Isolation of Oxidase-Negative *Pseudomonas aeruginosa* from Sputum Culture

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Two isolates of *Pseudomonas aeruginosa* lacking characteristic indophenol oxidase were recovered from a sputum specimen. A discussion of the characteristic biochemical tests and antibiograms along with a possible explanation for this phenomenon is presented.

Isolation and identification of nonfermentative, gram-negative bacilli in the clinical laboratory are becoming more important as documented evidence of pathogenicity mounts against these organisms (6, 9). The test for the production of indophenol oxidase is included in many schemes for the identification of gram-negative bacilli (4, 5, 9, 10, 12). All *Pseudomonas aeruginosa* isolates characteristically produce indophenol oxidase (4, 5, 9, 10). Two atypical isolates were recovered from a sputum sample with consistently negative oxidase tests. All other biochemical and physical characteristics were consistent with *P. aeruginosa*.

A 77-year-old male, whose chief complaint was shortness of breath and difficulty in breathing, was admitted to North Carolina Baptist Hospital. The patient had a history of chronic obstructive pulmonary disease and required the assistance of a respirator. Sputum cultures were ordered because of the suspicion of pneumonia, and therapy was begun with erythromycin and tobramycin. All initial cultures revealed only normal throat flora. After several days in the hospital on antibiotic therapy, a sputum sample was submitted that resulted in the isolation of the bacteria described herein. Subsequently, the patient improved and was discharged with medication for his pulmonary deficiency.

The sputum sample was inoculated onto 5% sheep blood agar, chocolate agar, MacConkey agar, and Columbia CNA agar. After 24 h of incubation, various amounts of normal throat flora were present on the blood, chocolate, and CNA agar plates. In addition, the blood, chocolate, and MacConkey agar plates had moderate growth (10 to 200 colonies) of two morphologically distinct colony types of gram-negative bacilli. Type 1, designated as smooth, was circular and convex; type 2, designated as mucoid, was circular and convex, with a tendency for the colonies to coalesce. On MacConkey agar, both types were colorless, non-lactose fermenters.

Beta hemolysis was produced on blood agar. Biochemical tests were performed by conventional procedures (5, 10, 12). Antibiotic susceptibilities were determined by a microtube dilution method (3). Table 1 presents a summary of the biochemical data for both isolates compared with standard reactions of *P. aeruginosa* and *Pseudomonas fluorescens*.

The results shown in Table 1 indicate that both isolates have biochemical and physical characteristics consistent with *P. aeruginosa*. Both isolates utilized acetamide and produced the pigment pyocyanin, characteristic results which differentiate *P. aeruginosa* and *P. fluorescens*. *P. aeruginosa* utilizes acetamide (4) and is the only *Pseudomonas* species known that produces pyocyanin (9, 10). In addition to the lack of oxidase activity, these isolates were also nonmotile and only reduced nitrate to gas after 7 days of incubation. The mucoid isolate, which failed to grow at 42°C, appears to be a colonial variant of the smooth isolate based upon all other identical biochemical and antibiotic susceptibility patterns (Table 2). Both isolates appear to be oxidase-negative mutants of *P. aeruginosa*.

The oxidase test was performed with filter paper saturated with a 1% aqueous tetramethyl-*para*-phenylene-diamine solution. This indirect test technique (Kovacs) is accomplished by rubbing growth from a blood agar plate or other noninhibitory media onto the reagent-soaked filter paper. A dark purple color, appearing within 10 s, indicates a positive oxidase test. Because the test was negative at the end of 10 s, an extension was made to 60 s to allow for weak or borderline results (12). Further oxidase tests were performed with the PathoTec Co Strips (General Diagnostic Division, Warner-Lambert Co., Morris Plains, N.J.) and the Cepti-Seal reagent dropper (Marion Scientific Corp., Kansas City, Mo.). These results were also negative.

Both isolates were submitted to the Special

TABLE 1. Biochemical characteristics of *Pseudomonas* isolates compared with reactions of *P. aeruginosa* and *P. fluorescens*^a

Test	Type 1 (smooth)	Type 2 (mucoid)	<i>P. aeruginosa</i> ^b	<i>P. fluorescens</i> ^b
Indophenol oxidase	—	—	100	100
Pyocyanin (chloroform-soluble pigment)	+	+	58	0
Pyoverdinin	—	—	88	94
Growth at 42°C	+	—	100	0
Motility	—	—	93	100
Acid:Glucose	+	+	100	100
1% OFBM ^c				
D-Mannitol	+	+	81 (11)	94
Xylose	+	+	98 (2)	98
Maltose	—	—	0	70
Lactose	—	—	0	22
ONPG ^d	—	—	0	2
Citrate, Simmons	+	+	100	—
L-Lysine decarboxylase	—	—	0	0
L-Arginine dihydrolase	+	+	96 (3)	98
L-Ornithine decarboxylase	—	—	0	0
Nitrate to gas ^e	+	+	94	2
Urease	+	+	77	40
Gelatinase	+	+	80 (19)	100
Esculin hydrolysis	—	—	0	0
Deoxyribonuclease	—	—	13	0
Cetrimide	+	+	95	95
Acetamide	+	+	60	5
Hemolysis	+	+	44	16

^a See references 4, 5, and 10.

^b Figures indicate percent positive within 2 days; figures in parentheses indicate percent positive, delayed 3 or more days; —, no test.

^c OFBM, OF basal media (Difco).

^d ONPG, *o*-nitrophenyl- β -D-galactopyranoside.

^e Nitrate to gas in 7 days.

TABLE 2. Antibiotic minimum inhibitory concentrations for both *Pseudomonas* isolates

Antibiotic	MIC ^a	Antibiotic	MIC ^a
Amikacin	8	Nafcillin	>64
Ampicillin	>64	Nalidixic acid	>512
Carbencillin	32	Penicillin G	>64
Cephalothin	>64	Polymyxin B	1
Chloramphenicol	>64	Streptomycin	>64
Clindamycin	>64	Tetracycline	8
Erythromycin	>64	Ticarcillin	16
Gentamicin	2	Tobramycin	1
Kanamycin	64	Trimethoprim-sulfamethoxazole	256 ^b
Methicillin	>64	Vancomycin	>64

^a MIC, Minimum inhibitory concentration in micrograms per milliliter.

^b Ratio of trimethoprim to sulfamethoxazole contained in 256 μ g/ml is 12.8 to 243.2 μ g, respectively.

Bacteriology Section, Center for Disease Control, Atlanta, Ga., and the Laboratory Section, North Carolina Department of Human Resources, Raleigh, N. C. The results obtained

from both laboratories were in agreement with our findings, i.e., oxidase-negative *P. aeruginosa*.

The antibiotic minimum inhibitory concentrations for both isolates are shown in Table 2. These findings were subsequently confirmed by Clyde Thornsberry's laboratory at the Center for Disease Control, Atlanta, Ga. Tobramycin and polymyxin B had the lowest minimum inhibitory concentrations of all drugs tested, i.e., 1 μ g/ml. This susceptibility pattern is consistent with patterns obtained for *P. aeruginosa* (5).

P. aeruginosa, especially mucoid isolates, have been among the bacteria most often isolated from respiratory tracts of patients suffering from cystic fibrosis (7, 8). Unfortunately, this patient was not evaluated for cystic fibrosis during his hospital stay. However, because this patient's initial sputum cultures did not reveal the pseudomonads, it is likely that this represents a hospital-acquired strain, perhaps from the respiratory assistance equipment (1). Whether the pseudomonads were the cause of an infectious process or mere colonization cannot be determined.

The lack of oxidase and motility may have been due to the antibiotic therapy. Erythromycin and tobramycin had been initiated before the collection of the sample which yielded the isolates described above. Both antibiotics are noted for the successful inhibition of protein biosynthesis in bacteria (2, 11). Consequently, an alteration of the mechanism(s) responsible for these characteristics may have resulted from this therapy. It is interesting to postulate that the isolation of bacteria with aberrant biochemical patterns may be the consequence of conventional antimicrobial or cancer chemotherapy. Obviously, more studies are needed to affirm this theory.

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