The Similarities Between *Pseudomonas paucimobilis* and Allied Bacteria Derived from Analysis of Deoxyribonucleic Acids and Electrophoretic Protein Patterns

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The chromosomal DNA was isolated and purified from 17 strains of *Pseudomonas paucimobilis*, and from the type or reference strains of *Flavobacterium capsulatum*, *F. devorans*, *F. multivorum*, '*Chromobacterium lividum*', *Xanthomonas campestris* and seven species of *Pseudomonas*. The DNA base compositions (mol % G + C) of *P. paucimobilis* strains were between 62.2 and 68.6%, and typical strains had a mean value of 65.3 ± 0.4 mol %, determined from thermal denaturation temperature. DNA–DNA molecular hybridization with ³H-labelled probe DNA from NCTC 11030 *P. paucimobilis* (the type strain) indicated that the species comprised a core of 13 closely related strains (74 to 96%), which included *F. devorans* NCIB 8195 (= ATCC 10829). Four *P. paucimobilis* strains displayed lower levels of hybridization ($\leq 38\%$). The hybridization results showed that *P. paucimobilis* was not closely related to allied yellow-pigmented bacteria or to other reference pseudomonads. The electrophoretic protein patterns of representative strains were analysed by computer-assisted techniques and similarity coefficients were calculated. A high degree of congruence was obtained with the DNA hybridization data, and the protein analyses indicated that the four atypical *P. paucimobilis* strains were heterogeneous and not a single group within the species. The generic affinities of *P. paucimobilis*, *F. capsulatum*, '*P. azotocolligans*' and *P. echinoides* are uncertain, but available chemotaxonomic data indicate these species could provide the basis for a new genus.

INTRODUCTION

Holmes *et al.* (1977) proposed the name *Pseudomonas paucimobilis* for certain yellow-pigmented, Gram-negative, rod-shaped bacteria isolated from clinical sources and from the hospital environment. The species corresponded to a group of bacteria previously known as Group IIk, biotype 1 (Tatum *et al.*, 1974). Clinical bacteriologists sometimes refer to strains of Group IIk, biotype 1 as 'xanthomonad' or 'Xanthomonas-like', and Holmes *et al.* (1977) showed by a numerical analysis of phenotypic data that *P. paucimobilis* was more similar to species of *Xanthomonas* than to representatives of authentic *Pseudomonas* species. Even so, Holmes *et al.* (1977) found that the species differed from *Xanthomonas* in several important biochemical tests, notably in producing cytochrome oxidase. Additional evidence to exclude *P. paucimobilis* from *Xanthomonas* was subsequently obtained by Jenkins *et al.* (1979), who identified the main pigment of *P. paucimobilis* as a carotenoid (nostoxanthin), a different compound from the brominated aryl-polyenes present in *Xanthomonas* species.

Subsequent to the Holmes *et al.* (1977) proposal of the name *P. paucimobilis*, several cases of infections or colonization in man have been attributed to the species (Hajiroussou *et al.*, 1979; Peel *et al.*, 1979; Slotnick *et al.*, 1979; Crane *et al.*, 1981), and evidence has been found of similarities between it and various other species in the *Flavobacterium*–*Cytophaga* complex. Yabuuchi *et al.* (1979) reported that *Flavobacterium devorans* ATCC 10829 and *P. paucimobilis*

were identical to each other in a comprehensive range of biochemical characteristics, DNA base compositions and cellular fatty acid compositions. Oyaizu & Komagata (1981) using similar techniques also found a close relationship between the above two species and with *F. capsulatum*. Furthermore, Jenkins *et al.* (1979) identified nostoxanthin as the major pigment of *P. echinoides* and '*P. azotocolligans*', a fact they suggested might indicate taxonomic affinities to *P. paucimobilis*. In a study of rRNA cistron similarities amongst flavobacteria, Bauwens & De Ley (1981) found that *P. paucimobilis* grouped with *F. capsulatum*, *F. devorans*, '*P. azotocolligans*' and '*Chromobacterium folium*', which provided further evidence that the species was not typical of the genus *Pseudomonas*. The strains previously known as Group IIk, biotype 2 (Tatum *et al.*, 1974), which resembled *P. paucimobilis* in phenotypic characteristics, were named *F. multivorum* (Holmes *et al.*, 1981). The new species differed from *P. paucimobilis* both in its DNA base composition (Holmes *et al.*, 1977) and in its cellular fatty acid composition (Dees *et al.*, 1979).

The aim of the present study was to examine the relationships within *P. paucimobilis*, as well as its relationships to other species of yellow-pigmented bacteria for which there is evidence of possible affinities. Determinations of DNA base compositions and DNA-DNA molecular hybridization (S1 endonuclease assay) were used to investigate strains. As there is evidence (Kerstens & De Ley, 1980) that relationships between bacteria based on the numerical analysis of whole cell protein electrophoretic patterns agree well with DNA hybridization data, selected strains were examined to determine the extent of correlation between these two taxonomic approaches.

METHODS

Strains used and growth conditions. The bacterial strains used and their sources are listed in Table 1. Strain numbers prefixed with 'A', 'F', or 'CL' were previously received by the NCTC for identification and the name given here is the result of that identification. The names of the other strains are the designations with which they were received from the respective culture collections. The names in quotation marks are not on the Approved Lists of Bacterial Names (Skerman *et al.*, 1980) and have no standing in nomenclature. Full details of the sources, phenotypic characteristics and clinical significance of the strains of *P. paucimobilis* have been published previously (Holmes *et al.*, 1977; Hajiroussou *et al.*, 1979; Peel *et al.*, 1979; Slotnick *et al.*, 1979). The bacteria, except *P. echinoides*, were grown overnight at 30 °C in 200 ml nutrient broth (Oxoid no. 2) with aeration in an orbital incubator at 120 r.p.m., or on nutrient agar [Oxoid no. 2 nutrient broth and 1.2% (w/v) agar] in plastic Petri dishes. *Pseudomonas echinoides* ICPB 2835 was grown at 30 °C for 2 d on a medium which contained 5% (w/v) Bacto cystine agar (Difco) and 1% (w/v) Bacto haemoglobin (Difco). Bacteriological purity of the cultures was rigorously checked by subculture on nutrient agar and by microscopic examination of Gram-stained cells. Conventional biochemical characteristics were examined previously (Holmes *et al.*, 1977; B. Holmes, personal communication).

Isolation and purification of DNA. The bacteria were harvested, washed and suspended in 0.15 M-NaCl/0.1 M-EDTA buffer pH 8.0 and the DNA was isolated and purified as described previously (Owen & Holmes, 1978, 1980). In the preparation of labelled DNA, the bacteria were grown in the medium described by Owen & Snell (1976) which contained: 10 ml M9 salts solution (prepared at tenfold concentration; Na₂HPO₄, 60 g; KH₂PO₄, 30 g; NaCl, 5 g; NH₄Cl, 10 g; distilled water, 1 l); 1 ml 0.1 M-MgSO₄; 1 ml 0.01 M-CaCl₂; 1 g tryptone (Difco); 2.5 ml 40% (w/v) maltose and 20 µg thiamin (both sterilized separately by filtration); 85 ml distilled water. The bacteria were grown overnight at 37 °C in 5 ml medium, subcultured into 10 ml of fresh medium, and then incubated at 37 °C with aeration. After 2 to 3 h, [³H]thymidine (Amersham) was added [10 µCi ml⁻¹ (370 kBq ml⁻¹)], followed by two further additions (5 µCi ml⁻¹) at 1 h intervals, and the incubation continued for about 5 h. The cells were collected by centrifugation and suspended in about 1 ml NaCl/EDTA buffer. The cells were lysed and the DNA was extracted as described previously (Owen *et al.*, 1978), except that the aqueous phase containing the nucleic acids separated after the phenol/chloroform deproteinization was purified by gel filtration chromatography. A column (30 × 1.8 cm; Pharmacia) packed with Sepharose 4B (Pharmacia) was washed and equilibrated with 0.0168 M-NaCl. After loading with the aqueous supernatant (1.5 ml), the column was eluted at a flow rate of 1 ml min⁻¹ and 0.2 ml fractions were collected. Elution was monitored continuously at 254 nm by a UV optical unit and flow cell (LKB Uvicord type 470 1A). The fractions that contained ³H-labelled DNA were identified by their radioactivity and pooled. The concentration of double-stranded DNA was estimated assuming an A₂₆₀ of 1 = 50 µg DNA ml⁻¹. The DNA solutions were considered to be sufficiently purified if the absorbance ratios 230/260 and 280/260 (nm) were about 0.450 and 0.515, respectively. The DNA solutions for hybridization were stored at -30 °C.

Thermal denaturation. The DNA base composition (mol % G + C) was estimated from the thermal denaturation temperature (*T_m*), which was determined in a spectrophotometer (Owen *et al.*, 1978) by the method of Marmur &

Table 1. *Bacterial strains used and sources*

Received as:		Obtained from:*	Source of isolation
Name	Strain		
<i>Pseudomonas paucimobilis</i>	NCTC 11030†	1	Hospital environmental, U.K.
<i>P. paucimobilis</i>	NCTC 11031	1	Human, U.S.A.
<i>P. paucimobilis</i>	NCTC 11032	1	Human, U.S.A.
<i>P. paucimobilis</i>	A129/72	2	Human, U.K.
<i>P. paucimobilis</i>	CL623/72	2	Human, U.K.
<i>P. paucimobilis</i>	A32/67	2	Hospital environmental, U.K.
<i>P. paucimobilis</i>	A90/67	2	Hospital environmental, F.R.G.
<i>P. paucimobilis</i>	F92	2	Human, U.K.
<i>P. paucimobilis</i>	CL311/70	2	Hospital environmental, U.K.
<i>P. paucimobilis</i>	A47/65	2	Hospital, U.K.
<i>P. paucimobilis</i>	F143	2	Human, U.K.
<i>P. paucimobilis</i>	CL158/74	2	Hospital environmental, U.K.
<i>P. paucimobilis</i>	A97/68	2	Environmental, U.K.
<i>P. paucimobilis</i>	CL246/69	2	Hospital environmental, U.K.
<i>P. paucimobilis</i>	CL310/70	2	Human, U.K.
<i>P. paucimobilis</i>	CL311/70‡	2	Human, U.K.
<i>P. paucimobilis</i>	CL682/77	2	Human, Australia
<i>P. paucimobilis</i>	CL339/78	2	Human, U.K.
<i>P. paucimobilis</i>	CL8/79	2	Human, U.S.A.
<i>P. aeruginosa</i>	NCTC 10662†	1	Human, U.K.
' <i>P. azotocolligans</i> '	ATCC 12417	3	Soil, U.S.A.
<i>P. cepacia</i>	NCTC 10743	1	Onion, U.S.A.
<i>P. echinoides</i>	ICPB 2835	4	Plate contaminant, F.R.G.
<i>P. flava</i>	DSM 619†	5	Ditch mud, Holland
<i>P. maltophilia</i>	NCTC 10275	1	Human oral cavity, U.S.A.
<i>P. putida</i>	NCTC 10936	1	Source not known
' <i>Chromobacterium lividum</i> '	NCTC 10590	1	Plant material, U.K.
<i>F. capsulatum</i>	NCIB 9890†	6	Environmental, U.S.A.
<i>F. devorans</i>	NCIB 8195†	6	Environmental, U.S.A.
<i>F. multivorum</i>	NCTC 11343	1	Human, U.S.A.
<i>Xanthomonas campestris</i>	NCPPB 528†	7	Source not known

* 1, National Collection of Type Cultures (NCTC), London, U.K.; 2, field isolate previously received by NCTC for identification; 3, American Type Culture Collection (ATCC) Rockville, Md., U.S.A.; 4, International Collection of Phytopathogenic Bacteria (ICPB), University of California, Davis, U.S.A.; 5, Deutsche Sammlung von Mikroorganismen (DSM), Göttingen, F.R.G.; 6, National Collection of Industrial Bacteria (NCIB), Aberdeen, U.K.; 7, National Collection of Plant Pathogenic Bacteria (NCPPB), Harpenden, U.K.

† Type strain (Skerman *et al.*, 1980).

‡ Strain examined by protein electrophoresis only.

Doty (1962). The mol % G + C was calculated from the T_m determined in $0.1 \times$ SSC buffer (0.15 M-NaCl buffered with $0.015 \text{ M-trisodium citrate}$ pH 7.0) using the equation: $\% \text{ G} + \text{C} = 52.0 + 2.08 (T_{mx} - 75.3)$, where T_{mx} is the denaturation temperature of the test DNA, and 75.3°C is the denaturation temperature of reference DNA from *Escherichia coli* NCTC 10537.

Fragmentation of double-stranded DNA. The DNA concentration was adjusted to $70 \mu\text{g ml}^{-1}$ and sheared by sonication as described previously (Owen *et al.*, 1978).

DNA-DNA molecular hybridization. ^3H -labelled DNA ($1 \mu\text{g}$) was mixed with unlabelled DNA ($50\text{--}100 \mu\text{g}$) in 0.3 ml Pierce Reactivials, and heat denatured at 90°C for 10 min, then immediately cooled to near 0°C in an ice bath. The Na^+ concentration of the DNA solution was adjusted to 0.42 M in a final volume of $200 \mu\text{l}$ by adding $10 \mu\text{l}$ 4.2 M-NaCl . Each DNA mixture was incubated overnight (approximately 18 h) at 70°C (this temperature corresponds to $T_m - 26^\circ\text{C}$) to give homologous reassociations of at least 90%. The amount of double-stranded DNA present in the reaction mixture was determined by the S1 endonuclease assay of Crosa *et al.* (1973) with slight modifications. After incubation, $200 \mu\text{l}$ enzyme assay buffer ($0.062 \text{ M-sodium acetate}$ buffer pH 4.8, and $2.05 \text{ mM-zinc sulphate}$), $20 \mu\text{g}$ sheared, denatured calf-thymus DNA ml^{-1} (stock solution contains: 2 mg DNA ml^{-1} in 10 mM-Tris/HCl buffer pH 7.5), and 50 units S1 endonuclease (Sigma) were added and thoroughly mixed. A zero-time sample ($50 \mu\text{l}$) was removed and the mixture was incubated for 20 min at 60°C . The reaction was stopped by chilling in an ice bath, and $70 \mu\text{l}$ samples were placed on dry 2.4 cm DE81 filter discs (Whatman) in a scintillation vial. Each disc was washed in triplicate with 5 ml volumes of 5% (w/v) Na_2HPO_4 (Salzberg *et al.*,

1977; Popoff & Coynault, 1980), and in duplicate with 5 ml volumes of distilled water. Discs were dried thoroughly and radioactivity was determined in a scintillation counter as described previously (Owen & Snell, 1976).

Calculation of percentage DNA hybridization. At least four determinations were made for each DNA pair hybridized. Each set of hybridizations included a positive control (the labelled DNA with its corresponding unlabelled DNA), and a negative control [the labelled DNA with an unrelated unlabelled DNA from *Brevibacterium* sp. NCD0 2051 with a G + C content of 66% (D. Pitcher, personal communication)]. The reassociation of the label with itself was about 5%. Relative hybridization of heterologous strands of DNA, corrected for self-reassociation of the labelled DNA, was expressed as a percentage hybridization, with the reassociation of homologous strands at 100%.

Preparation of protein samples. Approximately 0.5 g wet weight of the bacteria were harvested, washed and suspended in 1 ml of electrophoresis buffer (0.09 M-Tris/HCl, 0.08 M-boric acid, 0.0025 M-EDTA, pH 8.35). The bacteria were disrupted by sonication (MSE Instruments ultra-sonicator) for 2 min in an ice bath. The sonicate was centrifuged at 4 °C for 30 min at 36 000 g and the supernatant was then centrifuged for a further 2 h at 36 000 g. The concentration of protein in the supernatant was determined by the Lowry method. The protein samples were stored at -20 °C.

Electrophoresis. Protein samples containing approximately 200 µg of protein were layered on to gels containing a gradient of 2.7 to 27% (w/v) polyacrylamide (Gradipore gels, Universal Scientific, London). The gels measured 120 × 70 × 0.3 mm and were cast in batches of 100. Thyroglobulin (Sigma; 10 µg) was added as a marker to each well. Electrophoresis was performed in a water-cooled tank (Pharmacia GE4 II) for 20 h at a constant voltage of 100 V at 15 °C. After electrophoresis, the gels were stained in 0.04% (w/v) Coomassie brilliant blue G250 (Sigma) in 3.5% (w/v) perchloric acid for 16 h and then destained in distilled water.

Scanning of gels. The stained protein patterns in the gels were scanned with a densitometer (Vitatron TLD 100; MSE Instruments) under the following conditions: slit width 250 µm, red filter 603 and scanning speed 1 mm s⁻¹. The complete scan, which included the thyroglobulin band, comprised 500 absorbance values (Y values) recorded at 0.2 mm intervals along the gel. The Y values were recorded as integers between 1 and 255 using an analogue to digital converter (Millhouse Designs, Alton, Hampshire) interfaced to a microcomputer (Commodore PET 3032) and finally stored on magnetic disk (Commodore 3040). The trace used for computations was started 5 points (1 mm) from the centre of the thyroglobulin band, which was the highest Y value in the first 30 points (6 mm) of the scan, and consisted of the next 400 points (80 mm). This original trace was then reduced to a 100 point trace by averaging the points in groups of four in order to shorten the time required for computing. The gels were routinely scanned at high resolution in case the data were needed for future analysis. A general background trend in each trace was removed to increase the discrimination between protein patterns. A grossly averaged trace of 10 points (the 100 point trace averaged in groups of ten) was constructed and a fraction (0.8) of this trace was then subtracted from the 100 point trace. The mathematical treatment of the background trend is dealt with elsewhere (Jackman, 1981).

Computations. The best fit between each pair of traces with background trends removed was computed and similarities were calculated using the Pearson product moment correlation coefficient (*r*) herein referred to as the correlation coefficient. Strains were then clustered by the method of unweighted pair-group average linkage (UPGMA). Details of the methods are given by Sneath & Sokal (1973). The best fit between each pair of traces was obtained by laterally shifting one trace with respect to the other in single point steps of ± 5 points either side of the initial alignment with respect to the thyroglobulin band. The correlation coefficient was calculated at each point and the highest value was used in the cluster analysis. All calculations were performed on a Commodore PET 3032 using programs written by and available from P. J. H. Jackson.

RESULTS

DNA characteristics

The thermal denaturation temperatures and the corresponding base compositions (mol % G + C) of 17 strains previously identified on phenotypic characteristics as *P. paucimobilis* are listed in Table 2. Fifteen strains had G + C contents of 64.9 to 65.9 mol % with a mean and standard deviation of 65.3 ± 0.4 mol %. Strains CL8/79 and A97/68 had slightly higher values of 66.4% and 67.4%, respectively. The majority of the strains gave high levels (≥ 74%) of hybridization with labelled DNA from NCTC 11030 as shown in Table 2. Four strains were atypical in having lower (≤ 38%) hybridization levels with NCTC 11030; three of these (NCTC 11032, CL158/74 and CL8/79) had DNA hybridization levels between 34 and 38%. The remaining strain (A97/68) was 10% related to NCTC 11030.

Table 2 also lists the DNA characteristics of the various strains allied to *P. paucimobilis* and the selected reference strains. *Flavobacterium devorans* NCIB 8195 was identical to the typical

Table 2. DNA characteristics and protein similarities of *P. paucimobilis* and allied bacteria

Reference no. in Fig. 1	Strain	T_m (°C)*	G + C content (mol %) [†]	NCTC 11030 reference strain	
				DNA-DNA hybridization (%) [‡]	Protein pattern similarity (%) [§]
1	<i>P. paucimobilis</i> NCTC 11030	ND	65.5	(100)	90
2	<i>P. paucimobilis</i> CL339/78	81.55 ± 0.05	65.0	96 ± 4	82
3	<i>P. paucimobilis</i> CL682/77	81.7 ± 0.00	65.3	95 ± 3	76
4	<i>P. paucimobilis</i> A90/67	81.8 ± 0.00	65.5	95 ± 1	76
5	<i>P. paucimobilis</i> CL623/72	81.65 ± 0.15	65.2	86 ± 8	71
6	<i>P. paucimobilis</i> A47/65	81.6 ± 0.10	65.1	85 ± 3	62
7	<i>P. paucimobilis</i> F143	81.55 ± 0.05	65.0	83 ± 6	78
8	<i>P. paucimobilis</i> CL310/70	ND	64.9 ^a	83 ± 1	ND
9	<i>P. paucimobilis</i> CL246/69	81.95 ± 0.05	65.8	82 ± 4	74
10	<i>P. paucimobilis</i> F92	81.8 ± 0.10	65.5	81 ± 7	ND
11	<i>P. paucimobilis</i> A129/72	81.95 ± 0.05	65.8	80 ± 1	ND
12	<i>P. paucimobilis</i> A32/67	81.75 ± 0.15	65.4	78 ± 1	ND
13	<i>P. paucimobilis</i> NCTC 11031	ND	64.9 ^a	74 ± 2	80
14	<i>P. paucimobilis</i> NCTC 11032	ND	64.9 ^a	38 ± 6	46
15	<i>P. paucimobilis</i> CL158/74	82.0 ± 0.10	65.9	35 ± 3	55
16	<i>P. paucimobilis</i> CL8/79	82.2 ± 0.10	66.4	34 ± 1	42
17	<i>P. paucimobilis</i> A97/68	ND	67.4 ^a	10 ± 0	38
18	<i>F. devorans</i> NCIB 8195	81.65 ± 0.15	65.2	93 ± 6	87
19	<i>F. capsulatum</i> NCIB 9890	81.6 ± 0.10	65.1	8 ± 3	ND
20	' <i>P. azotocolligans</i> ' ATCC 12417	82.25 ± 0.15	66.6	12 ± 2	40
21	<i>P. flava</i> DSM 619	83.3 ± 0.10	68.6	0	ND
22	<i>P. echinoides</i> ICPB 2835	81.95 ± 0.25	65.8	12	ND
23	<i>X. campestris</i> NCPPB 528	81.5 ± 0.10	64.9	0	ND
24	' <i>C. lividum</i> ' NCTC 10590	ND	61.0 ^b	10 ± 3	ND
25	<i>P. cepacia</i> NCTC 10743	ND	67.5 ^c	0	ND
26	<i>P. putida</i> NCTC 10936	80.25 ± 0.05	62.2	5 ± 1	ND
27	<i>P. maltophilia</i> NCTC 10257	ND	65.0 ^c	4 ± 3	ND
28	<i>F. multivorum</i> NCTC 11343	ND	38.2 ^d	2 ± 2	ND
29	<i>P. aeruginosa</i> NCTC 10662	ND	66.8 ^c	0	ND

ND, Not determined.

* Values for mean T_m are averages of two or three separate determinations + S.D. in 0.1 × SSC buffer.[†] Values not determined in this study are marked by superscript letters: *a*, recalculated (as described in Methods) from T_m data of Holmes *et al.* (1977); *b*, from Gillis & De Ley (1980); *c*, from De Ley *et al.* (1978); *d*, from Holmes *et al.* (1981).[‡] Mean value of at least two determinations in most cases. Pooled S.D. = ±3% (*n* = 23).[§] Average similarity was calculated relative to the duplicates of the reference strain.| These values were reported by Holmes *et al.* (1977).

strains of *P. paucimobilis* in its DNA base composition and level of molecular hybridization. The other strains of yellow-pigmented bacteria had similar base compositions to *P. paucimobilis*, except for '*C. lividum*' NCTC 10590 which had a slightly lower value of 61% (Gillis & De Ley, 1980), and *F. multivorum* NCTC 11343 (previously CDC Group IIk, biotype 2) with a much lower value of 38.2%; the latter species therefore could not share any common DNA with *P. paucimobilis*. The DNA-DNA hybridization results showed that all these species had low (≤12%) hybridization levels with the type strain of *P. paucimobilis*. The DNA results are summarized in Fig. 1.

Electrophoretic analysis of proteins

Electrophoresis in gradient gels resolved the cellular proteins of each of 18 investigated strains of *P. paucimobilis* and allied species into about 50 bands with molecular masses in the

Fig. 1. Overall DNA similarities (hybridizations and G + C contents) between *Pseudomonas paucimobilis* NCTC 11030, the type and DNA reference strain (reference no. 1) and other strains of *P. paucimobilis* (reference nos 2-17, Table 2) and allied bacteria (reference nos 18-29, Table 2). The shapes and areas outlined are not intended to have a precise taxonomic significance.

Fig. 2. Densitometric traces of the electrophoretic protein pattern of *P. paucimobilis* NCTC 11030. (a) Complete trace (500 points); (b) reduced trace (100 points); (c) final trace (background removed). Details of the computation of the traces are given in Methods.

Fig. 3. Dendrogram based on protein patterns using the correlation coefficient with 0.8 background subtracted and UPGMA clustering.

range 12–500 kDal. Figure 2 shows the original (500 point) densitometric trace of the electrophoretic protein pattern of *P. paucimobilis* NCTC 11030, as well as the grossly averaged trace with and without the background. Duplicate samples of NCTC 11030 were run on two different gels and shows 90% similarity, which is a measure of the reproducibility of the method.

The dendrogram derived from the numerical analysis based on the protein patterns of 17 strains is shown in Fig. 3. Eleven strains formed a cluster with similarities of 68% or above. These strains included *P. paucimobilis* NCTC 11030 (the type strain), 10 other strains identified on phenotypic characteristics as *P. paucimobilis*, and *F. devorans* NCIB 8195. The remaining six strains joined the main *P. paucimobilis* cluster at similarity levels below 42%, and included four strains identified on phenotypic characteristics as *P. paucimobilis*, '*P. azotocolligans*' and *F. capsulatum*. Table 2 lists the percentage similarity derived from the electrophoretic protein patterns between *P. paucimobilis* and other strains.

DISCUSSION

The results on G + C contents and DNA–DNA hybridization, and the electrophoretic analysis of cellular proteins provided evidence that *P. paucimobilis*, as currently defined on conventional morphological and biochemical characteristics (Holmes *et al.*, 1977), contains a core of highly related strains. Furthermore, the results obtained with *F. devorans* NCIB 8195 (= ATCC 10829) confirm the studies of Yabuuchi *et al.* (1979) in showing the strain to be almost identical to the type strain of *P. paucimobilis*. It is also clear from the results that *P. paucimobilis* contains some atypical strains with slightly different base sequences and protein profiles. *Pseudomonas paucimobilis* A97/68 is possibly incorrectly identified as it has low DNA similarity to the type strain. Likewise, low DNA similarity levels were found between *P. paucimobilis* and phenotypically allied species producing yellow pigments such as '*P. azotocolligans*', *F. capsulatum*, *P. echinoides* and *P. flava*.

Figure 4 shows that the similarities within and between *P. paucimobilis* and allied bacteria, derived from DNA hybridization and electrophoretic analysis of cellular proteins, had a high degree of congruence ($r = 0.92$). The overall results obtained thus agreed with the observation of Kersters & De Ley (1980) that strains with high DNA similarity tend to have similar protein patterns. It is generally accepted that bacteria with high levels ($\geq 70\%$) of DNA base similarity relative to a particular reference DNA, must also show DNA similarities to each other, and that is supported by the electrophoresis results. In the case of strains with lower DNA relatedness, no such assumption can be made. However, it was evident from the protein analysis that *P. paucimobilis* NCTC 11032 and CL8/79 were related. It is likely, therefore, that the two strains also have similar DNA base sequences to each other. Furthermore, both were $36 \pm 2\%$ related to NCTC 11030. The three remaining strains, *P. paucimobilis* A97/68 and CL158/74, and '*P. azotocolligans*' ATCC 12417, were not in general closely related in their proteins either to each other or to *P. paucimobilis*. The above four atypical *P. paucimobilis* strains did not show any consistent phenotypic differences from other members of the species (Holmes *et al.*, 1977), so

Fig. 4. Correlation of percentage protein similarity with percentage DNA-DNA hybridization.

their reclassification is not advocated at present. Likewise, '*P. azotocolligans*' was phenotypically similar to *P. paucimobilis* (R. J. Owen, unpublished results). In contrast, the DNA differences shown by *P. echinoides*, *P. flava* and *F. capsulatum* were reflected in phenotypic differences (Oyaizu & Komagata, 1981; R. J. Owen, unpublished results); for *F. capsulatum*, these were detected in the assimilation of various carbon compounds (Oyaizu & Komagata, 1981).

A feature of the relationship between DNA hybridization and protein similarities evident from Fig. 4 was that strains with zero DNA homology showed a calculated protein pattern similarity of about 30%. This characteristic was attributed to the best fit algorithm used in the protein analysis computation, which considers the highest similarity as the most appropriate. The value was similar to that found between completely unrelated bacterial groups and represents the inherent similarity in the general shape of electrophoretic patterns (Jackman, 1981). The fact that each peak may be a composite of several structurally and functionally different proteins with the same electrophoretic mobility, could contribute to this apparent high background similarity level.

Eleven of the *P. paucimobilis* strains were previously included by Holmes *et al.* (1977) in a numerical analysis of phenotypic characteristics. In the case of the strains with high levels of DNA similarity, there was an excellent agreement with similarities based on the phenetic analysis, but the linear congruence between the two approaches did not hold at lower levels of DNA relatedness. The data on these particular strains show that similarities derived from protein analysis are a more sensitive measure of taxonomic differences than those derived from phenetic analyses. Even so, the latter technique indicated that strains such as A97/68 and CL158/74 were atypical of *P. paucimobilis*, but there were no obvious reasons to argue for their exclusion from the new species (Holmes *et al.*, 1977). Jones & Sneath (1970) and Harvey & Pickett (1980) recognized the lack of linearity between similarities based on DNA and phenetic analyses. Harvey & Pickett (1980) concluded from their study of *Yersinia enterocolitica* that differences between the two approaches were due to the fact that DNA hybridization was based on the entire genome, whereas numerical phenetic analysis measured at best only 20% of the genome through phenotypic expression, and to problems in test reproducibility.

The generic and supra-generic affinities of *P. paucimobilis* are uncertain. There is strong evidence from rRNA cistron comparisons (Bauwens & De Ley, 1981), cellular fatty acid profiles (Dees *et al.*, 1979; Oyaizu & Komagata, 1981) and numerical phenetic analysis (Holmes *et al.*, 1977), that the species is not an authentic member of the genus *Pseudomonas*. On the basis of rRNA hybridization, Bauwens & De Ley (1981) found that *F. capsulatum* was a member of an rRNA superfamily which contained members of the '*Acetobacteriaceae*' (e.g. *Acetobacter*, *Gluconobacter*, *Agrobacterium* and *Rhizobium*) (Gillis & De Ley, 1980). The species with the closest affinities to *F. capsulatum* were '*P. azotocolligans*', '*C. folium*', *P. paucimobilis* and *F.*

devorans. There are additional chemotaxonomic data which point to the uniqueness of these bacteria: (a) *Pseudomonas paucimobilis*, '*P. azotocolligans*' and *P. echinoides* contain a yellow pigment which Jenkins *et al.* (1979) identified as a nostoxanthin; hitherto this pigment was reported to occur only in a few species of cyanobacteria; (b) *Pseudomonas paucimobilis* and *F. devorans* contain a novel sphingoglycolipid (ceramide gluconuric acid) as a major component of the cellular lipids (Yabuuchi *et al.*, 1979); (c) the major cellular fatty acid in *P. paucimobilis*, *F. devorans* and *F. capsulatum* is C_{18:1}, which represents at least 50% of the total fatty acid, as well as 2-OH-C_{14:0}, C_{16:0}, C_{16:1}, C_{17:1} acids (Dees *et al.*, 1979; Yabuuchi *et al.*, 1979; Oyaizu & Komagata, 1981); and (d) the main respiratory quinones in *P. paucimobilis* and *F. capsulatum* are ubiquinones (Q-10) (Oyaizu & Komagata, 1981).

These data and the DNA hybridization results obtained in the present study, which indicated a background similarity of 8–12% between *P. paucimobilis*, *F. capsulatum*, '*P. azotocolligans*' and *P. echinoides*, show that these species may eventually justify inclusion in a new genus.

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REFERENCES

- BAUWENS, M. & DE LEY, J. (1981). Improvements in the taxonomy of *Flavobacterium* by DNA:rRNA hybridizations. In *The Flavobacterium-Cytophaga Group*, pp. 27–31. Edited by H. Reichenbach & O. B. Weeks. Weinheim: Verlag Chemie.
- CRANE, L. R., TAGLE, L. C. & PALUTKE, W. A. (1981). Outbreak of *Pseudomonas paucimobilis* in an intensive care facility. *Journal of the American Medical Association* **246**, 985–987.
- CROSA, J. H., BRENNER, D. J. & FALKOW, S. (1973). Use of a single-strand specific nuclease for analysis of bacterial and plasmid deoxyribonucleic acid homo- and heteroduplexes. *Journal of Bacteriology* **115**, 904–911.
- DEES, S. B., MOSS, C. W., WEAVER, R. E. & HOLLIS, D. (1979). Cellular fatty acid composition of *Pseudomonas paucimobilis* and Groups IIk-2, Ve-1 and Ve-2. *Journal of Clinical Microbiology* **10**, 206–209.
- DE LEY, J., SEGERS, P. & GILLIS, M. (1978). Intra- and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. *International Journal of Systematic Bacteriology* **38**, 154–168.
- GILLIS, M. & DE LEY, J. (1980). Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acetobacter* and *Gluconobacter*. *International Journal of Systematic Bacteriology* **30**, 7–27.
- HAJIROUSSOU, V., HOLMES, B., BULLAS, J. & PINNING, C. A. (1979). Meningitis caused by *Pseudomonas paucimobilis*. *Journal of Clinical Pathology* **32**, 953–955.
- HARVEY, S. & PICKETT, M. J. (1980). Comparison of Adansonian analysis and deoxyribonucleic acid hybridization results in the taxonomy of *Yersinia enterocolitica*. *International Journal of Systematic Bacteriology* **30**, 86–102.
- HOLMES, B., OWEN, R. J., EVANS, A., MALNICK, H. & WILCOX, W. R. (1977). *Pseudomonas paucimobilis*, a new species isolated from human clinical specimens, the hospital environment, and other sources. *International Journal of Systematic Bacteriology* **27**, 133–146.
- HOLMES, B., OWEN, R. J. & WEAVER, R. E. (1981). *Flavobacterium multivorum*, a new species isolated from human clinical specimens and previously known as Group IIk, biotype 2. *International Journal of Systematic Bacteriology* **31**, 21–34.
- JACKMAN, P. J. H. (1981). *Taxonomy of aerobic axillary coryneforms based on electrophoretic protein patterns*. Ph.D. thesis, University of London, U.K.
- JENKINS, C. L., ANDREWES, A. G., MCQUADE, T. J. & STARR, M. P. (1979). The pigment of *Pseudomonas paucimobilis* is a carotenoid (nostoxanthin), rather than a brominated aryl-polyene (xanthomonadin). *Current Microbiology* **3**, 1–4.
- JONES, D. & SNEATH, P. H. A. (1970). Genetic transfer and bacterial taxonomy. *Bacteriological Reviews* **34**, 40–81.
- KERSTERS, K. & DE LEY, J. (1980). Classification and identification of bacteria by electrophoresis of their proteins. In *Microbiological Classification and Identification, SAB Symposium Series No. 8*, pp. 273–297. Edited by M. Goodfellow & R. G. Board. London: Academic Press.
- MARMUR, J. & DOTY, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *Journal of Molecular Biology* **5**, 109–118.
- OWEN, R. J. & HOLMES, B. (1978). Heterogeneity in the characteristics of deoxyribonucleic acid from *Flavobacterium odoratum*. *FEMS Microbiology Letters* **4**, 41–46.
- OWEN, R. J. & HOLMES, B. (1980). Differentiation between strains of *Flavobacterium breve* and allied bacteria by comparisons of deoxyribonucleic acids. *Current Microbiology* **4**, 7–11.
- OWEN, R. J. & SNELL, J. J. S. (1976). Deoxyribonucleic acid reassociation in the classification of flavobacteria. *Journal of General Microbiology* **93**, 89–102.
- OWEN, R. J., LEGROS, R. M. & LAPAGE, S. P. (1978). Base composition, size and sequence similarities of genome deoxyribonucleic acids from clinical isolates of *Pseudomonas putrefaciens*. *Journal of General Microbiology* **104**, 127–138.

- OYAIZU, H. & KOMAGATA, K. (1981). Chemo-taxonomic and phenotypic characterization of the strains of species in the *Flavobacterium-Cytophaga* complex. *Journal of General and Applied Microbiology* **27**, 57-107.
- PEEL, M. M., DAVIS, J. M., ARMSTRONG, W. L. H., WILSON, J. R. & HOLMES, B. (1979). *Pseudomonas paucimobilis* from a leg ulcer on a Japanese seaman. *Journal of Clinical Microbiology* **9**, 561-564.
- POPOFF, M. & COYNAULT, C. (1980). Use of DEAE-cellulose filters in the S1 nuclease method for bacterial deoxyribonucleic acid hybridization. *Annales de microbiologie* **131A**, 151-155.
- SALZBERG, G., LEVI, Z., ABOUD, M. & GOLDBERGER, A. (1977). Isolation and characterization of DNA-DNA and DNA-RNA hybrid molecules formed in solution. *Biochemistry* **16**, 25-29.
- SKERMAN, V. B. D., MCGOWAN, V. & SNEATH, P. H. A. (1980). Approved lists of bacterial names. *International Journal of Systematic Bacteriology* **30**, 225-420.
- SLOTNICK, I. J., HALL, J. & SACKS, H. (1979). Septic-aemia caused by *Pseudomonas paucimobilis*. *American Journal of Clinical Pathology* **72**, 882-884.
- SNEATH, P. H. A. & SOKAL, R. R. (1973). *Numerical Taxonomy: The Principles and Practice of Numerical Classification*. San Francisco: W. H. Freeman.
- TATUM, H. W., EWING, W. H. & WEAVER, R. E. (1974). Miscellaneous Gram-negative bacteria. In *Manual of Clinical Microbiology*, 2nd edn, pp. 270-294. Edited by E. H. Lennett, E. H. Spaulding & J. P. Truant. Washington, D.C.: American Society for Microbiology.
- YABUCHI, E., TANIMURA, E., OHYAMA, A., YANO, I. & YAMAMOTO, A. (1979). *Flavobacterium devorans* ATCC 10829: a strain of *Pseudomonas paucimobilis*. *Journal of General and Applied Microbiology* **25**, 95-107.