Assignment of Centers for Disease Control group IVc-2 to the genus *Ralstonia* as *Ralstonia paucula* sp. nov.

P. Vandamme,[1] J. Goris,[1] T. Coenye,[1] B. Hoste,[2] D. Janssens,[2] K. Kersters,[1] P. De Vos[1] and E. Falsen[3]

Author for correspondence: P. Vandamme. Tel: +32 9 264 51 13. Fax: +32 9 264 50 92. e-mail: peter.vandamme@rug.ac.be

[1,2] Laboratorium voor Microbiologie, Ledeganckstraat 35, B-9000 Gent[1] and BCCM/LMG Culture Collection[2], University of Ghent, Belgium

[3] Department of Clinical Bacteriology, University of Göteborg, S-413 46 Göteborg, Sweden

An integrated genotypic and phenotypic analysis of 12 Centers for Disease Control (CDC) group IVc-2 strains revealed that this taxon represents a novel species belonging to the genus *Ralstonia*. Comparative 16S rDNA sequence analysis allocated a representative CDC group IVc-2 strain to the *Ralstonia* branch of the β subclass of the *Proteobacteria*. DNA–DNA hybridizations did not detect significant binding levels towards any presently known *Ralstonia* species, including *Ralstonia pickettii*. Its DNA base ratio is between 65 and 67 mol%. The name *Ralstonia paucula* sp. nov. is proposed, with strain LMG 3244 (= CDC E6793), isolated from a human respiratory tract, as the type strain. *R. paucula* can be differentiated from other *Ralstonia* species by whole-cell protein analysis, amplified rDNA restriction analysis and a variety of classical biochemical tests. Strains have been isolated from various human clinical and environmental sources.

Keywords: CDC group IVc-2, *Ralstonia*, *β-Proteobacteria*

INTRODUCTION

Centers for Diseases Control (CDC) group IVc-2 is a Gram-negative non-fermentative rod sporadically associated with human infection. Cases of bacteraemia, peritonitis and tenosynovitis have been reported but an underlying disease, potentially affecting the immunocompetency of the patients was described in most cases (Anderson *et al*., 1997). This bacterium is believed to be an environmental organism and several infections associated with contaminated water have been described (Anderson *et al*., 1997). CDC group IVc-2 strains have also been reported in pool water (Aspinal & Graham, 1989), groundwater (Campbell Wyndham *et al*., 1994), and even in considerable percentages of different brands of bottled mineral water (Manaia *et al*., 1990).

Although known in scientific literature for a long time, few studies have focussed on the taxonomic affiliation of this organism and a proper binomial name has not been proposed. In 1987, Rossau and colleagues reported that CDC group IVc-2 strains belonged to rRNA superfamily III *sensu* De Ley or the β subclass of the *Proteobacteria* (De Ley, 1992), with the generically misnamed *Alcaligenes eutrophus* and *Pseudomonas solanacearum* as its closest neighbours (Kersters & De Ley, 1984; Rossau *et al*., 1987). Later, Yabuuchi *et al*. (1995) reclassified *Alcaligenes eutrophus*, *Pseudomonas solanacearum* and *Pseudomonas pickettii* in a novel genus *Ralstonia*, as *Ralstonia eutropha*, *Ralstonia solanacearum* and *Ralstonia pickettii*, respectively. The T_m(e) values of DNA of CDC group IVc-2 strains towards radioactively labelled rRNA of the *R. eutropha* type strain (Rossau *et al*., 1987) indicate that CDC group IVc-2 belongs to the same genus; this was recently confirmed by 16S rDNA gene sequence analysis as reported by Osterhout *et al*. (1998). In the present study, we performed an integrated genotypic and phenotypic analysis of CDC group IVc-2 strains to characterize their phenotype and genotype, and their relatedness towards established species of the genus *Ralstonia*. Apart from *R. pickettii*, *R. solanacearum* and *R. eutrophus*, we also included reference strains of *R. gilardii*, a recently delineated species from environmental and human clinical origin (Coenye *et al*., 1999).

Abbreviation: ARDRA, amplified rDNA restriction analysis.

The GenBank/EMBL/DDBJ accession number for the 16S rDNA sequence of strain LMG 3413^T is AF085226.

00950 © 1999 IUMS

METHODS

Bacterial strains and growth conditions. All strains were grown on Trypticase Soy agar (catalogue no. 11768; BBL) and incubated aerobically at about 37 °C, except when stated otherwise.

The strains and their sources are listed in Table 1. Bacteriological purity was checked by plating and examining living and Gram-stained cells.

Analysis of protein electrophoretic patterns. Strains were incubated for 48 h. Preparation of cellular protein extracts, PAGE, densitometric analysis, and normalization and interpolation of the protein profiles were performed as described by Pot *et al.* (1994); numerical analysis was performed using the GelCompar 4.0 software package (Applied Maths). The profiles were recorded and stored on a PC. The similarity between all pairs of traces was expressed by the Pearson product–moment correlation coefficient converted for convenience to a percentage value.

Amplified rDNA restriction analysis (ARDRA). DNA was prepared and 16S rDNA genes were amplified as described previously for *Alcaligenes* and *Bordetella* strains (Heyndrickx *et al.*, 1996) from 24-h-old cells. Restriction digestion of the amplified 16S rDNA was carried out as recommended by the manufacturer using 5 U of one of the restriction enzymes *Hae*III (Boehringer Mannheim), *Rsa*I (Pharmacia Biotech), *Hpa*II, *Taq*I or *Nla*IV (New England Biolabs). For each strain the normalized restriction patterns obtained with each of the five restriction enzymes were assembled into a combined profile and analysed using the Dice similarity coefficient (S_D) and the UPGMA clustering algorithm (GelCompar 4.0).

Preparation of high-molecular-weight DNA. High-molecular-weight native DNA was prepared as described before (Vandamme *et al.*, 1992).

DNA base compositions. All of the mean mol% guanine plus cytosine (mol% G+C) values were determined by

Table 1. List of strains studied

^{T} indicates type strain. Abbreviations: API, Appareils et Procédés d'Identification, Marcy-l'Etoile, France; ATCC, American Type Culture Collection, Manassas, VA, USA; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection University of Göteborg, Department of Clinical Bacteriology, Göteborg, Sweden; CDC, Centers for Disease Control and Prevention, Atlanta, GA, USA; CIP, Collection bactérienne de l'Institut Pasteur, Paris, France; LMG, BCCM/LMG Culture Collection, Laboratorium voor Microbiologie, Universiteit Gent, Gent, Belgium; NCPPB, National Collection of Plant-pathogenic Bacteria, Harpenden Laboratory, Hertfordshire, UK. Source of isolates are given if known.

Strain	Other strain no.	Received from:	Source
Ralstonia eutropha LMG 1199^T	CCUG 1776^T, ATCC 17697^T	ATCC	Soil (USA, 1957)
Ralstonia gilardii LMG 5886 ^{T}	API 141-2-84^T, CCUG 38401^T	D. Monget	Whirlpool
Ralstonia gilardii LMG 3400	API 119-04-76, CDC D2683	J. Gayral	Spinal fluid (USA)
Ralstonia gilardii LMG 15537	CCUG 24719	G. L. Gilardi	Bone marrow (USA)
Ralstonia pickettii LMG 5942^T	CCUG 3318^T, Pickett K-288 ^{T}	M. Pickett	Human (USA)
Ralstonia pickettii LMG 6871	CCUG 18841, CCM 2846	CCM	Soil (Senegal)
Ralstonia pickettii LMG 7001	CCUG 3314, Pickett K-214	M. Pickett	Human (USA)
Ralstonia pickettii LMG 7002	CCUG 3316, Pickett K-279	M. Pickett	Human (USA)
Ralstonia pickettii LMG 7005	CCUG 1467, CDC A5832	R. Weaver	
Ralstonia pickettii LMG 7008	CCUG 2165	K. Lincoln	Urine (Sweden, 1973)
Ralstonia pickettii LMG 7012	CCUG 12413, CDC F2780	D. G. Hollis	Human wound (USA)
Ralstonia pickettii LMG 7145	CCUG 3315, Pickett K-232	M. Pickett	Human (USA)
Ralstonia solanacearum LMG 2299^T	NCPPB 325^T, CCUG 14272^T	NCPPB	*Lycopersicon esculentum* (USA)
Ralstonia paucula LMG 3244^T	CDC E6793^T, CCUG 12507^T	R. Weaver	Human, respiratory tract (USA)
Ralstonia paucula LMG 3245	CDC E8967, CCUG 12411	R. Weaver	Human, sputum (USA)
Ralstonia paucula LMG 3317	CCUG 12446, API 99-7-76	D. Monget	Human (USA)
Ralstonia paucula LMG 3318	CCUG 12443, API 93-7-76	D. Monget	Human (USA)
Ralstonia paucula LMG 3319	CCUG 11264	PHLS, Gothenborg, Sweden	Wound newborn (Sweden, 1981)
Ralstonia paucula LMG 3320	CCUG 4424	PHLS, Vasteras, Sweden	Humidifier in nursery (Sweden, 1975)
Ralstonia paucula LMG 3413	CIP 62.31, CCUG 13723	CIP	
Ralstonia paucula LMG 3517	API 077-07-76, Gilardi 1810	D. Monget	USA
Ralstonia paucula LMG 3518	API 078-07-76, Gilardi 1913	D. Monget	USA
Ralstonia paucula LMG 15544	CCUG 24723, Gilardi 5172	G. L. Gilardi	Human urine (USA, 1985)
Ralstonia paucula L5L7		M. Da Costa	Mineral water (Portugal)
Ralstonia paucula L1L1		M. Da Costa	Mineral water (Portugal)

thermal denaturation and calculated by using the equation of Marmur & Doty (1962), as modified by De Ley (1970).

DNA–DNA hybridization experiments. The degree of DNA–DNA binding, expressed in percentage, was determined spectrophotometrically by the initial renaturation rate method of De Ley *et al.* (1970). Each value is the mean of at least two hybridization experiments. Values of 30% DNA binding and less do not represent significant DNA homology. The total DNA concentration was about 62 μg/ml, and the optimal renaturation temperature in 2× SSC was 81·0 °C.

16S rDNA sequencing. Part of the rDNA operon, comprising the nearly complete 16S DNA was amplified by PCR. The forward primer was AGA GTT TGA TCC TGG CTC AG, corresponding to positions 8–27 of the *Escherichia coli* 16S rRNA numbering system. The reverse primer was AAG GAG GTG ATC CAG CCG CA, complementary to positions 1541–1522 of the *Escherichia coli* 16S rRNA numbering system. PCR amplified 16S rDNAs were purified using the QIAquick PCR Purification Kit (Qiagen). Sequence analysis was performed using an Applied Biosystems 377 DNA Sequencer and the protocols of the manufacturer using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (with AmpliTaq DNA Polymerase, Fs). The sequencing primers were those given by Coenye *et al.* (1999). Sequence assembly was performed by using the program AutoAssembler (Perkin-Elmer, Applied Biosystems) and phylogenetic analysis was performed by using the GeneCompar 2.0 software package (Applied Maths). The consensus sequence and the sequences of strains belonging to the same phylogenetic group (retrieved from the EMBL database) were aligned and a phylogenetic tree was constructed based on the neighbour-joining method.

Phenotypic tests. Classical phenotypic tests were performed as described previously (De Vos *et al.*, 1985). The API 20NE and API ZYM microtest systems were used according to the recommendations of the manufacturer (bioMérieux).

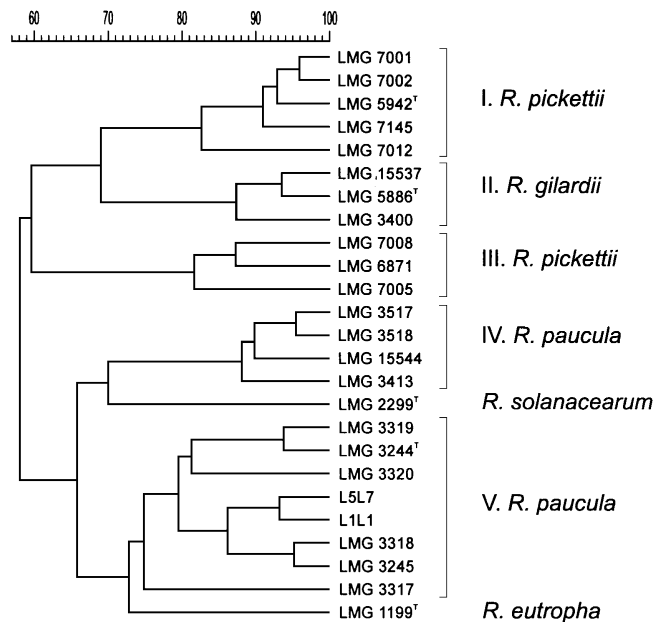

Fig. 1. Dendrogram derived from the unweighted pair group average linkage of correlation coefficients between the whole-cell protein patterns of all of the strains studied. Roman numerals are cluster numbers as discussed in the text.

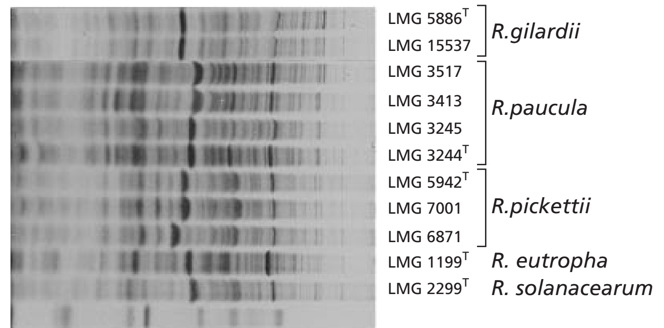

Fig. 2. Whole-cell protein profiles of a representative selection of strains examined. The molecular weight markers used (bottom lane) are indicated from left to right: lysozyme (14500), trypsin inhibitor (20100), trypsinogen (24000), carbonic anhydrase (29000), glyceraldehyde-3-phosphate dehydrogenase (36000), egg albumin (45000) and bovine albumin (66000).

RESULTS

PAGE of whole-cell proteins

Duplicate protein extracts of several strains were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. The similarity level between duplicate protein patterns was above 93% (data not shown).

The dendrogram resulting from the numerical analysis of the protein profiles of all of the strains examined is shown in Fig. 1; Fig. 2 shows the whole-cell protein patterns of a representative selection of the strains examined. CDC group IVc-2 strains formed two distinct clusters: a first cluster (cluster IV) grouping above a similarity level of 88% contained strains LMG 3517, LMG 3518, LMG 15544 and LMG 3413; and a second cluster (cluster V) grouping above a similarity level of 76%, contained strains LMG 3317, LMG 3318, LMG 3319, LMG 3320, LMG 3244^T, LMG 3245, L5L7 and L1L1. The differences between the whole-cell protein profiles of strains of the two clusters were primarily confined to the position and density of a dense protein band with molecular weight situated in the 29000–36000 molecular weight region (Fig. 2). In addition, some of the strains were characterized by a distortion of part of the protein pattern (compare the patterns of strains LMG 3245 and LMG 3244 in Fig. 2) which was a reproducible phenomenon.

Similarly, *R. pickettii* strains formed two distinct clusters (clusters I and III, grouping above similarity levels of 82% and 81%, respectively) which were characterized by a different position of a dense protein band with molecular weight situated in the 29000–36000 molecular weight region (compare the patterns

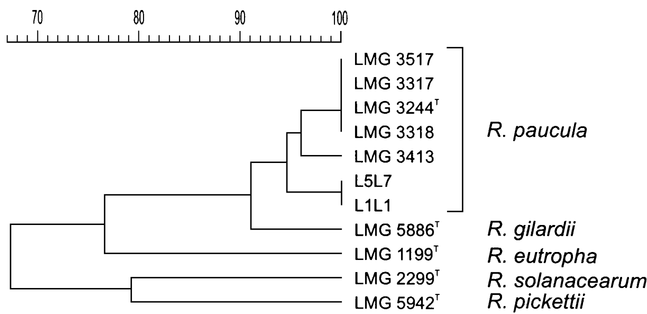

Fig. 3. Dendrogram derived from the unweighted pair group average linkage of Dice similarity coefficients (S_D) between the combined ARDRA patterns of all strains studied.

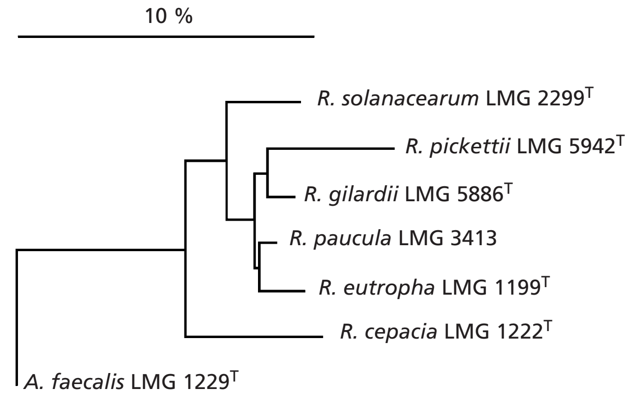

Fig. 4. Neighbour-joining phylogenetic tree of *R. paucula* LMG 3413 and related bacteria based on 16S rRNA sequence comparisons. Scale bar indicates 10% sequence dissimilarity.

of strains LMG 6871 and LMG 7001 in Fig. 2). The *R. gilardii* strains (cluster II, grouping above a correlation level of 87 %) and the type strains of *R. eutropha* and *R. solanaecearum* occupy distinct positions in the dendrogram (Fig. 1).

ARDRA analysis

Seven CDC group IVc-2 strains were examined along with the type strains of *R. eutropha*, *R. solanacearum*, *R. pickettii* and *R. gilardii*. In the numerical analysis of the combined ARDRA patterns (Fig. 3), all CDC group IVc-2 strains examined formed a single cluster above a similarity level of 95%. All of the type strains of the reference species occupied distinct positions.

DNA base composition

The DNA G+C content of all of strains examined is given in Table 2.

DNA–DNA hybridization results

The DNA–DNA hybridization results are listed in Table 2. Hybridization levels among CDC group IVc-2 strains representing both protein electrophoretic subclusters were between 67 and 100%. Values of 35% or less were measured towards and between type strains of *Ralstonia* reference species.

Phylogenetic analysis of the 16S rDNA sequence

Sequence similarity values towards 16S rDNA sequences of the closest phylogenetic neighbours of *R. paucula* LMG 3413 were: 97·5%, *R. gilardii* LMG 5886T (accession no. AF076645); 97·2%, *R. eutropha* LMG 1199T (M32021); 95·2% *R. pickettii* LMG 5942T (X67042); 94·2% *R. solanacearum* LMG 2299T (X67036). The similarity levels towards *Burkholderia cepacia* LMG 1222T (M22518), *Alcaligenes faecalis* LMG 1229T (M22508), and other representatives of the β subclass was below 90%. Fig. 4. shows the result of neighbour-joining cluster analysis of strain LMG 3413 and related bacteria.

Phenotypic tests

The results of the phenotypic analyses are listed below; some are shown in Table 3.

Table 2. DNA base ratio and DNA–DNA binding values (%) of strains examined

Strain	G+C content (mol%)	R. paucula LMG 3244T	R. paucula LMG 3413	R. pickettii LMG 5942T	R. eutropha LMG 1199T
R. paucula LMG 3244T	67	100			
R. paucula LMG 3413	65	82			
R. paucula LMG 3517		67			
R. gilardii LMG 5886T	68	21			
R. pickettii LMG 5942T	64	13		100	
R. eutropha LMG 1199T	69		24	35	100
R. solanacearum LMG 2299T	66	16		32	2

Table 3. Differential phenotypic characteristics of *R. paucula* and related or similar bacteria

+, Character is present in all strains; −, character is absent in all strains; w, weakly positive reaction; v, strain-dependent result.

Character	*R. paucula*	*R. pickettii*	*R. gilardii*	*B. bronchiseptica*
Catalase acitivity	+	−	+	+
Oxidase acitivity	+	−	−	+
Growth in the presence of:				
10 μg disk of penicillin	−	w	−	−
Tween 80	+	+	−	−
Growth at 42 °C	+	w	+	+
Oxidation/fermentation test for:				
D-Glucose	−	+	−	−
D-Fructose	−	v	−	−
D-Xylose	−	+	−	−
Nitrate reduction	−	+	−	+
Nitrite reduction	−	+	−	−
Denitrification	−	+	−	−
Urease activity	+	v	−	+
Assimilation of:				
D-Glucose	−	+	−	−
L-Arabinose	−	+	−	−
N-Acetylglucosamine	−	+	−	−
Caprate	+	w	−	v
Citrate	+	+	−	+
Adipate	+	w	w	+
Phenylacetate	+	w	−	+
Alkaline phosphatase activity	+	w	+	v
Acid phosphatase activity	+	w	+	v
Esterase C4 activity	+	w	+	+
Cystine arylamidase activity	+	−	−	−
Phosphoamidase activity	+	w	w	−
Lipase C14 activity	+	+	−	−

DISCUSSION

Classification of CDC group IVc-2 strains as a novel *Ralstonia* species

Previous studies have demonstrated that CDC group IVc-2 phylogenetically belongs to the genus *Ralstonia* as demonstrated by 16S rRNA sequence analysis and DNA–rRNA hybridization experiments (Rossau *et al*., 1987; Osterhout *et al*., 1998). In the present study, we examined the genotypic and phenotypic characteristics of 12 CDC group IVc-2 strains and compared them with those of the presently known species of the genus *Ralstonia*. The biochemical reactivity pattern, the whole-cell protein and the ARDRA patterns of all strains, including two spa water isolates (Manaia *et al*., 1990), were very similar and clearly distinct from those of strains representing the known *Ralstonia* species (Figs 1–3).

Analysis of the nearly complete 16S rDNA sequence of strain LMG 3413 revealed sequence similarity values of 94·2–97·5 % towards 16S rDNA sequences of *Ralstonia* species; the similarity levels towards *Burkholderia* or *Alcaligenes* species, the closest relatives of *Ralstonia*, were below 90 %. This confirmed that CDC group IVc-2 belongs to the genus *Ralstonia* as it clusters amidst the other named *Ralstonia* species and it is clearly separated from related genera. The DNA base ratio of about 66 mol % is within the range of the genus *Ralstonia* which is between 64 and 69 mol % (Coenye *et al*., 1999; Yabuuchi *et al*., 1995), and DNA–DNA hybridizations towards the type strains of *R. eutropha*, *R. solanacearum*, *R. pickettii* and *R. gilardii* revealed no significant DNA–DNA binding levels (Table 2). These data unambiguously indicate that CDC group IVc-2 represents a novel *Ralstonia* species for which we propose the name *Ralstonia paucula* sp. nov. below.

Identification of *R. paucula* sp. nov.

Thus far, two *Ralstonia* species have been isolated from human clinical specimens. *R. pickettii* is a well-established species that has been isolated from various human sources including urine, wounds, blood, cerebrospinal fluid and the nasopharynx (Riley & Weaver, 1975). *R. gilardii* is a novel species that has been

isolated from spinal fluid, bone marrow, a furuncle and environmental sources (Coenye *et al*., 1999). As discussed above, whole-cell protein and ARDRA analyses differentiate *R. paucula* from other *Ralstonia* species. In addition, phenotypic tests as listed in Table 3 readily allow differentiation of *R. paucula* from the other *Ralstonia* species. *Bordetella bronchiseptica*, an organism mainly isolated from respiratory tract infections in man and animals shares many characteristics with *R. paucula* but can be differentiated by its ability to reduce nitrate, inability to grow in the presence of Tween 80, and absence of cystine arylamidase, phosphoamidase and lipase C14 activity (Table 3).

Description of *Ralstonia paucula* sp. nov.

Ralstonia paucula (pau′cu.la. L. adj. *pauculus* rare, very few, to indicate that these strains only sporadically cause human infections).

Cells are Gram-negative, non-spore-forming and rod-shaped. After 24 h growth on Trypticase soy agar at 30 °C, the mean cell size was about 0·8 µm in width, and 1·2–2 µm in length. Strains produce convex, circular, and beige colonies with entire edge and smooth surface. Motile by means of peritrichous flagella. Catalase and oxidase activities are present. Non-pigmented. No haemolysis on horse blood agar. Grows at 30, 37 and 42 °C. No acid production from D-glucose, maltose, adonitol, D-fructose, or D-xylose. Growth in the presence of 0·5 and 1·5 % NaCl, but not in the presence of cetrimide, 10 % lactose, penicillin (10 µg disks) or 3, 4·5 or 6 % NaCl. Grows on Drigalski agar. Hydrolyses Tween 80. No fluorescence on King B medium. Negative for lysine or ornithine decarboxylases and for arginine dihydrolase activity. No amylase activity. Nitrate and nitrite are not reduced. No denitrification. Hydrolysis of urea, but not gelatin or aesculin. No β-galactosidase or DNase activity. No production of indole. No production of hydrogen sulfide or acid in triple sugar iron agar. No tryptophanase activity. Assimilates D-gluconate, caprate, adipate, L-malate, citrate, phenyl acetate and DL-lactate, but not D-glucose, trehalose, L-arginine, DL-norleucine, L-arabinose, D-mannose, D-mannitol, *N*-acetyl-D-glucosamine, maltose or sucrose. Alkaline and acid phosphatase, esterase C4, ester lipase C8, lipase C14, and leucine and cystine arylamidases, and phosphoamidase activity are present; valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-glucuronidase, α- and β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase and α-fucosidase activity are not detected.

Ubiquinone-8 was reported to be the respiratory quinone (Ferreira *et al*., 1996). Strains have been isolated from a variety of human clinical sources including blood, wounds, sputum, urine, eye, throat and peritoneal fluid. In addition, *R. paucula* strains have been isolated from pool water (Aspinal & Graham, 1989), groundwater (Campbell Wyndham *et al*., 1994) and bottled mineral water (Manaia *et al*., 1990). It should be noted that *R. paucula* strains were present in bottled mineral water only at the day of bottling; after 7 d storage, no strains could be recovered, suggesting that they did not survive or were, unlike several other components of the flora of bottled mineral waters, unable to multiply (Ferreira *et al*., 1996). The DNA G+C content is 65–67 mol %. The type strain is LMG 3244^T (= CCUG 12507^T = CDC E6793^T), which was isolated from a human respiratory tract in the USA. Its G+C content is 67 mol %. *Ralstonia paucula* reference strains are available from the BCCM/LMG and CCUG Culture Collections.

ACKNOWLEDGEMENTS

We thank D. Dewettinck, R. Coopman, L. Lebbe and K. Engelbeen for excellent technical assistance and are indebted to the Fund for Scientific Research – Flanders (Belgium) for positions as post-doctoral research fellow (P. V.), senior research associate (P. D. V.), and research and personnel grants (K. K.). We thank all depositors of strains listed in Table 1. T. C. acknowledges the support received from the Vlaams Instituut voor Bevordering van Wetenschappelijk-technologisch onderzoek in de Industrie (Belgium) in the form of a bursary for advanced study. We acknowledge the financial support received from the Cystic Fibrosis Trust (United Kingdom) (grant RS15), the Ministerie van de Vlaamse Gemeenschap, Bestuur Wetenschappelijk Onderzoek (Belgium) for a Concerted Research Action, and the Prime Minister's Services – Federal Office for Scientific, Technical and Cultural Affairs (Belgium).

REFERENCES

Anderson, R. R., Warnick, P. & Schreckenberger, P. C. (1997). Recurrent CDC group IVc-2 bacteremia in a human with AIDS. *J Clin Microbiol* **35**, 780–782.

Aspinal, S. T. & Graham, R. (1989). Two sources of contamination of a hydrotherapy pool by environmental organisms. *J Hosp Infect* **14**, 285–292.

Campbell Wyndham, R., Nakatsu, C., Peel, M., Cashore, A., Ng, J. & Szilagyi, F. (1994). Distribution of the catabolic transposon Tn*5271* in a groundwater bioremediation system. *Appl Environ Microbiol* **60**, 86–93.

Coenye, T., Falsen, E., Vancanneyt, M., Hoste, B., Govan, J. R. W., Kersters, K. & Vandamme, P. (1999). Classification of *Alcaligenes faecalis*-like isolates from the environment and human clinical samples as *Ralstonia gilardii* sp. nov. *Int J Syst Bacteriol* **49**, 405–413.

De Ley, J. (1970). Re-examination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J Bacteriol* **101**, 738–754.

De Ley, J. (1992). The *Proteobacteria*: ribosomal RNA cistron similarities and bacterial taxonomy, pp. 2111–2140. In *The Prokaryotes*, 2nd edn, vol. 2. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin: Springer.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

De Vos, P., Kersters, K., Falsen, E., Pot, B., Gillis, M., Segers, P. & De Ley, J. (1985). *Comamonas* Davis and Park 1962 gen. nov.,

nom. rev. emend., and *Comamonas terrigena* Hugh 1962 sp. nov., nom. rev. *Int J Syst Bacteriol* **35**, 443–453.

Ferreira, A. C., Morais, P. V., Gomes, C. & Da Costa, M. D. (1996). Computer-aided comparison of protein electrophoretic patterns for grouping and identification of heterotrophic bacteria from mineral water. *J Appl Bacteriol* **80**, 479–486.

Heyndrickx, M., Vauterin, L., Vandamme, P., Kersters, K. & De Vos, P. (1996). Applicability of combined amplified ribosomal DNA restriction analysis (ARDRA) patterns in bacterial phylogeny and taxonomy. *J Microbiol Methods* **26**, 247–259.

Kersters, K. & De Ley, J. (1984). Genus *Alcaligenes* Castellani and Chalmers 1919, 936[AL]. In *Bergey's Manual of Systematic Bacteriology*, vol. 1, pp. 361–373. Edited by N. R. Krieg & J. G. Holt. Baltimore: Williams & Wilkins.

Manaia, C. M., Nunes, O. C., Morais, P. V. & Da Costa, M. S. (1990). Heterotrophic plate counts and the isolation of bacteria from mineral waters on selective and enrichment media. *J Appl Bacteriol* **69**, 871–876.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Osterhout, G. J., Valentine, J. L. & Dick, J. D. (1998). Phenotypic and genotypic characterization of clinical strains of CDC group IVc-2. *J Clin Microbiol* **36**, 2618–2622.

Pot, B., Vandamme, P. & Kersters, K. (1994). Analysis of electrophoretic whole-organism protein fingerprints. In *Modern Microbial Methods. Chemical Methods in Prokaryotic Systematics*, pp. 493–521. Edited by M. Goodfellow & A. G. O'Donnell. Chichester: Wiley.

Riley, P. S. & Weaver, R. E. (1975). Recognition of *Pseudomonas pickettii* in the clinical laboratory : biochemical characterisation of 62 strains. *J Clin Microbiol* **1**, 61–64.

Rossau, R., Kersters, K., Falsen, E., Jantzen, E., Segers, P., Union, A., Nehls, L. & De Ley, J. (1987). *Oligella*, a new genus including *Oligella urethralis* comb. nov. (formerly *Moraxella urethralis*) and *Oligella ureolytica* sp. nov. (formerly CDC group IVe): relationship to *Taylorella equigenitalis* and related taxa. *Int J Syst Bacteriol* **37**, 198–210.

Vandamme, P., Vancanneyt, M., Pot, B. & 10 other authors (1992). Polyphasic taxonomic study of the emended genus *Arcobacter* with *Arcobacter butzleri* comb. nov. and *Arcobacter skirrowii* sp. nov., an aerotolerant bacterium isolated from veterinary specimens. *Int J Syst Bacteriol* **42**, 344–356.

Yabuuchi, E., Kosako, Y. Yano, I. Hotta, H. & Nishiuchi, Y. (1995). Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. nov.: proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. nov., *Ralstonia solanacearum* (Smith 1896) comb. nov. and *Ralstonia eutropha* (Davis 1969) comb. nov. *Microbiol Immunol* **39**, 897–904.