method of Bauer et al. (1) and by the agar dilution method (6). Tests were carried out at either 25 C or 35 C, depending on the temperature requirements of the strains.

RESULTS

Of the 216 *Pseudomonas* species, 15 were *P. fluorescens*, 17 were *P. putida*, and 184 were *P. aeruginosa* on the basis of biochemical and temperature tests. The results of the tests are shown in Table 1, along with reactions obtained with *P. aeruginosa* (pyocyanin negative). The shortened tests for nitrate reduction and gelatin liquefaction correlated with the standard tests with the exception of one strain of *P. fluorescens* which was negative in the shortened gelatin test.

TABLE 1. *Characteristics of P. fluorescens, P. putida, and atypical P. aeruginosa*

Characteristic	P. fluorescens (15 strains)	P. putida (17 strains)	P. aeruginosa (20 strains)
Pyocyanin	0[a]	0	0
Fluorescin	15	17	20
Oxidase	15	17	20
OF dextrose	15[b]	17	20
OF maltose	0	0	0
10% Lactose	0	6	18
Nitrate reduction	0	0	20
Gelatin liquefaction	15	0	20
Brown pigment	1	11	0
Growth on cetrimide	12	16	20
Growth:			
4 C	13	6	0
35 C	15	17	20
42 C	0	0	20
Polar flagella:			
1	1	3	20
>1	14	14	0

[a] Number of strains positive.
[b] All strains were oxidative.

Most of the strains of *P. fluorescens* and *P. putida* were isolated from respiratory tract specimens.

Fourteen of the 15 strains of *P. fluorescens* had two or more polar flagella, and 14 of the 17 *P. putida* had two or more polar flagella. The other three strains had only one polar flagellum; these strains grew at 4 C, however.

The results of disc sensitivity testing are shown in Table 2, along with the results obtained with strains of *P. aeruginosa* for comparison. Twelve strains of *P. fluorescens* and 16 *P. putida* were very sensitive to kanamycin; in contrast, most strains of *P. aeruginosa* were resistant to this agent. Only one strain of *P. fluorescens* was sensitive to carbenicillin; the other strains and the strains of *P. putida* were completely resistant to carbenicillin, showing no zone of inhibition at all. Most *P. aeruginosa* strains were sensitive to carbenicillin, and those which were not were intermediate in sensitivity. A majority of the strains of *P. putida* and *P. fluorescens* were susceptible to tetracycline, whereas almost all of the *P. aeruginosa* strains were resistant to this agent. More than half the strains of *P. fluorescens* were sensitive to sulfisoxazole.

The results of agar dilution testing are shown in Table 3; they agreed with the disc sensitivity results.

DISCUSSION

As shown by the data presented here, both *P. fluorescens* and *P. putida* are infrequently

TABLE 2. *Disc diffusion susceptibility results with P. fluorescens, P. putida, and P. aeruginosa*

Antibiotic	P. fluorescens (15 strains)	P. putida (17 strains)	P. aeruginosa (20 strains)
Carbenicillin	1[a]	0	14
Chloramphenicol	1	0	0
Colistin	14	15	18
Gentamicin	15	17	18
Kanamycin	12	16	3
Polymyxin B	14	17	18
Sulfisoxazole	9	3	1
Tetracycline	13	10	2

[a] Indicates number of strains sensitive.

isolated from clinical specimens, accounting for only 15% of fluorescin-producing pyocyanin-negative pseudomonads. About 10% of the *P. aeruginosa* strains we isolated produce only fluorescin. Therefore, *P. fluorescens* and *P. putida* make up less than 1% of all pseudomonads isolated in our laboratory. Our experience is similar to that of von Graevenitz and Weinstein (9) who found only 11 strains of *P. putida* and 4 of *P. fluorescens* in a 2-yr period. The infrequency of these organisms in clinical specimens is not unexpected, since they both have a predilection for growth at lower than body temperature, and media for isolation are routinely incubated only at 35 C.

Previous reports (7, 9) have shown that *P. fluorescens* and *P. putida* do not appear to be significant in causing disease, whereas *P. aeruginosa* is well-established as a pathogen. Because of this difference in pathogenicity, accurate differentiation of these organisms is meaningful in assessing their importance in a particular specimen. Identification would also be useful in future determinations as to whether *P. fluorescens* or *P. putida* truly do cause clinically significant disease. In addition, the differences in susceptibility to antibiotics of the fluorescent pseudomonads are further evidence as to the pertinence of accurate identification.

Characteristics which have been used to differentiate *P. aeruginosa* from *P. putida* and *P. fluorescens* are growth of the former at 42 and no growth at 4 C, and the presence of a single polar flagellum in the former (2, 8). To differentiate *P. putida* and *P. fluorescens*, gelatin liquefaction is useful (positive for the latter). Tests for growth at 4 and 42 C may not be practical in a clinical laboratory since not only is a special high-temperature incubator

TABLE 3. Agar dilution susceptibility results with P. fluorescens and P. putida

Antibiotic and organism	Minimum inhibitory concn (µg/ml)											
	≤0.4	0.8	1.6	3.1	6.3	12.5	25	50	100	200	400	≥800
Carbenicillin												
P. fluorescens									1		1	13
P. putida											5	12
Chloramphenicol												
P. fluorescens						3		9	3			
P. putida							1	3	13			
Colistin												
P. fluorescens	2	9	2		1		1					
P. putida		1	7	6	2		1					
Gentamicin												
P. fluorescens	14			1								
P. putida	4	5	6	2								
Kanamycin												
P. fluorescens	9	3				1	2					
P. putida	2	9	5				1					
Polymixin B												
P. fluorescens	2	5	6	1			1					
P. putida	1		14	2								
Tetracycline												
P. fluorescens			1	8	4	1		1				
P. putida					10	4	2	1				

ᵃ Fifteen strains of P. fluorescens and 17 strains of P. putida were tested.

needed, but, more importantly, 10 days of incubation is too long for a clinically meaningful report. Flagellar stains are time-consuming and require great care that the flagella do not become dislodged from the cells. However, these can be helpful in the laboratory when carefully done and in the context of resolving problems of diagnosis as indicated below.

All of our strains of P. aeruginosa reduced nitrate, whereas none of the other fluorescent strains did. Although P. fluorescens has been reported to have the ability to denitrify (8), it seems that those which are clinical isolates do not reduce nitrate (2). The shortened test for nitrate reduction, then, together with the marked sensitivity to kanamycin and resistance to carbenicillin would provide a rapid means of accurately identifying P. fluorescens and P. putida and separating them from P. aeruginosa in the clinical laboratory. By using both of these characteristics, one should not misidentify rare nitrate-negative P. aeruginosa or rare nitrate-positive P. fluorescens or P. putida. If strains should be isolated which do not fit these characteristics, these should then be tested further for flagellar type and for growth at 4 C.

To differentiate P. fluorescens from P. putida, the shortened test for gelatin liquefaction is useful and time-saving.

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