

# Characterization of *Pseudomonas* Species Isolated from Clinical Specimens<sup>1</sup>

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Received for publication 14 October 1970

More than 90 morphological and physiological characters of 227 strains of pseudomonads isolated from clinical specimens and 16 reference strains are described. The clinical isolates included *P. aeruginosa* (apycyanogenic), *P. fluorescens*, *P. putida*, *P. pseudomallei*, *P. cepacia*, *P. acidovorans*, *P. alcaligenes*, *P. pseudoalcaligenes*, *P. stutzeri*, *P. putrefaciens*, *P. maltophilia*, and *P. diminuta*.

With the appearance of recent reports (2, 3, 22-26) on the criteria for characterization of pseudomonads, identification of most pseudomonads isolated from clinical specimens is now possible in the diagnostic laboratory. This paper reports on a study that was undertaken to determine those morphological and physiological characters most useful in their identification. Diagnostic tests used primarily in the identification of fermentative bacteria, as well as nutritional tests applied to the nonfermentative bacteria, were examined.

## MATERIALS AND METHODS

Most isolates were recovered from clinical materials in this laboratory from September 1965 to September 1970. A few isolates were recovered from the hospital environment, received from other laboratories, or submitted to this laboratory for confirmation or assistance in identification. The strains of *P. aeruginosa* included in this study were atypical in that they were apycyanogenic and failed to produce the odor of trimethylamine. Pyocyanogenic strains were not included because of their ease in identification. The reference strains are listed in Table 1. The tests and media employed were previously described (10), except for the following: production of acid from glucose, fluorescein, and nitrogen gas, Sellers' Differential Agar F (Difco); fluorescein production, *Pseudomonas* Agar F (Difco); pyocyanine production, *Pseudomonas* Agar P (Difco); accumulation of poly- $\beta$ -hydroxybutyrate (PBHB), basal mineral medium (BMM) with DL- $\beta$ -hydroxybutyrate. PBHB was detected with Sudan Black B stain.

## RESULTS AND DISCUSSION

The reactions obtained are given in Tables 2 and 3. Those characteristics of particular interest

are emphasized below. The pseudomonads are characterized as gram-negative, aerobic, non-sporeforming bacilli with monotrichous or multitrichous flagella. [*P. mallei*, not examined here, is nonmotile (25).] They grow well on ordinary peptone medium, are indole-negative, are usually oxidase-positive, and either do not attack carbohydrates or attack them oxidatively with the production of acid but no gas.

**Fluorescent group:** *P. aeruginosa*, *P. fluorescens*, *P. putida*. Several features were common to all members of this group. They do not accumulate PBHB as a cellular reserve material, all strains possess the arginine dihydrolase system, and they are oxidase-positive. Although production of a fluorescent pigment is a group feature, several strains failed to demonstrate this pigment under the methods used.

The uniform characters for identification of apycyanogenic strains of *P. aeruginosa* include monotrichous flagella, growth at 42 C, inability to produce acid from disaccharides, and inability to assimilate arabinose, sucrose, trehalose, and inositol. Other features reported as universal for pyocyanogenic strains, including denitrification, gelatinase activity, hydrolysis of Tween 80 (26), gluconate oxidation (11), growth on triphenyl tetrazolium chloride (4), and assimilation of adipate, suberate, and acetamide (26), were variable features with the apycyanogenic strains.

The simple fluorescent pseudomonads (*P. fluorescens*, *P. putida*) are differentiated from *P. aeruginosa* since the former are multitrichous, do not grow at 42 C, and are characterized by variable acid production from disaccharides and variable assimilation of arabinose, sucrose, trehalose, and inositol. The classical features used to differentiate the simple fluorescent pseudomonads are

<sup>1</sup> Presented in part at the 69th Annual Meeting of the American Society for Microbiology, Miami Beach, 4-9 May 1969.

TABLE 1. *Reference strains*

Species	Designations	Source
<i>P. pseudomallei</i>	ATCC 11668	ATCC
<i>P. pseudomallei</i>	NCTC 1691, B-111, RH 2108	R. E. Hugh
<i>P. kingii</i> (EO-1)	B-3616	R. E. Weaver
<i>P. cepacia</i> ( <i>multivorans</i> )	382	M. Doudoroff
<i>P. acidovorans</i>	ATCC 15668, K176	M. J. Pickett
<i>P. acidovorans</i>	K620	M. J. Pickett
<i>P. testosteroni</i>	ATCC 11996, K177	M. J. Pickett
<i>P. alcaligenes</i>	ATCC 14909, K441	M. J. Pickett
<i>P. alcaligenes</i>	K517	M. J. Pickett
<i>P. pseudoalcaligenes</i>	K532	M. J. Pickett
<i>P. pseudoalcaligenes</i>	K564	M. J. Pickett
<i>P. stutzeri</i>	ATCC 11607	ATCC
<i>P. maltophilia</i>	ATCC 13637	ATCC
<i>P. diminuta</i>	ATCC 11568	M. J. Pickett
<i>P. diminuta</i>	K608	M. J. Pickett
<i>P. vesiculare</i>	ATCC 11426, K249	M. J. Pickett

gelatin liquefaction and the egg-yolk reaction, characteristics possessed by the former but not the latter species (17). The assimilation pattern differentiates these species, since both trehalose and inositol are utilized by *P. fluorescens* but not by *P. putida*. Although none of the strains of *P. fluorescens* examined produced nitrogen gas, certain biotypes are capable of denitrification (26).

**Pseudomallei group:** *P. pseudomallei*, *P. cepacia*. On the basis of nutritional studies of phytopathogenic pseudomonads, Ballard et al. (3) concluded that *P. cepacia* was the same as *P. multivorans* (26), and on the basis of deoxyribonucleic acid (DNA)-DNA hybridization studies it was further concluded that *P. cepacia* was related to the animal pathogens *P. pseudomallei* and *P. mallei*. The pseudomonad EO-1 of King (16), designated *P. kingii* by Jonsson (15), was shown (9, 23) to be indistinguishable from *P. cepacia*.

The principal characters of the pseudomallei group include accumulation of PBHB, multitrichous flagella, ability to utilize a wide range of organic compounds, variable oxidase activity, and resistance to antibiotics of the polymyxin group. The color of growth of the species in this group is highly variable, ranging from gray to yellow. The colonial morphology of *P. pseudomallei* is variable, ranging from smooth to wrinkled in structure. *P. pseudomallei* is distinguished from *P. cepacia* on the basis of the *o*-nitrophenyl- $\beta$ -D-galactopyranoside (ONPG) test, denitrification, arginine dihydrolase and lysine decarboxylase activity, growth on 2.5% NaCl, and assimilation of arabinose and maltose.

**Acidovorans group:** *P. acidovorans*, *P. testosteroni*. Pseudomonads with generally negative

physiological characteristics when examined with conventional diagnostic tests were described and named *P. acidovorans* by den Dorren de Jong (6). The acidovorans group is multitrichous, accumulates PBHB, and fails to grow at 42 C. *P. acidovorans* is differentiated from *P. testosteroni* on the basis of acid production from carbohydrates and assimilation of select organic compounds as sole sources of carbon. *P. testosteroni*, so named (20) on the basis of its ability to grow on testosterone and related steroids, was not isolated in this laboratory, and the results are based on examination of the reference strain.

**Alcaligenes group:** *P. alcaligenes*, *P. pseudoalcaligenes*. Other pseudomonads with generally negative physiological features, and similar to the acidovorans group, were described and named *P. alcaligenes* by Monias (21) and were further characterized by Hugh and Ikari (13). The new taxon, *P. pseudoalcaligenes*, was proposed by Stanier et al. (26). The alcaligenes group differs from the acidovorans group since it is monotrichous, grows at 42 C, generally fails to accumulate PBHB, and assimilates pelargonate but not norleucine. Acid production from fructose distinguishes *P. pseudoalcaligenes* from *P. alcaligenes*. In addition to the group features, *P. alcaligenes* is differentiated from *P. testosteroni* on the basis of their deamination of phenylalanine and assimilation of  $\beta$ -alanine, arginine, and adipate. Growth at 42 C is considered (26) a constant feature of the alcaligenes group, but biotypes of *P. alcaligenes* have been reported (12) which fail to grow at 42 C.

***P. stutzeri*.** This species is identified on the basis of its colonial morphology and production of nitrogen gas, features used historically (27) in its identification. The strains are diphasic and consist

TABLE 2. Biochemical features of *Pseudomonas* species<sup>a</sup>

Test or substrate	<i>P. aeruginosa</i> (48)	<i>P. fluorescens</i> (12)	<i>P. putida</i> (30)	<i>P. pseudomallei</i> (5)	<i>P. cepacia</i> (12)	<i>P. acidovorans</i> (6)	<i>P. testasteroni</i> (1)	<i>P. alcaligenes</i> (7)	<i>P. pseudocalcigenes</i> (6)	<i>P. stutzeri</i> (24)	<i>P. putrefaciens</i> (6)	<i>P. maltophilia</i> (81)	<i>P. diminuta</i> (4)	<i>P. vesicularis</i> (1)
D-Glucose, 1% (OFBM).....	48	12	30	5	12	6(w)	0	0	6(w)	24	6(d)	81(w)	0	1(w)
D-Fructose.....	46	11	29	5	12	6	0	0	6	24	3(d)	79	0	0
D-Galactose.....	45	12	30	5	12	0	0	0	3	22	0	32	0	0
D-Mannose.....	45	12	30	5	12	0	0	0	3	22	0	79	0	0
L-Rhamnose.....	15	9	18	4	0	0	0	0	0	18	2(d)	0	0	0
D-Xylose.....	43	12	29	5	12	0	0	0	3	24	0	45	0	0
Lactose.....	0	1	6	5	12	0	0	0	0	0	2(d)	75	0	0
Sucrose.....	0	9	3	4	11	0	0	0	0	0	0	77	0	0
Maltose.....	0	10	7	5	12	0	0	0	0	24	0	81	0	0
Mannitol.....	34	10	6	5	12	6	0	0	0	21	0	0	0	0
Lactose, 10% (PAB).....	9	6	11	5	12	0	0	0	0	0	0	0	0	0
Glucose (SDA).....	22	12	24	3	12	0	0	0	0	11	0	0	0	0
Gluconate oxidation (GS).....	35	8	22	0	1	0	0	0	0	0	0	0	0	0
ONPG.....	0	0	0	0	12	0	0	0	0	0	0	80	0	0
Hydrogen sulfide (KIA).....	0	0	0	0	0	0	0	0	0	0	6	0	0	0
Urea.....	43	11	19	3	5	0	0	1	0	5	3	0	0	0
Nitrite.....	11	0	0	4	6	6	1	7	6	7	6	27	0	0
Nitrogen gas.....	30	0	0	5	0	0	0	0	0	24	0	0	0	0
Oxidase.....	48	12	30	5	10	6	1	7	6	24	6	0	4	1
Arginine dihydrolase.....	48	12	30	5	0	0	0	0	0	0	0	0	0	0
Lysine decarboxylase.....	0	0	0	0	12	0	0	0	0	0	0	81	0	0
Ornithine decarboxylase.....	0	0	0	0	0	0	0	0	0	0	6	0	0	0
Phenylalanine deaminase.....	0	0	1	0	0	0	0	7	4	12	0	0	0	0
Esculin hydrolysis.....	0	0	0	3	11	0	0	0	0	0	0	81	0	0
Lipase.....	33	10	1	4	12	2	1	3	0	22	6	80	0	0
Starch hydrolysis.....	0	0	0	0	0	0	0	0	0	21	0	0	0	0
Deoxyribonuclease.....	8	0	0	0	0	0	0	0	0	2	6	81	4	0
Lecithinase.....	6	12	0	5	10	0	0	0	0	4	0	0	0	0
Gelatinase.....	29	12	0	5	7	0	0	1	0	0	6	81	4	1
Caseinase.....	29	12	0	5	8	0	0	1	0	3	6	81	4	1
Hemolysis.....	25	4	1	3	1	0	0	4	0	0	0	0	0	0
Growth on SS Agar.....	47	11	30	0	0	4	0	0	5	22	6	0	0	0
Growth on DC Agar.....	47	10	29	0	1	5	0	4	5	22	6	0	0	0
Growth on MacConkey Agar.....	48	12	30	5	12	5	0	7	6	24	6	81	4	1
Growth on TTC.....	39	8	28	0	4	0	0	0	2	0	0	0	0	0
Growth at 6.5% NaCl.....	3	0	4	0	0	0	0	0	0	24	6	0	0	0
Growth at 2.5% NaCl.....	48	11	30	0	11	0	1	4	6	24	6	81	4	1
Growth at pH 5.6 (SGA).....	47	12	30	5	12	6	1	7	6	0	0	81	4	1
Growth on cetrimide.....	48	11	29	0	10	0	0	3	5	2	0	3	0	0
Growth at 42 C.....	48	0	0	5	7	0	0	7	6	24	6	67	4	1
Brown color.....	1	0	0	0	0	0	0	0	3	0	0	81	4	0
Fluorescence.....	45	12	30	0	0	0	0	0	0	0	0	0	0	0
Wrinkled colonies.....	0	0	0	4	0	0	0	0	0	24	0	0	0	0
Polymyxin-resistant.....	0	0	0	5	12	0	0	0	0	0	0	0	4	1
Growth in BMM.....	48	12	30	5	12	6	1	7	6	24	6	0	0	0

<sup>a</sup> All strains were motile and indole-negative. *P. aeruginosa* strains were apocyanogenic. Reactions in OFBM refer to oxidation of the carbohydrate as indicated by production of acid. All strains failed to grow in sealed tubes of OFBM containing glucose. Abbreviations: OFBM, OF Basal Medium; PAB, Purple Agar Base; SDA, Sellers' Differential Agar; GS, gluconate substrate; ONPG, *o*-nitrophenyl- $\beta$ -D-galactopyranoside; KIA, Kligler Iron Agar; SS Agar, Salmonella Shigella Agar; DC Agar, Desoxycholate Agar; TTC, triphenyl tetrazolium chloride; SGA, Sabouraud's Glucose Agar; BMM, basal mineral medium; (w), weak; (d), delayed 3 or 4 days. Parenthetical values refer to number of strains examined; other values indicate number of strains giving reaction. All reactions were incubated for 3 weeks at 37 C before tests were discarded as negative. The majority of reactions were interpreted after 24 to 48 hr of incubation.

TABLE 3. Assimilation of organic compounds as sole carbon and energy source by *Pseudomonas* species<sup>a</sup>

Substrate	<i>P. aeruginosa</i> (48)	<i>P. fluorescens</i> (12)	<i>P. putida</i> (30)	<i>P. pseudomallei</i> (5)	<i>P. cepacia</i> (12)	<i>P. acidovorans</i> (6)	<i>P. testosteroni</i> (1)	<i>P. alcaligenes</i> (7)	<i>P. pseudocataligenes</i> (6)	<i>P. stutzeri</i> (24)	<i>P. putrefaciens</i> (6)
D-Glucose.....	48	12	30	5	12	0	0	0	0	24	0
D-Fructose.....	39	10	28	5	12	6	0	0	6	13	0
L-Arabinose.....	0	10	15	0	12	0	0	0	0	0	0
D-Xylose.....	0	9	13	0	11	0	0	0	0	0	0
Sucrose.....	0	11	12	5	12	0	0	0	0	20	0
Maltose.....	0	0	0	5	0	0	0	0	0	23	0
D-Trehalose.....	2	12	0	5	12	0	0	0	0	0	0
D-Mannitol.....	37	12	6	5	12	6	0	0	0	13	0
<i>i</i> -Inositol.....	0	12	0	5	12	0	0	0	0	0	0
Propionate.....	40	5	23	5	8	2	1	3	3	7	0
Butyrate.....	48	3	26	5	12	2	1	6	3	9	0
Pelargonate.....	48	12	30	5	12	0	0	7	6	24	0
Malonate.....	44	11	16	5	12	6	0	0	0	22	0
Adipate.....	37	0	0	5	12	6	1	0	2	10	0
Suberate.....	22	0	0	5	12	5	1	0	1	1	0
Citrate.....	48	12	30	5	12	6	1	7	5	24	0
$\beta$ -Alanine.....	48	12	30	5	12	6	0	7	6	0	0
L-Arginine.....	46	12	30	5	12	0	0	7	3	0	0
Asparagine.....	48	12	30	4	12	6	1	7	6	24	4
DL-Aspartate....	48	12	30	5	12	4	1	7	3	13	6
Glycine.....	27	0	22	0	1	6	1	0	4	16	0
L-Lysine.....	40	5	18	5	12	0	0	0	0	1	0
DL-Norleucine..	0	0	0	0	0	6	1	0	0	0	0
DL-Serine.....	7	8	10	5	10	0	0	0	2	2	0
DL-Valine.....	34	11	26	5	6	0	1	0	1	1	0
Acetamide.....	30	0	2	0	8	6	0	0	1	2	0
Betaine.....	48	12	30	5	12	0	0	0	4	18	0

<sup>a</sup> Preliminary studies showed that saline-washed cells and nonwashed cells gave similar results. Assimilation of the compound was indicated by growth. All cultures were incubated for 3 weeks at 37 C before tests were discarded as negative. The majority of reactions were interpreted after 24 to 48 hr of incubation. All strains assimilated acetate, succinate, fumarate, D-malate, DL-lactate, pyruvate, and L-glutamate and failed to assimilate DL-methionine.

of both rough and smooth colonial forms. Not all strains produce the characteristic yellow pigment, some being light brown in color. Other distinctive characters include starch hydrolysis, growth on 6.5% NaCl, assimilation of maltose and sucrose, inability to produce acid from sucrose and lactose, and inability to grow at pH 5.6. A pseudomonad with similar features, *P. mendocina*, is differentiated from *P. stutzeri* since the former is arginine dihydrolase-positive, assimilates arginine, and fails to hydrolyze starch (22).

*P. putrefaciens*. This monotrichous bacillus, which produces abundant hydrogen sulfide from KIA and a tan pigment, was first described by Derby and Hammer (5) and was later placed in the genus *Pseudomonas* by Long and Hammer (19). Organisms with similar features placed in group 1b by King (16) and described recently by von Graevenitz and Simon (28) were indicated by

Hugh (seminar on pseudomonads, 70th Annual Meeting, American Society for Microbiology, Boston, 1970) to be the same as *P. putrefaciens*. It produces decarboxylase for ornithine, is deoxyribonuclease-positive, grows on 6.5% NaCl, fails to grow at pH 5.6, and produces delayed acid from only a few carbohydrates.

*P. maltophilia* and the *diminuta-vesiculare* group. Three pseudomonads will not grow in the BMM unless supplemented with growth factors (2, 26). *P. maltophilia*, so named (14) because it readily produces acid from maltose, is multi-trichous and does not accumulate PBHB. It is oxidase-negative, produces decarboxylase for lysine, and is ONPG-positive. The brown coloration of the culture medium associated with *P. maltophilia* and previously described (8) as a pigment is probably due to a secondary chemical reaction among extracellular products which re-

TABLE 4. Simplified key with some salient features for the identification of pseudomonads<sup>a</sup>

Test or substrate	<i>P. aeruginosa</i>	<i>P. fluorescens</i>	<i>P. putida</i>	<i>P. pseudomallei</i>	<i>P. cepacia</i>	<i>P. actinovirans</i>	<i>P. testosteroni</i>	<i>P. alcaligenes</i>	<i>P. pseudocataligenes</i>	<i>P. stutzeri</i>	<i>P. putrefaciens</i>	<i>P. maltophilia</i>	<i>P. dimiwa</i>	<i>P. vesicularis</i>
Acid:														
Glucose.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose.....	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Lactose.....	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Maltose.....	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Mannitol.....	+	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
ONPG.....	V	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Hydrogen sulfide.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Denitrification.....	V	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arginine dihydrolase.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lysine decarboxylase.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ornithine decarboxylase.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Phenylalanine deaminase.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Esculin hydrolysis.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deoxyribonuclease.....	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-
Lecithinase.....	V	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatinase.....	V	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 6.5% NaCl.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 2.5% NaCl.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at pH 5.6.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 42 C.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fluorescence.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polymyxin-resistant.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth in BMM.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Assimilation:														
D-Fructose.....	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
β-Alanine.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DL-Norleucine.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pelargonate.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PBHB accumulation.....	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Wrinkled colonies.....	+	+	+	+	+	+	+	+	+	+	+	+	+	+
No. of flagella.....	1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1	<1

<sup>a</sup> Symbols: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; BMM, basal mineral medium; PBHB, poly-β-hydroxybutyrate; +, positive; -, negative; V, variable; (-), few strains negative; (+), few strains positive; w, weak; d, delayed; NT, not tested; 1, monotrichous; >1, multitrifidous.

act to form the brown color (1). *P. maltophilia* is not related to the diminuta-vesiculare group but is discussed with this group because of their unusual nutritional requirements.

The diminuta-vesiculare group is monotrichous, accumulates PBHB, and is oxidase-positive. The flagella are unusual, having a wavelength of only 0.6 to 0.98  $\mu\text{m}$  (7, 18). *P. vesiculare* produces weak acid from glucose. *P. diminuta* does not produce acid from carbohydrates but is reported (2, 18, 24) to form acid from ethanol. *P. diminuta* differs from *P. vesiculare* on the basis of growth factor requirements and the assimilation of select organic compounds, as demonstrated by Ballard et al. (2). *P. vesiculare* was not recovered in this laboratory, and the results are based on the reference strain.

**Simplified key for identification.** Table 4 is a condensed form of Tables 2 and 3 showing some salient features that can be used in the differentiation of pseudomonads isolated from clinical specimens. Of the pseudomonads examined in this laboratory, 94% were identified to species by using this table.

#### ACKNOWLEDGMENT

The technical assistance of Patricia McKay is gratefully acknowledged.

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