Emendation of the Description of *Pseudomonas cepacia* Burkholder (Synonyms: *Pseudomonas multivorans* Stainer et al., *Pseudomonas kingae* Jonsson; EO-1 Group)

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It has been reported that the names *Pseudomonas multivorans*, *P. kingae*, and *P. cepacia* are synonymous and that *P. cepacia* has priority. We have confirmed this synonymy by subjecting a larger number of strains (15 each) of *P. multivorans* and *P. kingae* to a wider variety of morphological, nutritional, physiological, and biochemical tests than employed in other studies. A total of 127 tests performed on each strain showed that the two groups of strains were 94% similar, differing only in the following nine characters: growth at 41 °C; pigmentation; growth on cetrimide agar; nitrate reduction; acid from sucrose and maltose on O-F medium; utilization of nicotinate and *n*-dodecane as sole carbon sources; and susceptibility to nalidixic acid. Salient properties for both groups of strains are nutritional versatility and accumulation of poly- β -hydroxybutyrate as a cellular reserve material.

Eugonic oxidizers group number one (EO-1) is a distinct group of nonfluorescent, gram-negative pseudomonads originally collected by E. O. King at the Center for Disease Control (CDC), Atlanta, Ga., and characterized by Jonsson (11), who proposed the species name *Pseudomonas kingae* for this group of bacteria. Strains of the EO-1 group were also described by Gilardi (8), who included a partial nutritional characterization of 10 strains.

Pseudomonas multivorans was first described, but not named, by Morris and Roberts (20), who studied 18 strains isolated from Trinidad soil and river water and cited the following as salient physiological characteristics: accumulation of poly- β -hydroxybutyric acid (PHB) as a cellular reserve material; utilization of citrate as a sole source of carbon; proteolysis of gelatin and milk; oxidation of numerous carbohydrates; strong lipolysis; hydrolysis of esculin; intense yellow to reddish-purple pigmentation; and polar multitrichous cells. Some of these Trinidad strains and isolates from clinical sources and soil were later studied by Stanier et al. (30), who proposed the name *Pseudomonas multivorans* for them on the basis of their nutritional, cytological, and biochemical similarities. Stanier et al. also noted that these strains accumulated PHB and were nutritionally versatile in the type and number of organic compounds utilized as carbon sources.

The importance of *P. kingae* and *P. multivorans* as etiological agents of clinical infection has been documented. *P. kingae* strains have been reported as causing necrotizing pneumonitis (7) and bacterial endocarditis (25, 29). In addition, *P. kingae* has been isolated from a benzalkonium chloride disinfecting solution which was responsible for numerous cases of urinary tract infection (9). Pedersen et al. (21) examined strains of *P. multivorans* from sputum, a tracheal aspirate, and blood cultures and concluded that they may be etiologically significant in blood infections. As with *P. kingae*, *P. multivorans* strains have been isolated from various disinfectant solutions in the hospital environment and have been responsible for numerous cases of clinical infection (2, 4, 19). Evidently, both *P. kingae* and *P. multivorans* are opportunistic pathogens potentially hazardous in their ability to contaminate and possibly grow in disinfectant solutions.

Several workers have noted the similarity between *P. kingae* and *P. multivorans*. Pickett and Pedersen (22) compared three strains of *P. kingae* and seven strains of *P. multivorans* on the basis of 52 characteristics and concluded that there was no feature for which one of these groups was uniformly positive and the other uniformly negative. Gilardi (8) also noted this similarity when he compared ten *P. kingae* strains with one strain of *P. multivorans*. However, no formal proposal was made concerning the synonymy of *P. kingae* and *P. multivorans*.

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Ballard et al. (1) established synonymy between *P. multivorans* and the phytopathogen *P. cepacia* Burkholder (5) on the basis of deoxyribonucleic acid homology and other characteristics. Snell et al. (28) examined morphological, biochemical, serological, and other properties of three strains of EO-1, the type strain of *P. kingae*, and two strains of *P. multivorans* (including the type strain) and found them similar to 35 field strains and the type strain of *P. cepacia*. They proposed that members of all of these species are similar and should be designated as *P. cepacia*, the name which has priority. Results obtained in the study reported here support this proposal by confirming the synonymy of *P. kingae* with *P. multivorans*. Fifteen strains of each of these two species, isolated from clinical and environmental sources, were compared on the basis of a wide variety of biochemical and nutritional properties. This report presents the results of a study of a considerably larger number of strains of *P. kingae* and *P. multivorans* than previously studied by other workers. Additional data are presented on resistance of these strains to various antibiotics and on the number and arrangement of their flagella.

MATERIALS AND METHODS

Bacterial strains. Fifteen strains of *P. kingae* (EO-1) were examined and compared with 15 strains of *P. multivorans*. All *P. kingae* strains were received from R. E. Weaver (CDC, Atlanta, Ga.). The type strain for *P. kingae* (A977 = ATCC 25416) designated by Jonsson (11) was not included in this study.

Fifteen of the 19 strains of *P. multivorans*, including the type strain (Stanier's #382 = ATCC 17759) characterized by Stanier et al. (30), were obtained from the American Type Culture Collection. Tables 1 and 2 show designations and sources for all strains used in this study. Initially, cultures were grown at 35 C for 18 to 24 h in flasks of tryptic soy (Difco) broth on a reciprocal shaker and then streaked on plates of yeast extract (YE) agar. The most prevalent colonial type was picked from these plates to establish stock cultures maintained on slants of YE agar, stored at 4 to 5 C, and transferred at 3- to 4-week intervals.

Nutritional (substrate utilization) tests. Nutritional tests were performed essentially as described by

TABLE 1. *Strains of P. kingae* studied

Strain no. ^a	CDC no.	Origin
1	B3720	Urine
2	B3586	Urine
3	B3619	Benzalkonium chloride disinfectant
4	B2118	Baby lotion (with hexachlorophene)
5	B1805	Blood
6	A9894	Cerebral spinal fluid
7	B2505	Lymph node
8	B1731	Baby lotion (with hexachlorophene)
9	A8807	Lung
10	A8653	Urine
11	A8352	Urine
12	A8619	Lung
13	A7734	Urine
14	A7214	Urine
15	B1267	Antiseptic solution

^a Assigned for present study.

TABLE 2. *Strains of Pseudomonas multivorans* studied^a

Strain no. ^b	ATCC no.	Serial no. ^c	Origin and other designations ^d
16	17460	85	River water, Trinidad; B2320
17	17478	104	Soil, California (D-mandelate enrichment at 30 C)
18	17616	249	Soil, California (anthranilate enrichment at 41 C); strain S-4-AN
19	17759	382	Soil, Trinidad (isolate 8); Sneath D335; NCIB 9085; type strain
20	17760	383	Forest soil, Trinidad (isolate 61); Sneath D336; NCIB 9086
21	17761	384	Rotting tree trunk, Trinidad (isolate 7c); Sneath D337; NCIB 9087
22	17763	386	Baby's thermometer, Bristol Royal Infirmary
23	17765	396	Urine (child), Bristol Royal Infirmary; B2394
24	17766	397	Urine (child), Bristol Royal Infirmary; B2443
25	17769	420	Soil, Trinidad; NCIB 9088; HCC 79
26	17770	421	Soil, Trinidad; NCIB 9089; HCC B175
27	17771	422	Soil, Trinidad; NCIB 9091; HCC B338
28	17772	423	Soil, Trinidad; NCIB 9092; HCC B339
29	17773	424	NCIB 9093; HCC B340
30	17774	425	Forest soil, Trinidad; Sneath T1 and B269; NCIB 9135

^a All cultures were purchased from the American Type Culture Collection, Rockville, Md.

^b Assigned for present study.

^c Assigned by Stanier et al. (30).

^d Abbreviations: NCIB, National Collection of Industrial Bacteria; HCC, Hankey Culture Collection.

Stanier et al. (30). Strains were tested for utilization of carbon sources, in both agar and broth media composed of a standard mineral base [Hutner's vitamin-free mineral base (6); 0.08 M potassium phosphate buffer, pH 6.8; 0.1% $(\text{NH}_4)_2\text{SO}_4$] plus 0.1 to 0.2% (wt/vol) of the carbon source. The agar media contained 1% (wt/vol) Noble agar (Difco), and plates were inoculated by the replica plating technique (17). Agar tests were repeated four to six times, and the data were analyzed statistically, using Student's *t* test for determining significant difference (27). Broth media were inoculated with cells grown for 18 to 24 h at 30 C in YE broth and rinsed three times with phosphate buffer (KH_2PO_4 , Na_2HPO_4 , 0.033 M, pH 6.8). The compounds tested are listed in Table 3.

Physiological and biochemical tests. The following tests were performed as described by Stanier et al. (30): denitrification; nitrate and atmospheric nitrogen as sole nitrogen sources; anaerobic growth in glucose medium; ability to grow at 4, 25, 37, 39, and 41 C in YE broth; motility; pigment production; aromatic ring (catechol) cleavage; constitutive arginine dihydrolase production; oxidase production (14); gelatin hydrolysis; lecithinase production (the egg yolk reaction); starch hydrolysis; and Tween 80 hydrolysis.

The following tests for the identification of nonfermentative, gram-negative rods were performed as described by King (12): acid production from six different carbohydrates in the O-F medium of Hugh and Leifson (10) and in a peptone broth medium; acidification of 10% glucose or lactose-phenol red agar slants; and growth on triple sugar-iron agar slants (TSI), MacConkey agar, *Salmonella-Shigella* agar, Simmons' citrate agar, and cetrimide agar (0.08% hexadecyltrimethylammonium bromide). Other tests used were: nitrate reduction; anaerobic growth and acid production in O-F glucose medium; pigment production on medium A (*Pseudomonas* "P" agar) and medium B (*Pseudomonas* "F" agar) of King et al. (13), heart infusion tyrosine, TSI, and motility agars; hydrogen sulfide production on TSI agar; esculin hydrolysis; catalase, urease, and indole production; gelatin liquefaction; and methyl red, Voges-Proskauer, and litmus milk reactions. Colonial morphology and hemolytic reaction were determined on blood agar plates (tryptic soy agar and 5% defibrinated sheep blood; Scott Laboratories, Fiskeville, R.I.) incubated for 48 h at 37 C. All media were from Difco unless otherwise stated.

Cytological observations (cellular morphology). Gram-staining reactions (Hucker's modification) were determined. All strains were examined for flagella arrangement and number by using the modification of Leifson's (18) flagella-staining method described in the CDC *Laboratory Methods Manual* (15). Flagella numbers were determined by the statistical method of Lautrop and Jessen (16). The accumulation of PHB as a cellular reserve material was determined by phase contrast microscopy of wet mounts as described by Stanier et al. (30). Photomicrographs of flagellated cells and PHB-containing cells were prepared by using a $\times 100$ oil immersion, phase contrast objective (numerical aperture, 1.25).

Antibiotic susceptibility tests. All strains were

tested for susceptibility to 17 different antibiotics by using the standardized single-disk diffusion method of Bauer et al. (3) and Ryan et al. (23). All antibiotics were from Difco except for carbenicillin, which was from Baltimore Biological Laboratories, Cockeysville, Md.

RESULTS AND DISCUSSION

Nutritional tests. The results of carbon-substrate utilization tests performed with both basal agar and basal broth media are listed in Table 3. The percentages of strains positive in this table have been converted to + (positive), - (negative), or V (variable) symbols on the basis of the percentage divisions as described by Pickett and Pedersen (22); i.e., + = 85 to 100% of strains positive, V = 16 to 84% of strains positive, and - = 0 to 15% of strains positive. The growth response patterns of *P. kingae* and *P. multivorans* strains were similar when compared by these percentage divisions. The results for substrate utilization tests performed with the basal agar medium represent responses for four to six trials (usually six) on each substrate. By comparison of the symbols assigned on the basis of the percentage divisions, *P. kingae* and *P. multivorans* showed no difference in growth response on 37 of 46 substrates tested on the basal agar medium. Substrates for which different growth responses were noted (Table 3) were *o*-hydroxybenzoate, *m*-hydroxybenzoate, phenol, acetamide, nicotinate, *n*-butanol, ethanol, *n*-propanol, and L(+)-mandelate. However, for the last four of these substrates, the differences between the percentage of positive *P. kingae* strains and the percentage of positive *P. multivorans* strains was minimal (14 to 27% difference) and can be excluded as insignificant. When the original raw data (i.e., number of strains positive for any given replica test on a given substrate) for the tests run on agar plates were analyzed statistically by Student's *t* test, the two sets of strains were not significantly different in ability to utilize 37 of the 46 substrates. The nine substrates for which significant differences ($P < 0.01$) in growth response were noted were D(+)-xylose, L(+)-rhamnose, butyrate, L(+)-mandelate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, phenol, testosterone, and acetamide. Five of these were the same compounds for which differences were found by comparison of the symbols assigned in Table 3.

On the basis of the symbols assigned in Table 3, *P. kingae* and *P. multivorans* showed no difference in growth response on 41 of 49 substrates tested in the basal broth medium. Substrates for which different growth responses were obtained were sucrose, cellobiose, D(+)-

TABLE 3. *Nutritional properties of P. kingae and P. multivorans*^a

Substrate	<i>P. kingae</i> (n = 15)				<i>P. multivorans</i> (n = 15)			
	Agar	% positive ^b	Broth	% positive ^c	Agar	% positive ^d	Broth	% positive ^e
Carbohydrates and sugar derivatives								
D(+)-Xylose	V	80	+	100	V	47	+	93
D-Glucose	NT		+	100	NT		+	100
D(+)-Mannose	+	100	+	100	+	100	+	100
Sucrose	+	100	+	100	+	100	V	67
Maltose	V	20	V	40	V	27	V	67
Cellobiose	+	100	V	73	+	100	+	93
Starch	-	0	-	0	-	0	-	0
Gluconate	+	100	+	100	+	100	+	100
Saccharate	+	100	+	100	+	100	+	100
Mucate	+	100	+	100	+	100	+	100
D-Arabinose	+	100	+	100	+	100	+	100
D(+)-Fucose	V	40	-	7	V	67	V	20
L(+)-Rhamnose	-	0	-	0	-	7	-	7
Fatty acid								
Butyrate	+	93	+	100	+	100	+	100
Dicarboxylic acids								
Glutarate	+	100	+	100	+	100	+	100
Sebacate	+	100	+	100	+	100	+	100
Hydroxyacids								
L(+)-Tartrate	-	0	-	0	-	7	-	0
meso-Tartrate	+	93	+	100	+	87	+	93
DL-β-Hydroxybutyrate	NT		+	100	NT		+	100
Glycolate	V	60	V	60	V	60	+	87
Miscellaneous organic acids								
Pyruvate	+	100	+	100	+	100	+	100
Citraconate	+	100	+	100	+	93	+	100
Glycol								
2,3-Butyleneglycol	+	100	+	100	+	100	+	100
Alcohols								
Ethanol	-	7	V	60	V	33	V	33
n-Propanol	-	0	V	27	V	20	V	27
n-Butanol	+	87	+	93	V	73	+	87
Isobutanol	-	0	-	13	-	0	-	0
Geraniol	-	7	NT		-	0	NT	
Non-nitrogenous and other cyclic compounds								
L(+)-Mandelate	-	0	+	100	V	27	+	100
o-Hydroxybenzoate	-	13	+	100	+	87	+	100
m-Hydroxybenzoate	V	67	+	100	+	100	+	100
p-Hydroxybenzoate	+	100	+	100	+	100	+	100
Non-nitrogenous and other cyclic compounds								
Phthalate	-	0	-	0	-	7	-	7
Phenol	-	0	+	100	V	53	+	93
Testosterone	V	60	NT		V	80	NT	
Aliphatic amino acids								
L(+)-Lysine	+	93	+	100	+	93	+	100
L-Threonine	+	100	+	100	+	100	+	100
L-Isoleucine	V	40	V	47	V	47	V	67
L(+)-Valine	+	93	V	47	+	93	V	80
L-Arginine	NT		+	100	NT		+	100
DL-Ornithine	+	93	+	93	+	93	+	100
Amino acids containing a ring structure								
L(+)-Histidine	+	100	+	100	+	100	+	100
D-Tryptophan	-	0	-	0	-	0	V	40

TABLE 3—Continued

Substrate	<i>P. kingae</i> (n = 15)				<i>P. multivorans</i> (n = 15)			
	Agar	% positive ^a	Broth	% positive ^a	Agar	% positive ^a	Broth	% positive ^a
Amines								
Histamine	V	60	+	100	V	60	+	93
Tryptamine	+	93	+	100	+	100	+	100
Miscellaneous nitrogenous compounds								
Betaine	+	100	+	100	+	100	+	100
Acetamide	V	67	+	100	+	100	+	100
Nicotinate	+	100	+	100	V	53	V	67
Trigonelline	—	0	—	7	—	13	V	20
Paraffin								
Hydrocarbons								
n-Dodecane	NT		—	0	NT		V	47
n-Hexadecane	NT		V	33	NT		V	53

^a Percent positive indicates the percentage of strains able to utilize the substrate. Single tests were performed for each strain on broth media; four to six replica tests were performed for each strain on agar media. Symbols assigned on the basis of the percentage values: +, 100 to 85%; V, 84 to 16%; —, 15 to 0%; NT, Not tested.

^b Strain numbers of positive strains for each substrate in agar medium used by a fraction of the strains of *P. kingae* are the following: xylose, all except 5, 11, 13; maltose, 2, 6, 11; D-fucose, 2, 4, 5, 9, 12, 14; butyrate, all except 13; meso-tartrate, all except 13; glycolate, all except 4, 8, 12, 13, 14, 15; ethanol, 14; n-butanol, all except 11, 13; geraniol, 14; o-hydroxybenzoate, 11, 14; m-hydroxybenzoate, all except 5, 6, 9, 12, 14; testosterone, all except 4, 8, 12, 13, 14, 15; L-lysine, all except 13; L-isoleucine, 1, 3, 6, 7, 11, 15; L-valine, all except 8; DL-ornithine, all except 13; histamine, all except 4, 5, 8, 10, 12, 13; tryptamine, all except 5; acetamide, all except 4, 8, 12, 13, 15.

^c Strain numbers of positive strains for each substrate in broth used by a fraction of the strains of *P. kingae* are the following: maltose, 5, 6, 8, 9, 10, 11; cellobiose, all except 4, 6, 9, 15; D-fucose, 14; glycolate, all except 4, 8, 12, 13, 14, 15; ethanol, all except 5, 7, 10, 11, 13, 15; n-propanol, 1, 2, 3, 12; n-butanol, all except 13; isobutanol, 3, 12; L-isoleucine, 1, 3, 8, 11, 12, 13, 14; L-valine, 1, 4, 8, 12, 13, 14, 15; DL-ornithine, all except 13; trigonelline, 14; n-hexadecane, 2, 3, 7, 10, 12.

^d Strain numbers of positive strains for each substrate in agar medium used by a fraction of the strains of *P. multivorans* are the following: xylose, 17, 18, 19, 22, 23, 24, 29; maltose, 21, 26, 27, 28; D-fucose, all except 16, 17, 18, 25, 27; L-rhamnose, 25; L-tartrate, 17; meso-tartrate, all except 19, 27; glycolate, all except 16, 18, 21, 23, 24, 30; citraconate, all except 18; ethanol, 17, 22, 23, 24, 29; n-propanol, 17, 22, 29; n-butanol, all except 18, 21, 25, 27; L-mandelate, 17, 19, 22, 29; o-hydroxybenzoate, all except 23, 24; phthalate, 18; phenol, all except 18, 19, 21, 22, 23, 24, 28; testosterone, all except 18, 23, 24; L-lysine, all except 30; L-isoleucine, 16, 17, 22, 23, 25, 28, 29; L-valine, all except 18; DL-ornithine all except 18; histamine, all except 18, 23, 24, 26, 27, 30; nicotinate, all except 17, 19, 20, 21, 22, 26, 29; trigonelline, 18, 28.

^e Strain numbers of positive strains for each substrate in broth used by a fraction of the strains of *P. multivorans* are the following: xylose, all except 28; sucrose, all except 16, 18, 20, 25, 30; maltose, all except 16, 17, 18, 23, 24; cellobiose, all except 17; D-fucose, 22, 23, 24; L-rhamnose, 25; meso-tartrate, all except 17; glycolate, all except 23, 24; ethanol, 17, 22, 23, 24, 29; n-propanol, 17, 22, 23, 29; n-butanol, all except 18, 21; phthalate, 18; phenol, all except 28; L-isoleucine, all except 18, 20, 21, 24, 27; L-valine, all except 16, 28, 29; D-tryptophan, 16, 20, 21, 25, 27, 28; histamine, all except 30; nicotinate, all except 17, 20, 21, 26, 29; trigonelline, 18, 23, 28; n-dodecane, 16, 20, 25, 26, 27, 28, 30; n-hexadecane, all except 17, 18, 19, 21, 22, 23, 29.

fucose, glycolate, D-tryptophan, nicotinate, trigonelline, and n-dodecane. Considering all the data represented by the symbols in Table 3, nicotinate and n-dodecane were the only compounds for which gross differences were found between the two groups of strains. In most cases where there were differences between the two groups on one basal medium, there were no differences on the other. For most compounds tested there was a higher percentage of *P. kingae* and *P. multivorans* strains positive for growth when tested with the broth medium than with the agar medium. These apparent discrepancies in growth response on agar or broth media are possibly due to differences in the availability of the carbon substrate in agar as opposed to broth media. Another possible explanation might relate to the manner in

which growth response was scored on agar media. If growth on a particular substrate was very slight in comparison with a positive control, the plate was scored negative. However, slight turbidity in liquid media was also scored as a negative response. Obviously, there is considerable subjectiveness in growth evaluation for nutritional studies. In our experience, responses with broth media were considerably easier to score and most tubes were either densely turbid or obviously negative compared with controls.

Biochemical, physiological, and morphological characteristics. *P. kingae* and *P. multivorans* had similar biochemical, physiological, and morphological properties (Table 4). The following are tests for which differences were noted: growth at 41 C; pigmentation on

TABLE 4. Comparison of characters of *P. kingae* strains with *P. multivorans* strains^a

Character	<i>P. kingae</i> (n = 15)	<i>P. multivorans</i> (n = 15)
Denitrification	— (0)	— (0)
Nitrate reduction	V (47) ^b	— (13) ^c
Gas production in nitrate reduction tests	— (0)	— (0)
Utilization of sole nitrogen sources		
Nitrogen	— (0)	— (0)
Nitrate	+ (100)	+ (100)
Anaerobic growth in glucose medium of Stanier et al. (30)	— (0)	— (0)
Anaerobic growth in O-F glucose medium	— (0)	— (0)
Growth in YE broth at:		
4 C	— (0)	— (0)
25 C	+ (100)	+ (100)
37 C	+ (100)	+ (100)
39 C	+ (100)	+ (100)
41 C	+ (93) ^d	V (60) ^e
Growth on:		
MacConkey agar	+ (100)	+ (87) ^f
<i>Salmonella-Shigella</i> agar	— (0)	— (7) ^g
Simmons' citrate agar	+ (100)	+ (100)
Cetrimide agar	+ (93) ^h	V (40) ⁱ
Pyocyanin, medium A, King et al. (13)	— (0)	— (0)
Fluorescein, medium B, King et al. (13)	— (0)	— (0)
Yellow pigment on TSI agar	V (67) ^j	— (13) ^k
Other pigments on:		
Medium A	V (20) ^l	V (80) ^m
Medium B	— (0)	V (73) ⁿ
YE agar	+ (100)	+ (93) ^o
Heart infusion tyrosine agar	— (0)	V (73) ^p
Semisolid medium	— (0)	V (80) ^q
Intracellular accumulation of poly- β -hydroxybutyrate	+ (100)	+ (100)
Production of catalase	+ (100)	+ (100)
Production of oxidase	+ (100)	+ (100)
Production of indole	— (0)	— (0)
Methyl red test	— (0)	— (0)
Production of acetoin (Voges-Proskauer test)	— (0)	— (0)
Production of H ₂ S (TSI agar)	— (0)	— (0)
Production of opalescence (lecithinase) on egg yolk agar	V (80) ^r	+ (100)
Liquefaction of gelatin, CDC medium (12)	V (33) ^s	V (47) ^t
Hydrolysis of gelatin, Skerman's medium (26)	— (7) ^u	V (47) ^v
Hydrolysis of urea (Christensen)	+ (100)	+ (100)
Hydrolysis of Tween 80	+ (100)	+ (100)
Hydrolysis of esculin	V (80) ^w	V (67) ^x
Hydrolysis of starch	— (0)	— (0)
Peptonization reaction in litmus milk	+ (100)	+ (93) ^y
Constitutive arginine dihydrolase	— (0)	— (0)
<i>ortho</i> cleavage of catechol	+ (100)	+ (100)
Motility	+ (100)	+ (93) ^z
Multitrichous polar flagellation (>1 flagellum/cell)	+ (100)	+ (93) ^{aa}
Oxidative reaction in the Hugh and Leifson O-F test (10)	+ (100)	+ (100)
In peptone broth media, acid from glucose	V (60) ^{bb}	V (60) ^{cc}
In peptone broth media, acid from xylose, mannitol, lactose, sucrose and maltose	— (0)	— (0)
On O-F medium, acid from:		
Glucose	+ (100)	+ (100)
Xylose	+ (93) ^{dd}	+ (87) ^{ee}
Mannitol	V (53) ^{ff}	V (80) ^{gg}
Lactose	+ (100)	+ (100)
Sucrose	+ (93) ^{hh}	V (47) ⁱⁱ
Maltose	+ (100)	V (73) ^{jj}
Acid production from 10% (wt/vol):		
glucose agar	+ (100)	+ (100)
lactose agar	+ (100)	+ (100)

^a Symbols: +, 100 to 85% of strains positive; V, 84 to 16% of strains positive; -, 15 to 0% of strains positive. Numbers in parentheses indicate the percentages of strains positive. All percentages were based on a study of 15 strains each of *P. kingae* and *P. multivorans*, except for arginine dihydrolase percentages, which represent 7 strains from each group.

^b Positive strains: 1, 2, 3, 6, 7, 9, 11.

^c Positive strains: 17, 18.

^d Negative strain: 11.

^e Negative strains: 16, 21, 25, 27, 28, 30.

^f Negative strains: 16, 28.

^g Positive strain: 20.

^h Negative strain: 11.

ⁱ Positive strains: 16, 17, 20, 22, 23, 24.

^j Negative strains: 1, 5, 6, 9, 10.

^k Positive strains: 19, 23.

^l Positive strains: 7, 13, 15.

^m Negative strains: 18, 21, 24.

ⁿ Negative strains: 18, 21, 23, 24.

^o Negative strain: 18.

^p Negative strains: 18, 21, 23, 24.

^q Negative strains: 18, 23, 24.

^r Negative strains: 4, 13, 15.

^s Positive strains: 2, 3, 7, 11, 13.

^t Positive strains: 16, 20, 22, 23, 25, 28, 29.

^u Positive strain: 11.

^v Positive strains: 16, 20, 25, 27, 28, 29, 30.

^w Negative strains: 4, 8, 15.

^x Negative strains: 17, 18, 26, 27, 30.

^y Negative strain: 18 (weakly acid).

^z Negative strain: 23.

^{aa} Negative strain: 23.

^{bb} Negative strains: 2, 3, 5, 7, 13, 15.

^{cc} Negative strains: 16, 20, 22, 28, 29, 30.

^{dd} Negative strain: 11.

^{ee} Negative strains: 16, 28.

^{ff} Negative strains: 3, 4, 6, 7, 8, 9, 10.

^{gg} Negative strains: 17, 18, 27.

^{hh} Negative strain: 8.

ⁱⁱ Positive strains: 17, 19, 22, 23, 24, 26, 29.

^{jj} Negative strains: 19, 22, 25, 29.

various media (medium B, heart infusion tyrosine, TSI, and motility agars); gelatin hydrolysis (Skerman's plated medium; 26); lecithinase production (egg yolk agar); growth on cetrimide agar; nitrate reduction; and acid production from sucrose and maltose in O-F basal medium. The difference noted for gelatin hydrolysis on plates of Skerman's medium was not evident when strains were tested for gelatin liquefaction by using the CDC medium. The difference was probably related to the fact that the CDC medium, which showed a higher number of positive strains for *P. kingae*, was incubated for 7 days, whereas Skerman's medium was incubated for only 48 h. In addition, it should be noted that the difference between the two groups in ability to produce lecithinase was minimal (20% difference).

The differences in pigmentation noted are worthy of explanation. *P. multivorans* (but not *P. kingae*) strains produced a variety of pigments, including greenish-yellow, yellow, pur-

ple, and mixtures of these colors on medium A and medium B. Neither group of strains produced fluorescent pigment (fluorescein) on medium B or pyocyanin on medium A. Production of specific colored pigments by *P. multivorans* seemed to be a function of the medium on which it was grown and the incubation temperature. Even the same strain grown on various carbon substrates in a defined mineral base produced a variety of colored pigments. These findings support the conclusion of Stanier et al. (30) that differences in pigmentation from strain to strain in *P. multivorans* are not correlated with differences in other phenotypic characters and, therefore, are devoid of taxonomic significance. Of interest also is the production by all *P. kingae* strains of yellow and/or buff pigments on YE agar. *P. multivorans*, again, produced a variety of pigments on this medium. Jonsson (11) reported that 66.2% of his *P. kingae* isolates produced yellow pigment only on media containing iron (i.e., TSI agar). In the present study,

67% of *P. kingae* strains produced yellow pigment on TSI agar. Perhaps the production of yellow and/or buff pigments on YE agar is due to the iron content in the standard mineral base or in autolyzed yeast cells.

Colonial morphology and hemolytic reaction. Colonial morphologies for both groups of strains were highly similar. All colonies showed the following characteristics: circular form; convex elevation; entire margin; punctiform (<1 mm) to 1 mm in diameter; smooth, glistening surface; translucent to transparent

by transmitted light; grayish by reflected light; and butyrous consistency. Five strains of *P. multivorans* differed slightly from this description. Colonies of one of these strains had a yellow coloration. Colonies from four strains had slightly mottled surfaces, slightly undulate margins, and a cream-gray coloration, and were slightly larger in diameter (1 to 2 mm). All strains of *P. kingae* and *P. multivorans* showed "lysis" (blood cell lysis but not hemoglobin destruction or decolorization [15]) on blood agar under confluent areas of growth but not under

FIG. 1. Phase contrast photomicrographs ($\times 1,500$) of cells stained for flagella by Leifson's method. (a) *P. kingae* strain no. 1; (b) *P. kingae* strain no. 8; (c) *P. multivorans* strain no. 18; (d) *P. multivorans* strain no. 21.

isolated colonies. The edges of "lysis" zones were ill defined. Most *P. multivorans* strains also showed a slight greening of these zones.

Motility and flagella arrangement and number. All strains of *P. kingae* were motile as determined in semisolid medium. All but one strain (23 = ATCC 17765) of *P. multivorans* were motile. Flagella-stained preparations showed all strains from both groups to be polar multitrichous, with most cells showing two to five polar flagella (Fig. 1). The only exception was the nonmotile strain of *P. multivorans*, which showed few flagellated cells; some of

these cells showed polar flagella and some showed lateral flagella. However, lack of motility and the presence of a small number of laterally flagellated cells was not related to other phenotypic differences. The statistical method of Lautrop and Jessen (16) was applied to evaluate the strains as monotrichous or multitrichous and to demonstrate the similarity in flagella numbers between the two sets of strains. The number of polar flagella was counted on 50 to 100 cells of each strain, and a flagella index was computed by combining the percentages of all flagellated cells possessing more than one

Fig. 1. C-D.

flagellum per pole. The results of these counts (Table 5) showed *P. kingae* and *P. multivorans* to be multitrichous and closely similar in flagella numbers and indexes. The computed flagella indexes for *P. kingae* and *P. multivorans* were 64.3 and 69.7, respectively.

PHB accumulation. Phase microscopy of wet mounts of *P. kingae* and *P. multivorans* grown for 48 h at 30 C in the basal broth medium [0.02% (NH₄)₂SO₄] with DL- β -hydroxybutyrate as carbon source showed all strains to contain massive intracellular inclusions of PHB (Fig. 2).

Antibiotic susceptibilities. The antibiotic susceptibilities of *P. kingae* and *P. multivorans* as determined by standardized single-disk diffusion tests are listed in Table 6. *P. kingae* strains were resistant to 12 of 17 antibiotics tested, whereas *P. multivorans* strains were resistant to 11. Antibiotics for which both groups exhibited variable sensitivities were neomycin, chloramphenicol, kanamycin, gentamicin, and nalidixic acid. In addition, *P. multivorans* showed variable sensitivity to carbenicillin. Considering only differences in all three susceptibility groups, *P. kingae* and *P. multivorans* differed only in their susceptibility to nalidixic acid and carbenicillin. However, the difference in susceptibility to carbenicillin between the two groups of strains was minimal (27%).

On the basis of the assigned symbols (+, -, V), there was, overall, a 90% similarity between the 15 strains of *P. kingae* and the 15 strains of *P. multivorans* in response to the 127 tests performed. A total of 26 differences were detected on the basis of these symbols, but 17 can be excluded as not different for the reasons discussed earlier. If these 17 reactions are excluded, then the two groups showed an overall

similarity of 94%. A listing of the remaining nine test reactions that differed between the two groups as compared with the reactions found for the type strain of *P. multivorans* is shown in Table 7. Three of these characteristics for *P. kingae*, although different from the composite result for the 15 strains of *P. multivorans*, were the same as those found for the type strain.

In the taxonomic study of Stanier et al. (30), 14 characters were selected that seemed to be of greatest differential value in the recognition of *P. multivorans*. Table 8 shows a comparison of strains of *P. kingae* and *P. multivorans* used in this study with the ideal phenotype of *P. multivorans* based on these 14 selected characters. The strains of *P. kingae* and *P. multivorans* tested in this study were identical when compared on the basis of these 14 characters. The ideal phenotype of Stanier et al. for *P. multivorans* differs from strains of *P. kingae* in the utilization of D-fucose; however, it also differs from the 15 test strains of *P. multivorans*.

On the basis of the similarities in the patterns of the test results, we have concluded that *P. kingae* (EO-1) and *P. multivorans* are indistinguishable, thus confirming the results of Snell et al. (28). The data presented here have extended their studies by inclusion of a larger number of strains and of additional characters not previously used for comparison of these organisms. These additional tests included determination of flagella number and arrangement and antibiotic susceptibility, as well as ability to utilize numerous additional carbon compounds. Ballard et al. (1) and Sands et al. (24) have established that the names *P. multivorans* and *P. cepacia* are synonymous and, according to Snell et al., the name *P. cepacia* has priority over all later synonyms.

TABLE 5. Flagella counts in strains of *P. kingae* and *P. multivorans*

Organism	No. of strains	Total no. of cells counted ^a	Percentage of flagellated cells having the indicated number of flagella ^b								Flagella index ^c
			1	2	3	4	5	6	7	8	
<i>P. kingae</i>	15	1,450 ^d	35.7	33.9	19.5	7.4	1.9	1.1	0.2	0.3	64.3
<i>P. multivorans</i> . . .	14	1,300 ^e	30.3	26.7	23.8	10.8	5.0	2.1	1.1	0.2	69.7

^a Unless otherwise indicated, 100 cells/strain were counted.

^b Percentages calculated on the basis of 4 to 8 flagella/cell are approximations.

^c Combined percentages of all flagellated cells having more than one flagellum/pole.

^d Strain number 14; 50 cells counted.

^e Strain numbers 18 and 26; 50 cells counted. For strain number 23, no counts were performed because most cells were aflagellate or poorly stained.

FIG. 2. Phase contrast photomicrographs ($\times 1,500$) of wet mounts of cells grown for 48 h in a medium containing *DL*- β -hydroxybutyrate. (a) *P. kingae* strain no. 5; (b) *P. kingae* strain no. 11; (c) *P. multivorans* strain no. 16; (d) *P. multivorans* strain no. 29; (e) *P. aeruginosa*. Note accumulation of poly- β -hydroxybutyrate as intracellular inclusions in all strains (a-d) except the negative control (e).

Fig. 2. C-D.

Fig. 2. *E.*TABLE 6. Antibiotic susceptibilities of *P. kingae* and *P. multivorans* strains^a

Antibiotic	Potency ^b	<i>P. kingae</i>			<i>P. multivorans</i>		
		R	S	I	R	S	I
Ampicillin	10	+	(100)	—	(0)	—	(0)
Neomycin	30	V	(67)	—	(13) ^d	V	(20) ^e
Chloramphenicol	30	—	(0)	V	(67)	V	(33) ^h
Kanamycin	30	V	(60)	V	(27) ^k	—	(13) ⁱ
Tetracycline	30	+	(100)	—	(0)	+	(93)
Gentamicin	10	V	(73)	V	(27) ^p	—	(0)
Nalidixic acid	30	V	(20) ^r	V	(27) ^s	V	(53)
Nitrofurantoin	300	+	(100)	—	(0)	+	(100)
Lincomycin	2	+	(100)	—	(0)	+	(100)
Colistin	10	+	(100)	—	(0)	+	(93)
Penicillin G	10	+	(100)	—	(0)	+	(100)
Methicillin	5	+	(100)	—	(0)	+	(100)
Erythromycin	15	+	(100)	—	(0)	+	(100)
Cephalothin	30	+	(100)	—	(0)	+	(100)
Dihydrostreptomycin	10	+	(93)	—	(0)	+	(100)
Cephaloglycin	30	+	(100)	—	(0)	+	(100)
Carbenicillin	50	+	(100)	—	(0)	V	(73)

^a Abbreviations: R, Resistant; S, susceptible; I, intermediate. Parenthetical values refer to percentages of strains showing indicated susceptibility. For the purpose of comparison, these values have been converted to the following symbols: +, 100 to 85%; V, 84 to 16%; —, 15 to 0%.

^b Disk concentration in micrograms except for penicillin G, which is expressed in international units.

^c Sensitive strain: 21.

^d Sensitive strains: 11, 13.

^e Intermediate strains: 4, 8, 12.

^f Sensitive strains: 21, 22.

^g Intermediate strains: 16, 18, 28, 30.

^h Intermediate strains: 1, 2, 11, 13, 14.

ⁱ Resistant strain: 22.

^j Intermediate strains: 23, 24, 27.

^k Sensitive strains: 4, 8, 11, 13.

^l Intermediate strains: 12, 15.

^m Sensitive strains: 17, 18, 21, 22.

ⁿ Intermediate strains: 19, 29.

^o Intermediate strain: 18.

^p Sensitive strains: 4, 8, 11, 13.

^q Sensitive strains: 16, 18, 21, 22, 28.

^r Resistant strains: 6, 9, 13.

^s Sensitive strains: 2, 4, 8, 15.

^t Resistant strains: 18, 22, 23, 24.

^u Intermediate strains: 17, 27.

^v Sensitive strain: 21.

^w Intermediate strain: 13.

^x Sensitive strains: 20, 21, 26, 30.

TABLE 7. Differences between strains of *P. kingae* and *P. multivorans* compared with data for the type strain of *P. multivorans*^a

Character	<i>P. kingae</i>	<i>P. multivorans</i>	<i>P. multivorans</i> ATCC 17759
Biochemical tests			
Growth at 41 C	+	V	+
Pigmentation	—	+	+
Growth on cetrinide agar	+	V	—
Nitrate reduction	V	—	—
On O-F medium, acid from:			
Sucrose	+	V	+
Maltose	+	V	—
Utilization tests			
Nicotinate	+	V	—
<i>n</i> -Dodecane	—	V	—
Antibiotic susceptibility			
Nalidixic acid	I	S	S

^a Symbols: +, Positive; —, negative; V, variable; I, intermediate; S, sensitive.

TABLE 8. Comparison of *P. kingae* and *P. multivorans* on the 14 selected characters of greatest differential value in the recognition of *P. multivorans*^a

Character	<i>P. multivorans</i> ^b		<i>P. kingae</i> ^c	<i>P. multivorans</i> ^c
	No. of positive strains	Ideal phenotype		
Poly- β -hydroxybutyrate as cellular reserve material	19	+	+	+
Utilization of:				
D-Arabinose	19	+	+	+
D(+)-Fucose	19	+	V	V
Cellobiose	19	+	+	+
Saccharate and mucate	19	+	+	+
2,3-Butyleneglycol	19	+	+	+
Sebacate	19	+	+	+
meso-Tartrate	19	+	+	+
Citraconate	17	+	+	+
<i>o</i> -Hydroxybenzoate	14	+	+	+
<i>m</i> -Hydroxybenzoate	19	+	+	+
L-Threonine	19	+	+	+
DL-Ornithine	18	+	+	+
Tryptamine	19	+	+	+

^a Symbols: +, Positive; V, variable.

^b Data from the study of Stanier et al. (30) of 19 strains of *P. multivorans*.

^c Data from the present study based on 15 strains each of *P. kingae* and *P. multivorans*. Substrate utilization tests were performed on agar media except for *o*-hydroxybenzoate and *m*-hydroxybenzoate, which were performed in broth media.

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