Identification of *Pseudomonas cepacia* Burkholder and Its Synonymy with *Pseudomonas kingii* Jonsson

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Thirty-five strains of *Pseudomonas cepacia* Burkholder, isolated over the last 6 years, were compared with three EO-1 strains, two strains of *P. multivorans* Stanier et al., and the type strains of *P. cepacia* and *P. kingii* Jonsson, the name of the latter having been given to the EO-1 group. The strains were examined in a range of morphological, biochemical, and carbon substrate utilization tests, and, for selected strains, by electron microscopy, deoxyribonucleic acid base composition, serotyping, and phage typing. From the results of these tests, *P. kingii* Jonsson 1970 (EO-1 group) is considered a later subjective synonym of *P. cepacia* Burkholder 1950 (*P. multivorans*). The outstanding property of *P. cepacia* is its nutritional versatility, which is reflected in the large number of carbohydrates from which acid is produced by this organism. A table is given for differentiating *P. cepacia* from *P. pseudomallei*, *P. mallei*, *P. aeruginosa*, *P. fluorescens*, *P. stutzeri*, and *P. maltophilia*.

In recent years, a number of papers have appeared concerning a nonfluorescent, nutritionally versatile pseudomonad variously called *Pseudomonas cepacia* Burkholder 1950, *P. multivorans* Stanier, Palleroni and Doudoroff 1966, *P. kingii* Jonsson 1970, or EO-1 group ("eugonic oxidizers, group 1"; King, 18). Ballard et al. (1) established the synonymy of *P. multivorans* with Burkholder's (4) earlier-described *P. cepacia*, a phytopathogenic species, the latter name having nomenclatural priority. Other workers have noted the similarity between *P. cepacia* and *P. multivorans* (37) and between these and EO-1 strains, later called *P. kingii* by Jonsson (17; see also 8, 9, 23, 33). However, no formal proposal appears to have been made establishing *P. kingii* (EO-1) as another junior synonym of *P. cepacia*.

During the last 6 years we have identified 35 strains as *P. cepacia*. These strains, and two strains of *P. multivorans*, were found to be very similar to three representative EO-1 strains, in agreement with above-quoted authors. The same range of tests used in characterizing the *P. cepacia* strains was then applied to the type strain of *P. cepacia* (ATCC 25416; NCTC 10743) and the type strain of *P. kingii* (ATCC 25609; NCTC 10744) to establish whether the latter is a synonym of *P. cepacia*.

P. cepacia is of considerable clinical importance. Under one or other of the above-mentioned names, it is referred to by Hardy, Ederer, and Matsen (12) and by Phillips et al. (32) as a low-grade pathogen, but its ability to contaminate and perhaps grow in certain disinfectants in current use in hospitals (2) makes it a potentially dangerous organism. Reports of the isolation of this organism (again, variously named) from the hospital environment and from pathological material have been made by a number of authors (2, 3, 7-9, 12, 23, 27, 32, 42, 44, 48). Both Jonsson (17) and Hugh (15) listed many sources from which this organism has been isolated.

In addition to establishing formally the synonymy of *P. kingii* with *P. cepacia*, the present paper gives a full characterization of *P. cepacia* to assist differentiation from other pseudomonads likely to be encountered from hospital sources and adds to the clinical documentation of this pathogen.

MATERIALS AND METHODS

Organisms. Thirty-five field strains of *P. cepacia* were examined and compared with three EO-1 strains (see Table 1) and with the following NCTC strains: NCTC 10661 (strain Hayward 8; ATCC 17759; Stanier's

TABLE 1. *Field strains used in the present work*

No. of strains	Strain designations	Received from	Source or clinical condition	References
1	A73/66	R. L. McCorry, Belfast, UK	Blood culture	2
7	A28-34/69	K. J. Stokes, Surrey, UK	Infected wounds	
4	A38-41/69	K. J. Stokes, Surrey, UK	Savlon	
2	A46-47/69	K. J. Stokes, Surrey, UK	Waters	
4	A64-67/69	D. C. Jenkins, Kansas, USA	Urine	
1	A102/69 ^a	R. E. Weaver, Atlanta, USA	Urine	7
1	A103/69 ^a	R. E. Weaver, Atlanta, USA	Detergicide	
1	A104/69 ^a	R. E. Weaver, Atlanta, USA	Pneumonitis	
1	A107/69 ^b	R. E. Weaver, Atlanta, USA	Endocarditis	11
3	A61-62/70, A115/70	I. Phillips, London, UK	Blood cultures	32
4	A99/70, A109/70, A111/70, A113/70	I. Phillips, London, UK	Respirators	
1	A98/70	E. Lockey, London, UK	Blood culture	
1	A105/70	E. Lockey, London, UK	Intravenous fluid	42
2	A104/70, A116/70	E. Lockey, London, UK	Cetavlon	
1	A117/70	E. Lockey, London, UK	Savlon	
4	A106-108/70, A136/70	D. C. E. Speller, Bristol, UK	Blood cultures	

^a Received as EO-1 strains.^b Received as a IVD strain.

number 382), the type strain designated for *P. multivorans* by Stanier et al. (43); NCTC 10734 (strain Sneath B269, TI; ATCC 17774; Stanier 425), received from P. H. A. Sneath ("Chromobacterium ianthinum," Sneath [40, 41]) and identified as *P. multivorans* by Stanier et al. (43); NCTC 10743 (strain Stanier 717; ATCC 25416), the type strain designated for *P. cepacia* by Ballard et al. (1); NCTC 10744 (strain Jonsson A977, ATCC 25609), the type strain designated for *P. kingii* by Jonsson (17).

The episodes from which most of the field strains were obtained are described in the references included in Table 1. The following episodes have not been previously described: (i) One strain from R. L. McCorry, isolated from a blood culture of a hydrocephalic child in whom a Wade valve had been inserted. (ii) Four strains from D. C. Jenkins, isolated from urine and sent to us for identification with no further details. (iii) Five strains from E. Lockey, from a 42-year-old male who was admitted to hospital with a history of mild chest pain of 2 years' duration, which became more severe. Continuous intravenous infusion of heparin was started, but in a few days the patient developed a temperature of 104 F with rigors. A strain of *P. cepacia* was isolated from a blood culture taken at this time. After initial failure with gentamicin, Septrin was administered, and the patient became afebrile. Subsequently, further strains of *P. cepacia* were isolated twice from Cetavlon (centrimonium bromide) and from Savlon (chlorhexidine and cetrimide) in use in the ward, and from intravenous fluid being given to another patient.

A summary of the sources of the strains examined, excluding the NCTC strains, is given in Table 2.

Bacteriological investigations. Biochemical tests in routine use in the National Collection of Type Cultures (NCTC) and Computer Trials Department

TABLE 2. *Summary of the sources of the field strains*

Source	Origin		Totals
	UK	USA	
Human			
Blood cultures	9	2	23
Wounds	7		
Urine		5	
Miscellaneous			
Disinfectants	7	1	15
Water	2		
Respirators	4		
Drip-line	1		
Totals	30	8	38

were used for bacteriological investigations. Most of these tests were described by Cowan and Steel (5). The tests used are listed in Table 3 and comprise the same range of tests used by Lapage, Hill, and Reeve (22), with the following changes or additions. Nitrate reduction aerobically and anaerobically was tested by Crosby's (6) modification of the Griess-Ilosvay method, in which 1-naphthylamine-7-sulfonic acid (Cleve's acid) replaces 1-naphthylamine; selenite reduction (32); Tween 80 hydrolysis (38); hydrolysis of esculin (5) and tyrosine (10); Richard's (36) method of detecting breakdown of lysine and ornithine as well as Møller's (28) method; and growth on deoxycholate agar and serum liquefaction (5). In addition, carbon substrate utilization tests (43) were carried out on 30 of the strains, using the chemically defined medium of Owens and Keddle (31) and with the carbon substrates (listed in Table 4) at a final concentration of 0.2% (w/v).

TABLE 3. Characters of the field strains of *Pseudomonas cepacia* compared with EO-1 strains and the four NCTC strains

Characters	Field strains ^a	EO-1 strains ^a	NCTC strains				Composite result for <i>P. cepacia</i> ^b
			10661	10734	10743	10744	
Motility	31/35	3/3	+	+	+	+	+
> 1 Flagellum/cell.	17/17	3/3	+	NT	NT	NT	+
Growth on nutrient agar, at 5 C	0/35	0/3	—	—	—	—	—
Growth on nutrient agar, at 44 C	13/35	3/3	—	—	—	—	<i>d</i>
Growth on MacConkey agar	29/29	3/3	+	+	+	+	+
Growth on deoxycholate agar	27/34	1/3	+	—	NT	NT	<i>d</i>
Growth in citrate media (Koser, Simmons, and Christensen)	35/35	3/3	+	+	+	+	+
Growth in KCN media	6/32	0/3	+	—	NT	NT	—
Growth in 0.4% (w/v) selenite	0/23	0/3	—	—	—	—	—
Yellow pigment on nutrient agar	4/30	0/3	+	—	+	—	—
Yellow pigment on medium A of King et al. (19)	4/34	0/3	+	—	—	—	—
Pyocyanin, medium A, King et al. (19)	0/34	0/3	—	—	—	—	—
Fluorescein, medium B, King et al. (19)	0/34	0/3	—	—	—	—	—
Intracellular accumulation of poly- β -hydroxybutyrate	35/35	3/3	+	+	+	+	+
Production of catalase	35/35	3/3	+	+	+	+	+
Production of cytochrome oxidase (Kovacs, 20)	35/35	3/3	+	+	+	+	+
Production of β -galactosidase	19/27	3/3	+	—	+	+	<i>d</i>
Amino-acid breakdown:							
Arginine (Møller, 28)	0/35	0/3	—	—	—	—	—
Lysine (Møller, 28)	6/35	1/3	—	—	+	+	<i>d</i>
Ornithine (Møller, 28)	1/35	0/3	—	—	—	+	—
Arginine (Thornley, 45)	0/35	0/3	—	—	—	—	—
Lysine (Richard, 36)	23/23	3/3	+	+	+	+	+
Ornithine (Richard, 36)	9/23	3/3	—	+	—	+	<i>d</i>
Production of indole	0/35	0/3	—	—	—	—	—
Production of H ₂ S (lead acetate papers)	0/35	0/3	—	—	—	—	—
Production of H ₂ S (triple-sugar-iron agar)	0/23	0/3	—	—	—	—	—
Production of opalescence on egg-yolk-agar	27/35	3/3	+	+	+	+	+
Production of acetoin	0/35	0/3	—	—	—	—	—
Methyl red test	0/35	0/3	—	—	—	—	—
Nitrate reduction, aerobically	10/35	2/3	+	—	—	+	<i>d</i>
Nitrate reduction, anaerobically	12/35	2/3	—	—	—	+	<i>d</i>
Nitrite reduction, aerobically	0/35	0/3	—	—	—	—	—
Gas production in nitrate reduction tests	0/35	0/3	—	—	—	—	—
Liquefaction of gelatin	31/35	3/3	+	+	+	+	+
Liquefaction of serum	0/34	0/3	—	—	NT	NT	—
Hydrolysis of urea (Christensen)	34/35	3/3	+	+	+	+	+
Hydrolysis of Tween 80	24/24	3/3	+	+	+	+	+
Hydrolysis of esculin	9/23	3/3	—	+	+	—	<i>d</i>
Hydrolysis of tyrosine	24/24	3/3	+	+	+	+	+
Hydrolysis of starch	0/35	0/3	—	—	—	—	—
Oxidation of gluconate	1/35	0/3	—	—	—	—	—
Utilization of malonate	3/35	3/3	—	—	—	—	—
Deamination of phenylalanine	0/35	0/3	—	—	—	—	—
Alkaline reaction in purple milk	31/32	3/3	+	+	+	+	+
Casein digestion	32/35	3/3	+	+	—	—	+
Oxidative reaction in the Hugh and Leifson (16) O/F test	35/35	3/3	+	+	+	+	+
In peptone water media, acid from glucose	21/35	0/3	—	—	+	+	<i>d</i>
In peptone water media, acid from other carbohydrates	0/24	0/3	—	—	—	—	—

TABLE 3—Continued

Characters	Field strains ^a	EO-1 strains ^a	NCTC strains				Composite result for <i>P. cepacia</i> ^b
			10661	10734	10743	10744	
On ammonium salt media, acid from:							
Glucose	35/35	3/3	+	+	+	+	+
Arabinose	34/34	3/3	+	+	+	+	+
Cellobiose	20/20	3/3	+	+	+	+	+
Dulcitol	28/28	3/3	NT	+	+	+	+
Ethanol	6/24	0/3	+	—	—	—	<i>d</i>
Galactose	20/20	3/3	+	+	+	+	+
Glycerol	20/20	3/3	+	+	+	+	+
Inositol	20/20	3/3	+	+	+	+	+
Inulin	0/20	0/3	—	—	—	—	—
Lactose	35/35	3/3	+	+	+	+	+
Fructose	20/20	3/3	+	+	+	+	+
Maltose	35/35	3/3	+	+	+	+	+
Mannitol	35/35	3/3	+	+	NT	NT	+
Mannose	20/20	3/3	+	+	+	+	+
Rhamnose	0/20	0/3	—	—	—	—	—
Salicin	33/35	3/3	+	+	+	+	+
Sorbitol	35/35	3/3	+	+	+	+	+
Sucrose	28/35	3/3	+	—	+	+	+
Trehalose	20/20	3/3	+	+	+	+	+
Xylose	35/35	3/3	+	+	+	+	+
Acid production from 10% (w/v) lactose agar	33/35	3/3	+	—	+	+	+

^a Fractional entries are the number of strains positive out of the number of strains tested. NT, Not tested.

^b Symbols; +, 100 to 80% of strains positive; *d*, 79 to 21% of strains positive; —, 20 to 0% of strains positive.

Twenty-two strains were examined for arrangement of flagella by Plimmer and Paine's (34) staining technique, and two additional strains (NCTC 10661 and no. A32/69) were examined by electron microscopy. Deoxyribonucleic acid (DNA) base compositions, expressed as moles percent of guanine plus cytosine (% GC) of the moles of total bases, were determined for these same two strains and for NCTC 10743 and 10744. DNA was extracted by the method of Marmur (25). The % GC values were estimated from melting temperatures, (T_m ; Marmur and Doty, 26), determined in 0.1 M NaCl plus 0.01 M phosphate buffer. The equation relating % GC to T_m for this buffer, derived from the general equation of Owen, Hill, and Lapage (30), was: % GC = $2.362 T_m - 155.32$.

Selected strains (see Results) were examined in the Cross-Infection Reference Laboratory, Colindale, for susceptibility to *P. aeruginosa* bacteriophages and agglutination with *P. aeruginosa* antisera and with a *P. cepacia* antiserum, supplied by courtesy of D. Taplin, Miami.

RESULTS

The results obtained with the field strains, EO-1 strains, and NCTC strains are listed in

Table 3 for most of the tests and in Table 4 for the carbon substrate utilization tests. Following the symbols used by Cowan and Steel (5; i.e. 100 to 80% of strains positive = +; 79 to 21% of strains positive = *d*; 20 to 0% of strains positive = —), the fractional entries in the first columns of results in Tables 3 and 4 reduce to 17 "d" entries in the last columns out of a total of 112 tests. Of these 17 tests, 14 gave different results between the four NCTC strains; for example, aerobic reduction of nitrate was scored positive for 10 out of 35 strains tested, and 2 positive out of the three EO-1 strains, but NCTC 10661 and 10744 were positive whereas NCTC 10734 and 10743 were negative.

No further comment on most of the above results is required except the following points.

Viability. During the course of this work, a number of strains died. Loss of viability appeared erratic but has been noted also by Mackel (23) with reference to EO-1 strains. No loss of viability was found in the present series of strains when cultures were deliberately left at room temperature in the dark and periodically subcultured.

TABLE 4. Results of the carbon substrate utilization tests^a

Substrate ^b	Field strains ^c	EO-1 strains ^c	NCTC strains				Composite result for <i>P. cepacia</i> ^d
			10661	10734	10743	10744	
<i>m</i> -Hydroxybenzoate	23/23	2/3	+	+	+	+	+
Pelargonate	16/23	3/3	+	—	+	+	<i>d</i>
Hippurate	20/23	1/3	+	+	+	+	+
D-Pantothenate	19/23	3/3	+	+	+	+	+
Nicotinate	11/23	3/3	—	+	—	+	<i>d</i>
Benzylamine	24/23	2/3	+	—	—	—	<i>d</i>
DL-Serine	16/23	3/3	+	+	+	+	<i>d</i>
D-Xylose	16/23	3/3	+	—	+	+	<i>d</i>
Propionate	29/23	3/3	+	—	+	+	<i>d</i>
Glyoxylic acid	22/23	3/3	+	+	+	+	+
Glycolate	14/23	2/3	+	+	+	+	<i>d</i>

^a Substrates utilized by all the strains examined: α -ketoglutarate, pyruvate, succinate, acetate, fumarate, citrate, lactate, malonate, D-glucose, *n*-valerate, *meso*-inositol, L-valine, mannitol, betaine, sucrose, L-arginine, adipate, sarcosine, DL-tryptophan, L-hydroxyproline, DL-ornithine, DL-glutamic acid, L-histidine, L-asparagine, DL-aspartic acid, L-proline. Substrates not utilized by any of the strains examined: maleate, creatinine, urea, DL-methionine, catechol, oxalate.

^b Substrates utilized by some of the strains examined.

^c Fractional entries are the number of strains positive out of the number of strains tested.

^d Symbols: +, 100 to 80% of strains positive; *d*, 79 to 21% of strains positive; —, 20 to 0% of strains positive.

Motility and flagella arrangement. Four strains (A106/70, A107/70, A117/70, A115/70) appeared nonmotile by the hanging-drop method, but further tests for motility were not made, as these strains were identified from their biochemical reactions as *P. cepacia*. Flagella staining by the method of Plimmer and Paine (34) showed a multitrichous polar arrangement, though in some cases there also appeared to be some lateral flagella. Two strains (NCTC 10661 and no. A32/69) were examined in the electron microscope by negative staining with 2% (w/v) ammonium molybdate. The polar arrangement of the flagella was then clearly seen (Fig 1, 3). Most cells possessed two or three flagella, but occasionally up to 10 flagella per cell were observed (Fig. 2). In Fig. 2, there is an example of a flagellum probably winding back upon the cell and appearing to emerge laterally. The wavelengths of the extended flagella were 2.05 to 2.16 μ m. Strain NCTC 10661 also showed peritrichous fimbriae (Fig. 3), though none were seen with A32/69. The fimbriae were 10 nm in diameter, which is in good agreement with the figure given by Tweedy, Park, and Hodgkiss (46), who also observed fimbriae on another strain of *P. cepacia* (*P. multivorans*, NCIB 9691).

Pigmentation. No fluorescent pigments were observed. Burkholder (4), Morris and Roberts (29), Mackel (23), and Taplin et al. (44) reported that strains of *P. cepacia* produce

various pigments: yellow, brown, red, violet, and purple. Stanier et al. (43), Jonsson (17), Gilardi (8), and Pickett and Pedersen (33) all recognized that pigmentation was variable and that nonpigmented strains could be found. A yellow pigment was produced by only four of the field strains and by NCTC 10661 and 10743 on nutrient agar. This yellow pigment did not diffuse into the surrounding agar, unlike that described by Hugh (15). No other pigments were observed.

Acid from carbohydrates. The outstanding property of *P. cepacia* is its nutritional versatility. This is shown by the very wide range of carbon substrates it can utilize (*see below*) and by its ability to produce acid from most carbohydrates tested. As with most pseudomonads, results are usually negative in peptone-water medium (J. J. S. Snell, Thesis, Fellowship of the Institute of Medical Laboratory Technology, 1970). Although 21 strains out of 35 tested scored as positive for acid from glucose in peptone-water medium (Table 3), the reaction obtained was weak, and no positive reactions were recorded for the other 18 carbohydrates tested (not shown in the table). On the ammonium salt medium of Smith, Gordon, and Clark (39), *P. cepacia* is readily recognized by production of acid from most of the wide range of carbohydrates tested: glucose, arabinose, cellobiose, dulcitol, galactose, glycerol, inositol, lactose, fructose, maltose,

FIG. 1. *Electron photomicrograph of field strain no. A32/69, showing the polar arrangement of the flagella. $\times 20,000$.*

FIG. 2. Electron photomicrograph of field strain no. A32/69, showing 11 to 12 polar flagella and a few flagella apparently winding back upon the cell and appearing to emerge laterally. $\times 30,000$.

FIG. 3. *Electron photomicrograph of NCTC 10661, showing polar flagella and fimbriae. $\times 30,000$.*

mannitol, mannose, sorbitol, trehalose, xylose, and usually salicin and sucrose. Only a few strains produced acid from ethanol, and no strains produced acid from inulin or rhamnose.

Utilization tests. Of the 42 different carbon substrates tested, 36 were also used by Stanier et al. (43), who had tested NCTC 10661. For 34 of these substrates, our results for this strain were the same as those obtained by Stanier et al. In our laboratory, NCTC 10661 utilized pantothenate and valine, whereas Stanier et al. obtained negative results with these two substrates. Similarly, 33 substrates of the present set were used by Ballard et al. (1), who had tested NCTC 10743. Again, only two dis-

crepant results were obtained: in our laboratory this strain utilized sarcosine and did not utilize benzylamine, whereas Ballard et al. (1) obtained the opposite results for NCTC 10743 and these two substrates. Therefore, our results are sufficiently well correlated with those of Stanier et al. and Ballard et al. to allow valid comparisons to be made. Of the 11 carbon substrates for which fractional entries are made in Table 4, one (glyoxylic acid) was not used by Stanier et al., whereas seven (hippurate, nicotinate, benzylamine, DL-serine, D-xylose, propionate, and glycolate) also gave variable results with the 19 strains of *P. cepacia* characterized by Stanier et al., although only one of these

(benzylamine) gave variable results with the 18 strains later characterized by Ballard et al.

DNA base compositions. The % GC values determined were as follows: NCTC 10661, 69.3; A32/69, 69.5; NCTC 10743, 69.2; NCTC 10744, 69.0. These values can be considered the same, within the limits of experimental error ($\pm 0.4\%$ GC, Owen et al., 30). The values for NCTC 10661 and 10743 are a little higher than those given by Mandel (24) and Ballard et al. (1) for these same two strains: + 2.5% GC and + 1.4% GC, respectively. However, their determinations were made by a different technique (buoyant density), and differences of this magnitude from one laboratory to another are not uncommon (13, 14).

Phage typing. None of the strains tested were susceptible to any of the *P. aeruginosa* bacteriophages in use at Colindale.

Serotyping. Basset et al. (2) reported that the strains isolated from the outbreak they described gave a cross-reaction with *P. aeruginosa* antiserum 13 (47). Other strains from the present series did not give this cross-section. Only four out of 13 strains tested agglutinated in a slide test with a *P. cepacia* antiserum (positive: A73/66, A46/69, A104/69, A98/70; negative: 10661, A66/69, A67/69, A102/69, A103/69, A107/69, A61/70, A62/70, A99/70).

DISCUSSION

The characteristics of the 35 field strains and the three EO-1 strains agree with those found for the type strain of *P. cepacia* (NCTC 10743), the two NCTC strains of *P. multivorans* (NCTC 10661 and 10734) and the type strain of *P. kingii* (NCTC 10744). When different results were obtained between the field and NCTC strains, in most cases either the NCTC strains also differed from each other or the results were for tests which Stanier et al. (43) also

found to be variable for this species. The description of *P. cepacia*, as amended by Stanier et al. (43) and Ballard et al. (1), lays particular emphasis on the very wide range of carbon substrates this species is able to utilize. In this respect, agreement was very close between the field strains, the NCTC strains, and with the published data of Stanier et al. and Ballard et al.

The synonymy of *P. cepacia* Burkholder 1950 and *P. multivorans* Stanier et al. 1966 was established by Ballard et al. (1) on the basis of indistinguishable patterns of test results and of similar levels of molecular homology as shown by in vitro DNA-DNA molecular hybridization techniques. In addition to this synonymy, we propose that *P. kingii* also be considered a synonym of *P. cepacia*, the latter name having priority. This proposal is also made on the basis of indistinguishable patterns of test results. The only differences between the type strain of *P. cepacia* (NCTC 10743) and the type strain of *P. kingii* (NCTC 10744) are given in Table 5. Of the seven tests listed, five gave differences between the other two NCTC strains of *P. cepacia* (*P. multivorans* NCTC 10661 and 10734). With the remaining two tests, the three EO-1 strains gave different results from the type strain of *P. kingii*.

The in vitro molecular hybridization experiments of Ballard et al. (1) also established genetic relationships between *P. cepacia* and several other pseudomonads: *P. pseudomallei*, *P. mallei*, and the phytopathogenic species *P. marginata* (synonym: *P. alliicola*) and *P. caryophylli*. These relationships were also reflected in the nutritional versatility of all five species (*P. cepacia* being the most versatile), and all five accumulate poly- β -hydroxybutyrate intracellularly. Ballard et al. (1) consider these five species to be a "species cluster," which they provisionally call the "pseudomallei group."

TABLE 5. Differences between the type strains of *Pseudomonas cepacia* and of *P. kingii*, compared with data for the field and other NCTC strains

Characters	<i>P. cepacia</i> 10743	<i>P. kingii</i> 10744	10661	10734	Field strains	EO-1 strains
Biochemical tests						
Yellow pigment on nutrient agar	+	—	+	—	4/30	0/3
Breakdown of ornithine (Møller, 28)	—	+	—	—	1/35	0/3
Breakdown of ornithine (Richard, 36) . . .	—	+	—	+	9/23	3/3
Nitrate reduction, aerobically	—	+	+	—	10/35	2/3
Nitrate reduction, anaerobically	—	+	—	—	12/35	2/3
Hydrolysis of esculin	+	—	—	+	9/23	3/3
Utilization tests						
Nicotinate	—	+	—	+	11/23	3/3

This grouping together of species within the genus *Pseudomonas* appears realistic, for there are not many properties which can reliably separate, for identification purposes, *P. cepacia* from *P. pseudomallei* or *P. mallei*, although *P. cepacia* is very different from the other pseudomonads likely to be met in clinical circumstances. Table 6 summarizes the data available to us to differentiate *P. cepacia* from the above two species and also from *P. aeruginosa*, *P. fluorescens*, *P. stutzeri*, and *P. maltophilia*. The table was compiled from Lapage et al. (22), Snell (Thesis, Fellowship of the Institute of Medical Laboratory Technology, 1970), results of this present study, and NCTC records. Denitrification capacities reported for *P. pseudomallei* by Redfearn, Paleroni, and Stanier (35) and for *P. aeruginosa* and *P. fluorescens* by Stanier et al. (43) are greater than we give for these species in Table 6. However, Stanier's group uses a different technique from the one employed by us (see Materials and Methods).

The only consistent differences between *P. cepacia* and *P. pseudomallei* are breakdown of arginine (Møller [28] or Thornley [45]; negative for *P. cepacia*, positive for *P. pseudomallei*) and of lysine (Richard's method [36]; positive for *P. cepacia*, negative for *P. pseudomallei*) although there are several "d" entries in Table 5 which should also assist identification. In the hope of improving the separation of these two species, the wider range of carbohydrates shown in Table 3 for *P. cepacia* (acid production from carbohydrates in the ammonium salt medium of Smith et al. [39]) was also used with *P. pseudomallei* strains. However, the pattern of results obtained for *P. pseudomallei* was the same as that given by *P. cepacia* (see Table 3). Not recorded in Table 6 is the very characteristic colonial appearance of *P. pseudomallei* illustrated by Lapage et al. (22), which is not shown by *P. cepacia*.

Carbon substrate utilization results have not been included in Table 6, because they are unlikely to be used in hospital laboratory

TABLE 6. *Differentiation of Pseudomonas cepacia*

Character	<i>P. cepacia</i> (41) ^a	<i>P. pseudo-</i> <i>mallei</i> (11)	<i>P. mallei</i> (8)	<i>P. aeru-</i> <i>ginosa</i> (13)	<i>P. fluo-</i> <i>rescens</i> (4)	<i>P. stut-</i> <i>zeri</i> (8)	<i>P. malto-</i> <i>philia</i> (3)
Number of flagella per cell	>1	>1	0	1	>1	1	>1
Oxidase	+	+	d ^b	+	+	+	d
Growth at 5 C	—	—	—	—	+	d	—
Citrate utilization (Christensen)	+	+	d	+	+	+	+
Citrate utilization (Koser or Simmons)	+	+	—	+	+	+	—
Nitrate reduction	d	+	+	+	d	+	+
Nitrate reduction, with gas production	—	—	—	d	—	+	—
Breakdown of arginine (Møller, 28, and Thornley, 45)	—	+	+	+	+	d	—
Breakdown of lysine (Møller, 28)	d	—	—	—	—	—	+
Breakdown of lysine (Richard, 36)	+	—	NT ^b	NT	NT	NT	NT
Breakdown of ornithine (Richard, 36)	d	—	NT	NT	NT	NT	NT
Gelatin liquefaction	+	+	d	+	+	—	+
Accumulation of poly- β -hydroxybutyrate	+	+	+	—	—	—	—
Starch hydrolysis	—	d	—	—	—	+	—
Tween 80 hydrolysis	+	+	d	+	—	NT	—
Acid produced from ^c							
Glucose	+	+	+	+	+	+	—
Dulcitol	+	+	—	—	—	—	—
Maltose	+	+	d	—	—	—	+
Mannitol	+	+	+	+	+	d	—
Salicin	+	—	—	—	—	—	—
Sorbitol	+	+	+	—	d	—	—
Xylose	+	+	—	+	+	NT	—
Acid produced from 10% lactose agar	+	+	+	d	+	—	—

^a Figures in parentheses following species names indicate number of strains examined.

^b Abbreviations: NT, not tested; d, variable results.

^c On the ammonium salt media of Smith et al. (39).

routines. However, even with these tests added, the differentiation of *P. cepacia* and *P. pseudomallei* is not much improved. Our results on 30 and 11 strains, respectively, were that *P. cepacia* utilizes 30 out of 43 substrates tested, and 29 of these 30 are utilized also by *P. pseudomallei*.

The increasing number of reports in the literature of the occurrence of *P. cepacia* in hospital infections, including the episodes reported here, indicates that this organism is creating serious problems. The common sequence of events appears to be contamination, possibly from water supplies, of disinfectant solutions. This is followed by infection of the patients, frequently mediated by the use of indwelling catheters. This sequence of events emphasizes the need for proper supervision of the preparation and use of disinfectants.

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