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Classification of *Pseudomonas diminuta* Leifson and Hugh 1954 and *Pseudomonas vesicularis* Büsing, Döll, and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov., Respectively

P. SEGERS,[1] M. VANCANNEYT,[1] B. POT,[1] U. TORCK,[1] B. HOSTE,[1] D. DEWETTINCK,[1] E. FALSEN,[2] K. KERSTERS,[1] AND P. DE VOS[1]*

Laboratorium voor Microbiologie, Universiteit Gent, B-9000 Ghent, Belgium,[1] and Culture Collection, Department of Clinical Bacteriology, University of Göteborg, S-413 46 Göteborg, Sweden[2]

The taxonomic positions of strains previously assigned to *Pseudomonas diminuta* and *Pseudomonas vesicularis* were investigated by a polyphasic approach. The results of DNA-rRNA hybridization studies indicated that these two species belong to a separate genus in the α subclass (rRNA superfamily IV) of the *Proteobacteria*, for which the name *Brevundimonas* is proposed. Genus delineation and species delineation were determined by comparing the results of numerical analyses of whole-cell protein patterns, fatty acid compositions, and phenotypic characteristics and by measuring DNA base ratios and degrees of DNA relatedness. Taxonomic characteristics of *Brevundimonas diminuta* and *Brevundimonas vesicularis* strains were compared with characteristics of reference strains belonging to the following phylogenetically related taxa: a group of organisms gathered in Enevold Falsen group 21, the genera *Sphingomonas* and *Rhizomonas*, and the generically misclassified organisms [*Pseudomonas*] *echinoides* and "[*Pseudomonas*] *riboflavina*."

The original description of the genus *Pseudomonas* Migula 1894 allowed the inclusion of an extremely wide variety of aerobic gram-negative bacteria (24). The use of rRNA-based techniques, such as DNA-rRNA hybridization (12–14, 26) and 16S rRNA oligonucleotide cataloging (42), revealed the phylogenetic distribution of members of the genus *Pseudomonas* sensu Palleroni (24) in the α, β, and γ subclasses of the *Proteobacteria* (6). The phylogenetic heterogeneity of the genus *Pseudomonas* was also supported by the finding that there are enzymatic differences in aromatic amino acid biosynthesis (4). Therefore, the authentic genus *Pseudomonas* should be restricted to Palleroni's rRNA group I (12, 24). Various pseudomonads belonging to the other rRNA groups have previously been allocated to other genera (33) or elevated to genus rank (25, 38, 40, 43, 44).

The rRNA group IV members [*Pseudomonas*] *diminuta* and [*Pseudomonas*] *vesicularis*, often referred to as the diminuta group (1, 16), occupy a unique taxonomic position among the pseudomonads (genus names in brackets indicate that an organism is generically misclassified). Both of these species can be distinguished from most gram-negative bacteria by a combination of characteristics, including flagellar morphology, nutritional properties, acid tolerance, type of pigmentation, nature of carbon reserve materials, and DNA base composition (1, 16, 24). The results of DNA-rRNA hybridization experiments (12), 16S rRNA cataloging (42), and 16S rRNA sequencing (30) have indicated that [*P.*] *diminuta* occupies a separate branch in rRNA superfamily IV (the α-2 subclass) of the *Proteobacteria*.

Although [*P.*] *diminuta* and [*P.*] *vesicularis* are genotypically easily differentiated from other taxa belonging to rRNA superfamily IV, genus delineation and species delineation based on phenotypic characteristics are less clear. Therefore, some strains were preliminarily classified into EF group 21, a heterogeneous group containing organisms that are phenotypically similar to [*P.*] *vesicularis* and *Sphingomonas paucimobilis* (formerly [*Pseudomonas*] *paucimobilis*) (15). The taxonomic positions of these taxa and of representatives of the genera *Sphingomonas* and *Rhizomonas* (34, 44) were examined in this study. The phylogenetic positions of two other misclassified organisms, [*Pseudomonas*] *echinoides* and "[*Pseudomonas*] *riboflavina*," were also determined (names in quotation marks are invalid names).

On the basis of both genotypic and phenotypic characteristics, we propose that [*P.*] *diminuta* and [*P.*] *vesicularis* should be classified in the new genus *Brevundimonas*. The names *Brevundimonas diminuta* comb. nov. and *Brevundimonas vesicularis* comb. nov. are used below for [*P.*] *diminuta* and [*P.*] *vesicularis*, respectively.

MATERIALS AND METHODS

Strains used. The designations and sources of the strains which we used are listed in Table 1. All strains were checked for purity by plating and microscopic examination. The cells were cultivated on nutrient agar at 28°C for 1 or 2 days.

Polyacrylamide gel electrophoresis of whole-cell proteins. The methods used for preparation and electrophoresis of sodium dodecyl sulfate protein extracts have been described previously (35). The gel electrophoretic profiles were recorded densitometrically, stored as normalized records in a PC-AT computer, and analyzed numerically with the GelCompar software package (Applied Maths, Kortrijk, Belgium). The levels of similarity between pairs of traces were calculated by using the Pearson product-moment correlation coefficient (*r*),

* Corresponding author. Mailing address: Laboratorium voor Microbiologie, Universiteit Gent, K.L. Ledeganckstraat, 35, B-9000 Ghent, Belgium.

TABLE 1. Strains used, other strain designations, and sources of isolation

Strain[a]	Other designation(s)[a]	Name as received	Source
Brevundimonas diminuta strains			
LMG 2089t1^T	ATCC 11568^T, CCEB 513^T, CCUG 1427^T, LMG 1793^T	*Pseudomonas diminuta*	Fresh water
LMG 2337	NCTC 9239, CCUG 1797, ATCC 14736	*Pseudomonas diminuta*	Blood, patient with endocarditis
LMG 3503	NCIB 9407, CCUG 28327	*Pseudomonas diminuta*	
LMG 9559	CCUG 269C	*Pseudomonas diminuta*	Human urine (Göteborg, Sweden)
LMG 9561	ATCC 13184, CCUG 2031	*Pseudomonas diminuta*	Buccal cavity
LMG 9562	CCUG 2868, NCIB 10329	*Pseudomonas diminuta*	Unknown
LMG 9563	CCUG 14202	*Pseudomonas diminuta*	Blood plasma (Slagelse, Denmark)
LMG 10743	CCUG 24715, ATCC 19146, DSM 1635	*Pseudomonas diminuta*	Contaminant of *Bacillus cereus* culture
LMG 11061	CCUG 154	*Pseudomonas diminuta*	Contaminated egg yolk (Göteborg, Sweden)
LMG 11062	CCUG 1376	*Pseudomonas diminuta*	Unknown (Orebro, Sweden)
LMG 11063	CCUG 14332	*Pseudomonas diminuta*	Humidifier, water (Strasbourg, France)
LMG 11064	CCUG 25811	*Pseudomonas diminuta*	Human ear (Boras, Sweden)
LMG 11065	CCUG 1189	*Pseudomonas diminuta*	Unknown
LMG 11136	CCUG 28328	*Pseudomonas diminuta*	Hemoculture (Hospital, Brussels, Belgium)
LMG 11137	CCUG 28329	*Pseudomonas diminuta*	Hemoculture (Hospital, Brussels, Belgium)
LMG 11138	CCUG 28330	*Pseudomonas diminuta*	Pleural liquid (Hospital, Brussels, Belgium)
LMG 11139	CCUG 28331	*Pseudomonas diminuta*	Hemoculture (Hospital, Brussels, Belgium)
LMG 11147	CCUG 248	*Pseudomonas diminuta*	Water (Göteborg, Sweden)
LMG 11149	CCUG 1364	*Pseudomonas diminuta*	Unknown (Orebro, Sweden)
Brevundimonas vesicularis strains			
LMG 2350^T	ATCC 11426^T, CCUG 2032^T, NCTC 10900^T	*Pseudomonas vesicularis*	Leech (*Hirudo* sp.), urinary bladder, epithelium
LMG 9565	CCUG 14431A	*Pseudomonas vesicularis*	Leech (*Hirudo* sp.), urinary bladder?
LMG 9566	CCUG 14807	*Pseudomonas vesicularis*	Leg wound (United States)
LMG 11067	CCUG 14205	*Pseudomonas vesicularis*	Denmark
LMG 11068	CCUG 14806	*Pseudomonas vesicularis*	Environment (United States)
LMG 11140	CCUG 28336	*Pseudomonas vesicularis*	Vaginal swab (Hospital, Brussels, Belgium)
LMG 11141	CCUG 28337	*Pseudomonas vesicularis*	Pleural liquid (Hospital, Brussels, Belgium)
LMG 11142	Hansen 1220	*Pseudomonas vesicularis*	Hemoculture (Hospital, Brussels, Belgium)
LMG 11148	CCUG 1314	*Pseudomonas vesicularis*	Unknown (Denmark?)
LMG 9554	CCUG 14808	EF group 21	Blood (United States)
Brevundimonas sp. strains			
LMG 9564	CCUG 14203	*Pseudomonas vesicularis*	Denmark
LMG 9567t1	CCUG 14827, CCM 3400	*Pseudomonas vesicularis*	Oral contraceptives
LMG 11070	CCUG 22949	*Pseudomonas vesicularis*	Water, deep bore hole (Göteborg, Sweden)
Brevundimonas-like strain LMG 11050	CCUG 26751	EF group 21	Human blood (Göteborg, Sweden)
Sphingomonas paucimobilis LMG 1227^T	NCTC 11030^T, CCUG 6518^T, DSM 1098^T	*Sphingomonas paucimobilis*	Hospital respirator (London, United Kingdom)
[*Sphingomonas*] *capsulata* LMG 2830^T	ATCC 14666^T, CCUG 17697^T, DSM 30196^T, IFO 12533^T	*Sphingomonas capsulata*	Stocked distilled water
Sphingomonas adhaesiva LMG 10922^T	CCUG 27290^T	*Sphingomonas adhaesiva*	Sterile water used before surgery
Sphingomonas parapaucimobilis LMG 10923^T	CCUG 27291^T	*Sphingomonas parapaucimobilis*	Urine
Sphingomonas sanguis LMG 10925^T	CCUG 27654^T	*Sphingomonas* genospecies 1	Blood (United States?)
Sphingomonas terrae LMG 10924	CCUG 27293	*Sphingomonas* genospecies 2	Sterile water used before surgery
[*Sphingomonas*] *yanoikuyae* strains			
LMG 11252^T	CCUG 28380^T	*Sphingomonas yanoikuyae*	Human (Copenhagen, Denmark)
LMG 9552	CCUG 14335	EF group 21 D	Hospital dialysis equipment, water
LMG 9553	CCUG 14336A	EF group 21 D	Hospital dialysis equipment, water
LMG 11066	CCUG 14204	*Pseudomonas vesicularis*-like	Denmark
Rhizomonas-Sphingomonas-like strains			
LMG 11049	CCUG 25702	EF group 21	Cerebrospinal fluid (Göteborg, Sweden)
LMG 11069	CCUG 25098	*Pseudomonas vesicularis*-like	Water, shower in hospital (Göteborg, Sweden)
LMG 9555	CCUG 18318	EF group 21 B	Pleural fluid (Göteborg, Sweden)
LMG 11151	CCUG 18056	*Pseudomonas paucimobilis*	Tap water (Göteborg, Sweden)
"[*Pseudomonas*] *riboflavina*" LMG 2277^T	ATCC 9526^T	*Pseudomonas riboflavina*	Riboflavin-rich soil
Sphingomonas sp. strain LMG 2181^T	ATCC 14820^T	*Pseudomonas echinoides*	Laboratory contaminant of nutrient agar plate

[a] ATCC, American Type Culture Collection, Rockville, Md.; CCM, Czech Collection of Microorganisms, Brno, Czech Republic; CCUG, Culture Collection of the University of Göteborg, Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany; IFO, Institute for Fermentation, Osaka, Japan; LMG, Culture Collection, Laboratorium voor Microbiologie, University of Ghent, Ghent, Belgium; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCTC, National Collection Type Cultures, Central Public Health Laboratory, London, England.

and a cluster analysis was performed by using the unweighted average linkage algorithm (36).

Gas chromatographic analysis of methylated fatty acids. Bacteria were grown at 28°C on Trypticase soy agar containing 3.0% (wt/vol) Trypticase soy broth (BBL) and 1.5% (wt/vol) Bacto Agar (Difco). After 24 h of growth approximately 60 mg (wet weight) of cells was harvested from the most dilute quadrant exhibiting confluent growth. The methods used to extract fatty acids, prepare methyl esters, and separate methyl esters by gas chromatography have been described previously (37). Fatty acid methyl ester fingerprints were identified with the Microbial Identification System software package (MIS version 3.7; Microbial ID, Inc., Newark, Del.). A statistical program, CLUS (Microbial ID, Inc.), was used to perform a cluster analysis of the strains.

Phenotypic characterization. All 105 conventional tests used were performed as described previously (15). The carbon assimilation tests were performed with API 50CH, API 50AO, and API 50AA galleries (API Systems, Montalieu-Vercieu, France) according to a standardized procedure (19). Levels of similarity between isolates were calculated by using the simple matching coefficient of Sokal and Michener (29), and a cluster analysis was performed by the unweighted average pair group method (28), using the Clustan 2.1 program (41) and the Siemens model 7551 computer of the Centraal Digitaal Rekencentrum, Universiteit Gent, Ghent, Belgium.

DNA-DNA hybridization. Degrees of DNA-DNA binding, expressed as percentages, were determined spectrophotometrically by using the initial renaturation method of De Ley et al. (7) and the equipment and method described by Willems et al. (38).

DNA base composition. High-molecular-weight DNAs from representative strains of each of the clusters were isolated by the method of Marmur (21). The average guanine-plus-cytosine (G+C) contents were determined by the thermal denaturation method (10) and were calculated according to the equation of De Ley (5).

DNA-rRNA hybridization. DNAs were made single stranded and fixed on cellulose nitrate filters (9). ^{3}H-labeled rRNAs from the type strains of *B. vesicularis* and *S. paucimobilis* were isolated and purified as described by De Ley and De Smedt (8). Hybridization between the 16S rRNA probes and filter-fixed DNAs was carried out as described previously (8). The temperature at which one-half of each DNA-rRNA hybrid was thermally denatured ($T_{m(e)}$) was used to construct an rRNA dendrogram.

Analysis of cellular polyamines. Polyamine profiles were determined by a high-performance liquid chromatography-based technique described by Busse and Auling (3), which was slightly modified as described by Yang et al. (45). The polyamine contents are reported below in micromoles per gram (wet weight).

RESULTS

Polyacrylamide gel electrophoresis of whole-cell proteins. The results of a cluster analysis of the protein profiles of *B. diminuta*, *B. vesicularis*, *Sphingomonas*, *Rhizomonas*, and some related strains are shown in Fig. 1. Considerable heterogeneity was observed among the profiles of *B. vesicularis* strains. At an *r* level of 0.82 two subgroups could be delineated; one of these two subgroups contained five strains (LMG 2350^{T} [T = type strain], LMG 11068, LMG 9566, LMG 11067, and LMG 9565), and the other contained four strains (LMG 9554, LMG 11142, LMG 11148, and LMG 11140). The profiles of one *B. vesicularis* strain (LMG 11141), three *Brevundimonas* sp. strains

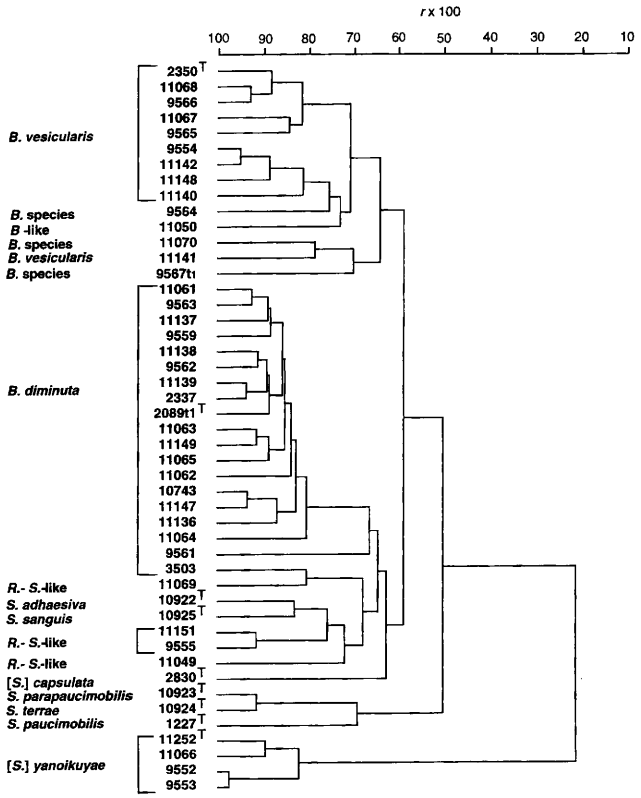

FIG. 1. Dendrogram based on the results of an unweighted pair group average linkage cluster analysis of correlation coefficients (*r*) obtained by using the sodium dodecyl sulfate-polyacrylamide gel electrophoresis protein patterns of all of the strains studied. Abbreviations: *B.*, *Brevundimonas*; *R.*, *Rhizomonas*; *S.*, *Sphingomonas*.

(LMG 9564, LMG 9567t1, and LMG 11070), and one unclassified strain (LMG 11050) were linked at *r* levels ranging from 0.65 to 0.75. A more homogeneous protein pattern was found for the strains of *B. diminuta*; 17 strains were grouped in a single cluster ($r \geq 0.82$), and only 2 strains (LMG 9561 and LMG 3503) occupied a separate position. In the genus *Sphingomonas* the reference strains of some species could not be characterized individually; similar protein profiles were found for the type strains of *Sphingomonas adhaesiva* and *Sphingomonas sanguis* ($r = 0.82$) and for *Sphingomonas parapaucimobilis* and *Sphingomonas terrae* ($r = 0.91$). The type strains of [*Sphingomonas*] *capsulata* and *S. paucimobilis* produced unique protein patterns. Two of four strains related to the genera *Sphingomonas* and *Rhizomonas* (strains LMG 11069 and LMG 11049) produced unique protein fingerprints, while the other two strains (LMG 11151 and LMG 9555) formed a distinct electrophoretic cluster. The four strains of [*Sphingomonas*] *yanoikuyae* produced very similar protein fingerprints that were clearly distinct from the fingerprints of the other strains studied.

Gas chromatographic analysis of methylated fatty acids (fatty acid methyl esters). The cellular fatty acid compositions of *Brevundimonas* strains and reference strains of phylogenetically related taxa are shown in Table 2. As shown in Fig. 2, a numerical analysis of the results revealed several clusters. All *Brevundimonas* strains grouped into two large clusters. One of these contained all *B. vesicularis* strains, three *Brevundimonas* sp. strains (LMG 9564, LMG 11070, and LMG 9567t1), and

TABLE 2. Fatty acid compositions of the taxa studied

Taxon[b]	Fatty acid composition (%)[a]														
	12:0 3OH	14:0	15:0	14:0 2OH	16:1 ω7c	16:1 ω5c	16:0	15:0 2OH	17:1 ω8c	17:1 ω6c	17:0	18:1 ω5c	Summed feature 7[c]	ECL 18.080[d]	19:0 cyclo ω8c
B. diminuta[e]	2.6 ± 0.4	1.5 ± 0.2	1.3 ± 0.5	–[f]	2.2 ± 0.7	–	30.4 ± 2.2	–	tr[g]	tr	1.2 ± 0.5	–	45.3 ± 6.5	1.3 ± 1.5	10.1 ± 3.7
B. vesicularis	3.1 ± 0.6	3.6 ± 0.7	0.9 ± 0.6	–	7.3 ± 1.9	–	21.2 ± 2.6	–	tr	tr	tr	–	59.0 ± 4.0	2.0 ± 1.3	tr
Brevundimonas sp. strain LMG 9564	2.3	2.8	4.0	–	7.3	tr	18.9	–	3.6	2.0	2.8	–	49.6	5.0	–
Brevundimonas sp. strain LMG 9567t1	2.8	3.4	1.5	–	4.2	–	24.3	–	1.2	–	1.4	–	56.8	4.5	–
Brevundimonas sp. strain LMG 11070	2.4	1.6	2.9	–	16.2	–	18.6	–	2.4	1.3	1.4	–	42.9	6.5	2.3
Brevundimonas-like strain LMG 11050[h]	1.1	tr	4.1	–	9.2	–	17.7	–	tr	1.6	2.6	tr	45.3	2.9	7.3
S. adhaesiva LMG 10922^{T}[i]	–	1.4	3.1	15.9	6.6	1.8	6.0	3.6	2.6	12.5	–	–	42.2	1.6	–
[*S.*] *capsulata* LMG 2830^{T}	–	–	–	19.8	7.2	1.5	7.2	1.4	tr	3.4	–	1.3	57.4	–	–
S. parapaucimobilis LMG 10923^{T}	–	1.7	–	9.5	10.1	1.6	9.0	–	–	1.7	–	2.6	63.9	–	–
S. paucimobilis LMG 1227^{T}	–	1.0	–	5.5	3.4	tr	7.9	–	–	1.2	–	3.7	76.5	–	–
S. sanguis LMG 10925^{T}	–	1.7	–	8.8	7.1	tr	14.4	–	–	1.2	–	1.7	64.3	–	–
S. terrae LMG 10924^{T}	–	tr	–	13.4	4.1	tr	12.6	–	–	–	–	2.1	66.4	–	–
[*S.*] *yanoikuyae*	–	tr	tr	7.8 ± 0.9	18.0 ± 1.6	1.9 ± 0.2	11.0 ± 2.7	tr		1.7 ± 0.5	–	1.4 ± 0.3	56.6 ± 3.7	tr	–
Rhizomonas-Sphingomonas-like strain LMG 11069[j]	–	–	–	16.8	26.7	1.4	5.9	–	–	–	–	–	45.3	1.7	–
Rhizomonas-Sphingomonas-like strain LMG 11049	–	–	–	15.4	20.6	1.5	5.0	–	–	1.7	–	–	54.3	1.4	–
Rhizomonas-Sphingomonas-like strains LMG 11151 and LMG 9555	–	tr	2.6 ± 0.3	1.0 ± 0.1	6.0 ± 0.2	tr	4.8 ± 0.3	5.3 ± 0.4	8.0 ± 0.1	39.9 ± 1.0	2.9 ± 0.1	tr	24.1 ± 0.6	2.1 ± 0.1	–

[a] The fatty acids that accounted for less than 1% of the total fatty acids in all of the strains studied are not shown.
[b] We determined the fatty acid compositions of 19 *B. diminuta* strains, 10 *B. vesicularis* strains, and 4 [*S.*] *yanoikuyae* strains. For these taxa and for *Rhizomonas-Sphingomonas*-like strains LMG 11151 and LMG 9555 the means ± standard deviations are shown.
[c] Summed feature 7 consisted of one or more of the following isomers which could not be separated by the Microbial Identification System: 18:1 ω7c, 18:1 ω9t, and 18:1 ω12t.
[d] ECL, equivalent chain length. The identity of the fatty acid is not known.
[e] *B. diminuta* strains also contain an unknown fatty acid with an equivalent chain length of 13.815; this fatty acid is present at a level of 1.3 ± 0.6%.
[f] –, not detected.
[g] tr, trace (less than 1.0%).
[h] *Brevundimonas*-like strain LMG 11050 also contains 2.8% 12:0 and 3.2% 16:1 ω11c.
[i] *S. adhaesiva* LMG 10922^{T} also contains 3.2% 18:1 2OH.
[j] *Rhizomonas-Sphingomonas*-like strain LMG 11069 also contains 1.0% 16:0 iso 3OH and 1.3% 16:0 2OH.

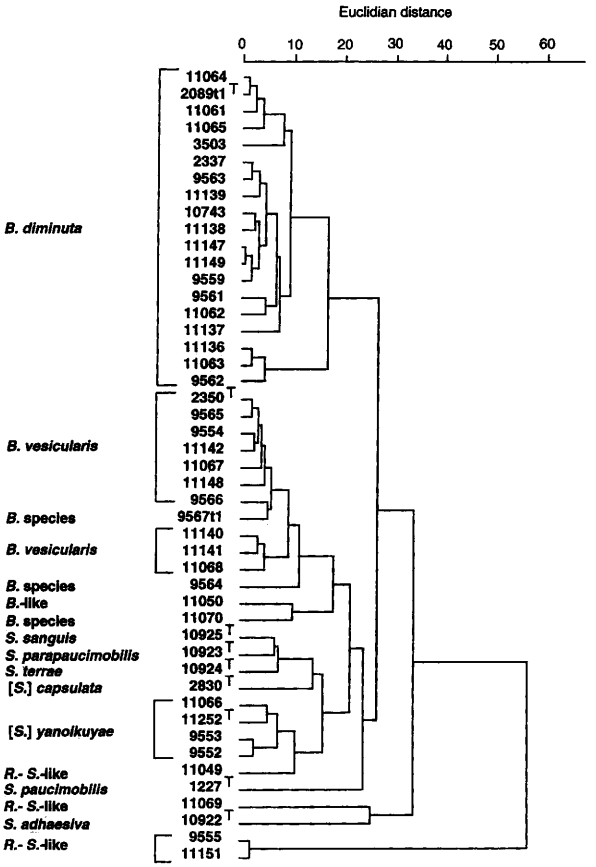

FIG. 2. Dendrogram obtained after numerical analysis of fatty acid methyl ester profiles. Abbreviations are explained in the legend to Fig. 1.

strain LMG 11050. The other large cluster contained all *B. diminuta* strains, including strain LMG 3503. All *Brevundimonas* strains contained two major fatty acids, 16:0 and 18:1 (summed feature 7), and the following minor components: 12:0 3OH, 14:0, 15:0, 16:1 ω7c, 17:0, and an unknown fatty acid with an equivalent chain length of 18.080. *B. diminuta* was easily differentiated from *B. vesicularis* strains by the presence of high percentages of 19:0 cyclo ω8c. *Brevundimonas* strains were differentiated from *Sphingomonas* and *Rhizomonas* strains by the presence of 12:0 3OH and by the absence of 14:0 2OH. The type strains of *S. parapaucimobilis*, *S. sanguis*, and *S. terrae* grouped together (Fig. 2), while those of *S. adhaesiva*, [*S.*] *capsulata*, and *S. paucimobilis* occupied distinct positions. The strains of [*S.*] *yanoikuyae* formed a separate cluster. Two of four strains related to the genera *Sphingomonas* and *Rhizomonas* formed a cluster (LMG 9555 and LMG 11151), while LMG strain 11049 was rather close to [*S.*] *yanoikuyae* and strain LMG 11069 occupied a separate position in the fatty acid methyl ester dendrogram.

Numerical analysis of phenotypic features. All *Brevundimonas*, *Sphingomonas*, *Rhizomonas*, and related strains studied were characterized phenotypically. Features that differentiate taxa within the genus *Brevundimonas* are shown in Table 3, and the results of a numerical analysis of the data are shown in a dendrogram in Fig. 3. All *B. diminuta* strains exhibited very similar, restricted growth on most carbohydrates (Table 3). A separate cluster was formed by all of the *B. vesicularis* strains (Fig. 3); these strains were differentiated from *B. diminuta* by the assimilation of particular sugars and by the lack of growth on several organic and amino acids (Table 3). The *Brevundimonas* sp. strains (LMG 11070, LMG 9564, and LMG 9567t1) grouped at the border of the *B. vesicularis* cluster. In this analysis, most of the *Sphingomonas* type strains occupied distinct positions; the exceptions were the type strains of *S. sanguis* and *S. terrae*, which grouped together. Well-defined clusters were formed by the four strains of [*S.*] *yanoikuyae* and by two strains related to the genera *Rhizomonas* and *Sphingomonas* (LMG 9555 and LMG 11151). The other strains (LMG 11050, LMG 11069, and LMG 11049) did not group in one of the clusters discussed above.

DNA-DNA hybridization. On the basis of the results of the numerical analyses described above, representative strains were selected for DNA-DNA hybridization experiments. Figure 4 shows that both *B. diminuta* and *B. vesicularis* are distinct and well-characterized genomic species; the DNA-binding values for these species ranged from 41 to 73% and from 52 to 68%, respectively. Two *Brevundimonas* sp. strains (LMG 11070 and LMG 9564) exhibited no significant levels of DNA binding with *B. diminuta* and *B. vesicularis* strains. Two other strains, *Brevundimonas* sp. strain LMG 9567t1 and *Brevundimonas*-like strain LMG 11050, exhibited no significant levels of DNA binding with *B. vesicularis*. Among the other strains investigated, two DNA homology groups could be differentiated: one of these groups contained [*S.*] *yanoikuyae* strains, and the other contained strains LMG 9555 and LMG 11151, which were rather distantly related to the genera *Sphingomonas* and *Rhizomonas* (see below).

DNA base composition. The average G+C contents of representative strains are shown in Table 4. The genera *Brevundimonas* and *Sphingomonas* and their respective species could not be differentiated on the basis G+C contents. The DNA base ratios ranged from 65.4 to 67.9 mol% for the genus *Brevundimonas* and from 65.2 to 67.9 mol% for the genus *Sphingomonas*. [*S.*] *yanoikuyae* strains had somewhat lower G+C contents (63 to 65 mol%).

DNA-rRNA hybridization. ^{3}H-labeled rRNAs from *B. vesicularis* LMG 2350^T and *S. paucimobilis* LMG 1227^T were hybridized with DNAs from strains selected after interpretation of different fingerprint data. Our results (Table 4 and Fig. 5) demonstrated that all of the *Brevundimonas* strains examined were located on a single, separate rRNA branch in rRNA superfamily IV (the α subclass) of the *Proteobacteria*. Two subgroups were delineated; one group contained all of the *B. vesicularis* strains and three *Brevundimonas* sp. strains (LMG 9567t1, LMG 9564, and LMG 11070) [$T_{m(e)}$ range, 81.4 to 79.3°C], and the other group contained all of the *B. diminuta* strains [$T_{m(e)}$ range, 78.0 to 75.9°C]. Strain LMG 11050 was located at the base of the *Brevundimonas* rRNA branch and fell outside the range of the genus.

The phylogenetic positions of the *Sphingomonas* and *Rhizomonas* reference strains have been discussed elsewhere (34), and the $T_{m(e)}$ values of strains LMG 9555, LMG 11049, and LMG 11069 indicated that these organisms are related to these genera. Additional hybridization data will be necessary to decide whether these strains are members of separate genera. [*P.*] *echinoides* LMG 2181^T was located on the *Sphingomonas* branch and should be assigned to this genus; its exact species status needs further study. The position of "[*P.*] *riboflavina*" LMG 2277^T remains unclear at the border of rRNA superfamily IV.

Analysis of cellular polyamines. The polyamine concentrations found in *Brevundimonas*, *Sphingomonas*, *Rhizomonas*, and related strains are shown in Table 5. All of the strains studied were characterized by the occurrence of significant

TABLE 3. Characteristics that differentiate *Brevundimonas* named and unnamed species and *Brevundimonas*-like strain LMG 11050[a]

Characteristic	*B. diminuta* (n = 19)[b]	*B. vesicularis* (n = 10)[b]	*Brevundimonas* sp. strain LMG 9564	*Brevundimonas* sp. strain LMG 9567t1	*Brevundimonas* sp. strain LMG 11070	*Brevundimonas*-like strain LMG 11050
Yellow or orange carotenoid pigment	−	v	−	+	−	−
Cystine requirement[c]	+	−	ND	ND	ND	ND
Hydrolysis of esculin[d]	−	+	+	+	+	+
Assimilation of[d]:						
D-Xylose	−	−	+	−	−	−
Adonitol	−	−	−	−	−	+
D-Mannose	−	−	+	−	−	−
D-Glucose	−	+	+	+	+	−
D-Galactose	−	+	+	+	+	−
Amygdalin	−	−	−	−	−	+
Arbutin	−	−	−	−	+	+
Salicin	−	−	−	−	+	+
Maltose	−	+	+	+	+	−
Trehalose	−	−	−	+	−	−
Propionate	−	−	−	+	−	−
Isobutyrate	−	−	−	+	−	−
n-Caproate	−	−	−	−	+	−
Adipate	−	−	−	−	−	+
Pimelate	−	−	−	−	−	+
Suberate	−	−	−	−	−	+
Azelate	−	−	−	−	−	+
Sebacate	−	−	−	−	−	+
Glycine	−	−	+	−	−	−
L-Histidine	+	−	−	−	−	−
API ZYM reactions						
Ester lipase (C8)	+	+	+	−	+	ND
α-Glucosidase	−	+	+	+	+	ND
β-Glucosidase	−	−	+	−	+	ND
Occurrence of fatty acid 19:0 cyclo ω8c	+	−	−	−	+	+
Occurrence of polyamines						
Homospermidine	+	+	−	+	+	+
Spermidine	tr	+	+	+	+	+
$T_{m(e)}$ with *B. vesicularis* LMG 2350^T rRNA (°C)	81.4–79.3	78–75.9	79.3	81.1	81.4	73.9

[a] −, 90% of more of the strains are negative; +, 90% or more of the strains are positive; v, variable; ND, not determined; tr, trace.
[b] n is the number of strains tested.
[c] Data from reference 1.
[d] Tests were performed with API 50CH, API 50AO, and API 50AA galleries.

amounts of homospermidine or spermidine or both. *B. diminuta* strains produced homospermidine as the major component (1.7 to 3.4 μmol/g [wet weight]) and trace amounts of spermidine. All of the *B. vesicularis* strains except strain LMG 9554 produced spermidine as the major component (1.6 to 2.5 μmol/g [wet weight]), homospermidine as a minor component (0.1 to 0.5 μmol/g [wet weight]), and, in some cases, trace amounts of norspermidine, putrescine, and spermine. *B. vesicularis* LMG 9554 produced less spermidine (0.6 μmol/g [wet weight]) and no homospermidine at all. *Brevundimonas* sp. strains LMG 9567t1 and LMG 11070 produced a polyamine pattern similar to that of the *B. vesicularis* strains. *Brevundimonas* sp. strain LMG 9564 produced only high amounts of spermidine (4.2 μmol/g [wet weight]). Strain LMG 11050 was differentiated from *B. vesicularis* by the higher amounts of homospermidine that it produced.

All of the *Sphingomonas* strains except the [*S.*] *capsulata* and [*S.*] *yanoikuyae* strains produced significant amounts of homospermidine (1.8 to 5.0 μmol/g [wet weight]) and only trace amounts of spermidine. [*S.*] *capsulata*, [*S.*] *yanoikuyae*, and the *Rhizomonas-Sphingomonas*-like strains were easily differentiated because they produced high levels of spermidine (2.5 to 4.0 μmol/g [wet weight]) and no homospermidine.

DISCUSSION

Previously, 16S rRNA cataloging revealed that [*P.*] *diminuta* and probably also [*P.*] *vesicularis* (although [*P.*] *vesicularis* was not included in the previous rRNA sequence studies) phylogenetically belong to the α subclass of the *Proteobacteria*, whereas the authentic pseudomonads belong to the γ subclass (42). Analogous data were obtained in DNA-rRNA hybridization studies in which workers placed the diminuta group in rRNA superfamily IV and not in rRNA superfamily II (12, 24, 26). These previous findings, together with the results of this study, support the proposal that [*P.*] *diminuta* and [*P.*] *vesicularis* deserve to be classified in a new genus, for which we propose the name *Brevundimonas*.

Recent 16S rRNA sequencing data, however, have shown that there is a close affiliation between *B. diminuta* and some *Caulobacter* strains (30). Definite conclusions concerning the taxonomic status of the genus *Caulobacter* cannot be drawn at this time because (i) on the basis of sequencing data (30) the *Caulobacter* strains that have been studied seem to be phylogenetically too heterogeneous to be members of one genus and (ii) rRNA sequence data for the type species (*Caulobacter vibrioides*) are not available.

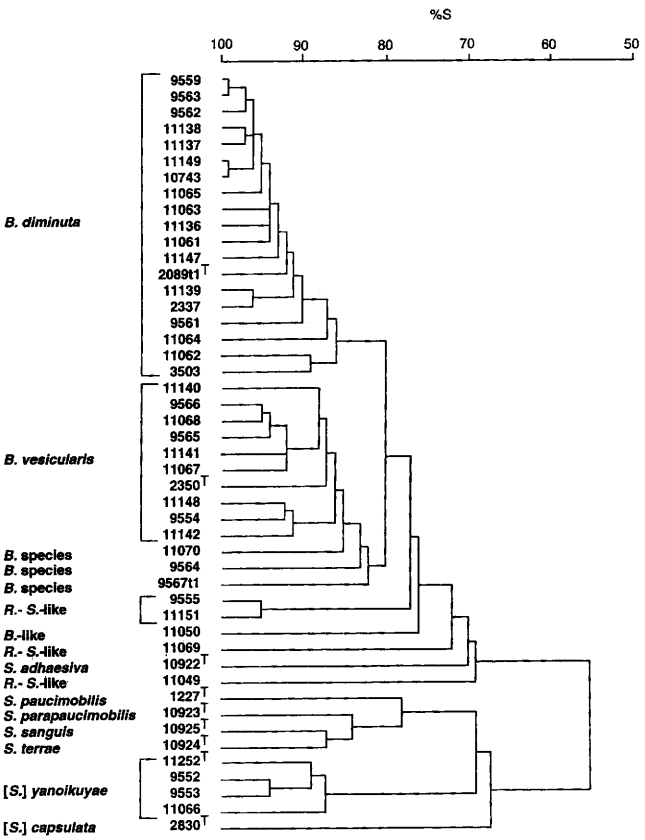

FIG. 3. Dendrogram obtained after an unweighted pair group cluster analysis of the similarity coefficients of the phenotypic data obtained for all of the strains investigated. We used 147 carbon assimilation tests to calculate the similarity coefficients (S_{SM}). Abbreviations are explained in the legend to Fig. 1.

Moreover, DNA-rRNA hybridization results obtained in this study indicated that *Brevundimonas* strains constitute a separate branch within rRNA superfamily IV (Fig. 5). This branch is equidistant from the following rRNA homology groups: (i) the family *Acetobacteraceae*, (ii) the genus *Rhodobacter*, (iii) the *Rhodospirillum-Azospirillum* group, (iv) the *Bradyrhizobium-Rhodopseudomonas* group, (v) the *Agrobacterium-Rhizobium* group, and (vi) the *Sphingomonas-Rhizomonas* group (18).

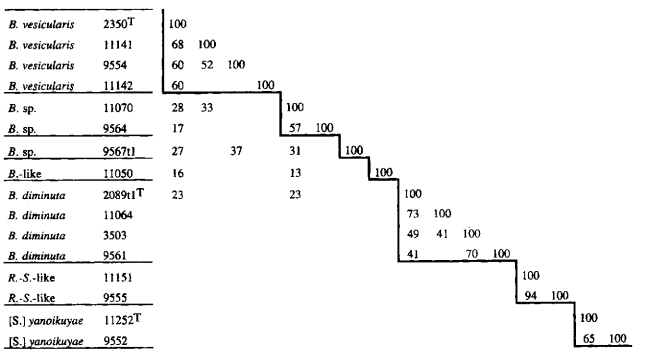

FIG. 4. DNA-DNA hybridization values for representative *Brevundimonas* strains. The levels of DNA relatedness are expressed as percentages of DNA binding. Abbreviations are explained in the legend to Fig. 1.

The rRNA homology group comprising the genera *Sphingomonas*, *Rhizomonas*, and *Zymomonas* has been discussed elsewhere (34). We found during this taxonomic study of the diminuta group that four strains (LMG 9555, LMG 11049, LMG 11069, and LMG 11151) were located at the lower border of the *S. paucimobilis* rRNA branch. Furthermore, according to the rRNA hybridization data [*P.*] *echinoides* belongs to the genus *Sphingomonas*, while "[*P.*] *riboflavina*" could not be classified more precisely in rRNA superfamily IV (Table 4) (14).

In addition to the genotypic evidence discussed above, a number of chemotaxonomic and other features confirmed the separate generic status of the genus *Brevundimonas*. Members of the genus *Brevundimonas* differ from the authentic pseudomonads by having short-wavelength polar flagella, restricted biochemical activity, and different polyamine and ubiquinone patterns, as well as different characteristic fatty acid compositions (Table 6). The first two characteristics also differentiate the genus from the family *Comamonadaceae* and the genus *Burkholderia*. *Brevundimonas* strains can be distinguished from the genus *Caulobacter* by the highly specific morphology of the organisms and by different biochemical activity and from the genera *Sphingomonas* and *Rhizomonas* by several phenotypic and genomic features. Previously published data indicate that the presence of sphingolipids and the fatty acid C14:0 2OH is highly specific for the genera *Sphingomonas* and *Rhizomonas* (Table 6). Thus, the results of our polyphasic study show that members of the genus *Brevundimonas* form separate taxonomic entities that are based not only on genotypic characteristics but also on phenotypic and chemotaxonomic characteristics.

On the *Brevundimonas* rRNA branch, we delineated two similarity groups (Table 4) with $T_{m(e)}$ values ranging from 81.4 to 79.3°C and from 78.0 to 75.9°C. The first group consists of the species *B. vesicularis* and three *Brevundimonas* strains which could not be classified as members of one of the recognized species, although a close resemblance to *B. vesicularis* could be deduced from the results obtained with several of the taxonomic methods used (see below). The second group contains *B. diminuta*. No significant differences in DNA base composition were observed between members of the two *Brevundimonas* species. The levels of DNA relatedness among *B. vesicularis* strains varied between 52 and 68%. *B. diminuta* strains yielded values that ranged from 41 to 73%. Genotypic species delineation became rather clear because of the low to moderate degrees of DNA binding among *B. vesicularis* strains, *B. diminuta* strains, and *Brevundimonas* sp. strains LMG 9564, LMG 9567t1, and LMG 11070 and because no significant DNA binding was measured between strain LMG 11050 and any of the other strains studied.

The results of a numerical analysis of phenotypic data corresponded well with the genotypic data. Homogeneous, separate phenotypic clusters were obtained for *B. diminuta* and *B. vesicularis*. Strains LMG 9564, LMG 9567t1, and LMG 11070 were closely linked to the *B. vesicularis* cluster, while strain LMG 11050 was significantly different (Fig. 3). Table 3 shows an overview of the distinguishing phenotypic features for members of the genus *Brevundimonas*. Numerical analyses of fatty acid patterns and protein profiles (Fig. 1 and 2) in general revealed separate clusters for *B. diminuta* and *B. vesicularis*. However, in both cases the three *Brevundimonas* sp. strains (LMG 9564, LMG 11070, and LMG 9567t1) and strain LMG 11050 grouped with the *B. vesicularis* strains. Except for two strains when the protein electrophoretic approach was used, a

TABLE 4. DNA base compositions of strains and $T_{m(e)}$ values of DNA-rRNA hybrids

Strain	G+C content (mol%)	$T_{m(e)}$ (°C) of hybrid with rRNA from:	
		B. vesicularis LMG 2350^T	*S. paucimobilis* LMG 1227^T
B. vesicularis LMG 2350^T	65.4	81.3	63.1
B. vesicularis LMG 9566	66.3	79.3	
B. vesicularis LMG 11067	65.4	81.1	63.1
B. vesicularis LMG 11148	66.0	81.1	63.1
B. vesicularis LMG 9554	65.6	81.4	
B. diminuta LMG 2089t1^T	65.9	77.5	64.4
B. diminuta LMG 1798	66.5	77.2	
B. diminuta LMG 3503	67.3		
B. diminuta LMG 10743	67.9	75.9	
B. diminuta LMG 11061	67.1	78.0	
B. diminuta LMG 11064	66.0	76.5	
B. diminuta LMG 11065	67.1	76.9	
Brevundimonas sp. strain LMG 9564	66.4	79.3	
Brevundimonas sp. strain LMG 9567t1	66.3	81.1	
Brevundimonas sp. strain LMG 11070	67.0	81.4	
Brevundimonas-like strain LMG 11050		73.9	
S. paucimobilis LMG 1227^T	65.4[a]	63.2	80.6[a]
[*S.*] *capsulata* LMG 2830^T	65.9[a]		71.1[a]
S. adhaesiva LMG 10922^T	67.9[a]		75.2[a]
S. parapaucimobilis LMG 10923^T	66.9[a]		79.5[a]
S. sanguis LMG 10925^T	66.2[a]		79.7[a]
S. terrae LMG 10924^T	65.2[a]		79.0[a]
[*S.*] *yanoikuyae* LMG 11252^T	63.7[a]		72.2[a]
[*S.*] *yanoikuyae* LMG 9552	65.0		72.7
[*S.*] *yanoikuyae* LMG 11066	63.2	65.1	71.8
Rhizomonas-Sphingomonas-like strain LMG 9555	65.3	64.0	71.6
Rhizomonas-Sphingomonas-like strain LMG 11049	64.2	65.1	71.8
Rhizomonas-Sphingomonas-like strain LMG 11069	63.4	63.8	71.1
"[*P.*] *riboflavina*" LMG 2277^T	61.5	64.0	64.2
[*P.*] *echinoides* LMG 2181^T	65.3	64.1	74.2

[a] Data from reference 34.

distinct homogeneous cluster was obtained for all *B. diminuta* strains. The presence of high percentages of the fatty acid 19:0 cyclo ω8c is characteristic of *B. diminuta* strains. The two recognized *Brevundimonas* species were also easily differentiated by their polyamine contents (Table 5).

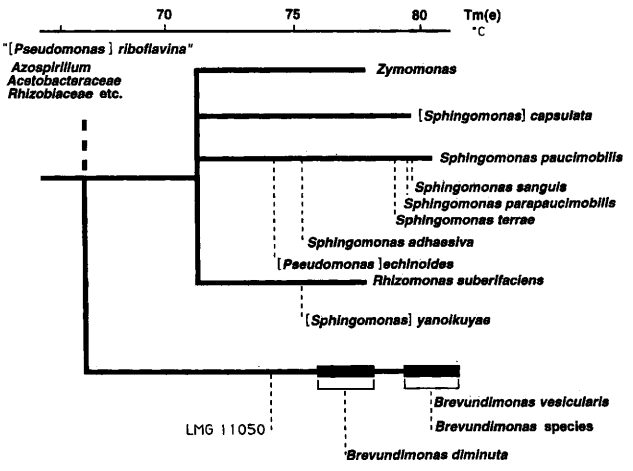

FIG. 5. Simplified rRNA dendrogram based on the $T_{m(e)}$ values of DNA-rRNA hybrids obtained with members of rRNA superfamily IV (the α subclass of the *Proteobacteria*). The data used are from this study and from reference 34.

Reference strains of most *Sphingomonas* and *Rhizomonas* species were included in this study because several of the strains studied (e.g., some EF group 21 strains) were phenotypically similar to members of these genera. The taxonomy of these genera is complex and continuously changing (11, 22, 32, 34). Reclassification of [*S.*] *yanoikuyae* in the genus *Rhizomonas* is probably correct (34). Moreover, [*S.*] *capsulata*, which was located on a separate rRNA branch, may be the core of a new genus. DNA-rRNA hybridization experiments revealed that the authentic *Sphingomonas* species are located on a separate rRNA branch (Fig. 5), and from this study it is clear that all of these species have a rather characteristic polyamine content (Table 5). All genotypic and phenotypic data confirmed that three strains (LMG 9552, LMG 9553, and LMG 11066) belong to [*S.*] *yanoikuyae*. As mentioned above, four strains (LMG 9555, LMG 11049, LMG 11069, and LMG 11151) were placed at the lower border of the *S. paucimobilis* rRNA branch and could, until now, not be classified in a particular genus. Two of these strains (LMG 9555 and LMG 11151) are phenotypically and chemotaxonomically very similar and probably should be classified in a new genus. However, we prefer to regard all four strains as *Sphingomonas-Rhizomonas*-like strains because the limited number of strains available does not allow reliable taxonomic descriptions.

In conclusion, on the basis of genotypic and phenotypic criteria, the genus *Brevundimonas* differs from its relatives, and separate generic status is justified. DNA-DNA hybridization studies revealed that there are at least two different species in

TABLE 5. Polyamine contents of strains

Taxon	No. of strains tested	Polyamine content (μmol/g [wet wt])[a]		
		HSPD	SPD	Other polyamines[b]
B. diminuta	19	1.7–3.4	tr[c]	
B. vesicularis[d]	9	0.1–0.6	1.6–2.5	NORSPD (4), PUT (1), SPM (1)
Brevundimonas sp. strain LMG 9564			4.2	
Brevundimonas sp. strain LMG 9567t1		0.6	2.9	PUT
Brevundimonas sp. strain LMG 11070		0.6	3.8	PUT
Brevundimonas-like strain LMG 11050		2.4	1.5	NORSPD
S. paucimobilis LMG 1227^T		4.8	tr	
S. parapaucimobilis LMG 10923^T		5.0	tr	
S. sanguis LMG 10925^T		4.8	tr	
S. terrae LMG 10924^T		3.5	tr	
S. adhaesiva LMG 10922^T		1.8		
[*S.*] *capsulata* LMG 2830^T			2.5	NORSPD, PUT, DAP
[*S.*] *yanoikuyae*	4		2.9–4.0	NORSPD (1), SPM (1), PUT (1)
Rhizomonas-Sphingomonas-like strain LMG 11069			3.2	
Rhizomonas-Sphingomonas-like strain LMG 11049			3.1	NORSPD
Rhizomonas-Sphingomonas-like strains	2		2.7–3.1	CAD(1)

[a] Abbreviations: HSPD, homospermidine; SPD, spermidine; NORSPD, norspermidine; PUT, putrescine; SPM, spermine; CAD, cadaverine.
[b] Some strains contain traces of other polyamines. The numbers in parentheses are the numbers of strains that produce the polyamines indicated.
[c] tr, trace.
[d] Strain LMG 9554 differs from the other *B. vesicularis* strains in that it produces only 0.6 μmol of spermidine per g (wet weight) and no homospermidine.

the genus. The genus and species descriptions below are based on the data described above and on previous data (1, 2, 17, 20, 24).

Description of *Brevundimonas* gen. nov. *Brevundimonas* (Brev.un.di'mo.nas. L. adj. *brevis*, short; L. fem. n. *unda*, wave; Gr. *monas*, a unit, monad; M.L. fem. n. *Brevundimonas*, bacterium with short-wavelength flagella). Gram-negative short rods that are 1 to 4 μm long and 0.5 μm in diameter. Motile by means of one polar flagellum that has a short wavelength (0.6 to 1 μm). Oxidase and catalase positive. Indole is not formed. Pantothenate, biotin, and cyanocobalamin are required as growth factors. Aerobic with a respiratory type of metabolism (Q10 is an intermediate electron carrier); never fermentative. Growth occurs on blood media at 30 and 37°C. No growth occurs at 4°C, and no autotrophic growth occurs with H_2. Poly-β-hydroxybutyrate is accumulated as reserve material and is not hydrolyzed extracellularly. Acid is produced from primary alcohols by all strains that can utilize alcohols. Lecithinase (egg yolk) and lipase (Tween 80) are not produced. Gelatin is not liquefied. The strains exhibit a limited nutritional spectrum; only DL-β-hydroxybutyrate, pyruvate, L-glutamate, and L-proline are used by 90% or more of the strains as sole carbon and energy sources.

As determined by API galleries, the following substrates are used by none of the strains or by at most 10% of the strains (although some of these characteristics are useful for differentiating between named and unnamed strains [Table 3]): glycerol, erythritol, D-arabinose, L-arabinose, D-ribose, D-xylose, L-xylose, adonitol, methyl-β-xyloside, D-fructose, D-mannose, L-sorbose, dulcitol, inositol, mannitol, sorbitol, methyl-α-D-mannoside, methyl-α-D-glucose, *N*-acetylglucosamine, amygdalin, arbutin, salicin, lactose, D-melibiose, sucrose, trehalose,

TABLE 6. Differentiation of the genus *Brevundimonas* and phenotypically similar taxa[a]

Taxon	Flagellation	Pigments	Carbon assimilation	Sphingolipids	Characteristic hydroxy fatty acid(s)	Major polyamines[b]	Ubiquinone[b]	G+C content (mol%)	Subclass of *Proteobacteria*
Brevundimonas	Polar, short wavelength	None, orange, or yellow	Restricted	ND[c]	12:0 3OH	SPD, HSPD	Q10	65–68	α-2
Sphingomonas and *Rhizomonas*	Polar	Deep yellow or whitish brown	Normal	+	14:0 2OH	HSPD, SPD	Q10	59–68	α-4
Authentic genus *Pseudomonas*	Polar, long wavelength	None, fluorescent, or yellow-orange	Normal	ND	10:0 3OH, 12:0 3OH	PUT, SPD	Q9	59–68	γ-3
Comamonadaceae	Polar or peritrichous	None or yellow	Restricted	ND	10:0 3OH[d]	PUT, HPUT	Q8	60–69	β-1
Burkholderia	Polar	Various (yellow, orange, or red-brown)	Normal	ND	14:0 3OH, 16:0 3OH	PUT, HPUT	Q8	64–68	β-3
Caulobacter	Prosteca or polar	None or yellow to golden red	Normal	ND	ND	ND	ND	62–67	α-2

[a] Data from this study and references 23, 24, 27, 31, 32, 34, 39, 43, and 44.
[b] Abbreviations: SPD, spermidine; HSPD, homospermidine; PUT, putrescine; HPUT, hydroxyputrescine; Q8, Q9, and Q10, ubiquinones with 8, 9, and 10 isoprene units, respectively.
[c] ND, not determined.
[d] Not present in some species (e.g., *Hydrogenophaga palleronii*) (23).

inulin, D-melezitose, D-raffinose, glycogen, xylitol, β-gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate, propionate, isobutyrate, heptanoate, *n*-caproate, caprylate, pelargonate, caprate, oxalate, malonate, maleate, glutarate, adipate, pimelate, suberate, azelate, sebacate, glycolate, D-malate, DL-glycerate, D-tartrate, L-tartrate, *meso*-tartrate, levulinate, citraconate, itaconate, mesaconate, citrate, phenylacetate, benzoate, *o*-hydroxybenzoate, *m*-hydroxybenzoate, *p*-hydroxybenzoate, D-mandelate, L-mandelate, phthalate, isophthalate, terephthalate, glycine, L-norleucine, DL-2-aminobutyrate, L-cysteine, L-methionine, L-phenylalanine, D-tryptophan, L-tryptophan, trigonelline, L-ornithine, L-lysine, L-citrulline, DL-kynurenine, betaine, creatine, β-alanine, DL-3-aminobutyrate, DL-4-aminobutyrate, DL-5-aminovalerate, 2-aminobenzoate, 3-aminobenzoate, 4-aminobenzoate, urea, acetamide, sarcosine, ethylamine, butylamine, amylamine, ethanolamine, benzylamine, diaminobutane, spermine, histamine, tryptamine, and glucosamine.

In addition, the following substrates are not used: ethyleneglycol, propyleneglycol, 2,3-butyleneglycol, eicosanedioate, benzoylformate, phenylethanediol, naphthalene, phenol, quinate, testosterone, kynurenate, anthranilate, hippurate, nicotinate, saccharate, mucate, DL-α-aminovalerate, methylamine, *n*-dodecane, *n*-hexadecane, methanol, isopropanol, *n*-butanol, isobutanol, and geraniol.

The following enzyme activities are always present, as determined by API ZYM tests: alkaline and acid phosphatase, ester lipase (C8) (except *Brevundimonas* sp. strain LMG 9567t1), leucine arylamidase, trypsin, and phosphoamidase.

The following enzyme activities are always absent, as determined by API ZYM tests: lipase (C14), cystine arylamidase, α-galactosidase, β-galactosidase, β-glucosidase (can be used to differentiate the unnamed *Brevundimonas* sp. [Table 3]), *N*-acetyl-β-glucosidase, α-mannosidase, and α-fucosidase.

Negative reactions are also obtained for lysine and ornithine decarboxylase, urease, arginine dihydrolase, and phenylalanine deaminase.

The genus belongs to the α subclass of the *Proteobacteria*; it constitutes a separate rRNA branch that is equidistant from the rRNA clusters consisting of the family *Acetobacteraceae*, the genus *Rhodobacter*, the *Rhodospirillum-Azospirillum* group, the *Bradyrhizobium-Rhodopseudomonas* group, the *Agrobacterium-Rhizobium* group, and the *Sphingomonas-Rhizomonas* group. The closest natural relatives are organisms that are now classified as members of the genus *Caulobacter*. Nitrate is not reduced or is rarely reduced. All strains are characterized by two major fatty acids, 16:0 and 18:1 (summed feature 7) and the following minor components: 12:0 3OH, 14:0, 15:0, 16:1, 17:0, 17:1, and an unknown fatty acid with an equivalent chain length of 18.080. Isolated from water and clinical specimens. The G+C contents range from 65 to 68 mol%. The type species is *Brevundimonas diminuta*.

Description of *Brevundimonas diminuta* (Leifson and Hugh 1954) comb. nov. The description of *Brevundimonas diminuta* (di.mi.nu' ta. L. adj. *minutus*, small, defective, minute) is the same as that given above for the genus, with the following additional characteristics. Cystine is a growth factor.

As determined by API galleries, the substrates that are used by 90% or more of the strains are acetate, butyrate, L-leucine, L-serine, L-histidine, DL-norvaline, and L-aspartate. The substrates that are used by none of the strains or by at most 10% of the strains are D-galactose, D-glucose, L-rhamnose, D-cellobiose, maltose, starch, DL-lactate, and aconitate. The substrates that are used by some strains (the reactions of the type strain are indicated in parentheses) are isovalerate (positive), *n*-valerate (positive), succinate (negative), fumarate (negative), L-malate (negative), 2-ketoglutarate (negative), D-α-alanine (negative), L-alanine (negative), L-isoleucine (positive), L-valine (negative), L-norleucine (negative), DL-norvaline (positive), L-threonine (positive), L-tyrosine (negative), and L-arginine (negative).

Esculin is not hydrolyzed. Negative for valine arylamidase and α-glucosidase activities. Chymotrypsin activity is present in some strains, including the type strain. The levels of DNA relatedness range from 41 to 100%. On tryptic soy agar, homospermidine is the major cellular polyamine component. Strains are characterized by the presence of high percentages of 19:0 cyclo ω8c. The type strain is LMG 2089 (= ATCC 11568 = CCUG 1427). The G+C content is 66 to 68 mol%.

Description of *Brevundimonas vesicularis* (Büsing, Döll, and Freytag 1953) comb. nov. The description of *Brevundimonas vesicularis* (ve.si.cu.la'ris. L. adj. *vesicularis*, pertaining to a vesicle) is the same as that given above for the genus, with the following additional characteristics.

As determined by API galleries, the substrates that are used by 90% or more of the strains are D-galactose, D-glucose, and maltose. The substrates that are used by none of the strains or by at most 10% of the strains tested are *n*-valerate, isovalerate, 2-ketoglutarate, L-isoleucine, *N*-norleucine, L-valine, L-tyrosine, L-histidine, and L-arginine. The substrates that are used by some strains (the reactions of the type strain are indicated in parentheses) are starch (positive), rhamnose (negative), D-cellobiose (positive), acetate (negative), butyrate (positive), succinate (positive), fumarate (positive), DL-lactate (positive), L-malate (positive), aconitate (negative), D-α-alanine (negative), L-alanine (negative), L-leucine (negative), DL-norvaline (negative), L-serine (negative), L-threonine (negative), and L-aspartate (negative).

Esculin is hydrolyzed. Positive for α-glucosidase activity and negative for chymotrypsin activity. Valine arylamidase is present in some strains but not the type strain.

Some strains can be distinguished from *B. diminuta* by the oxidation of glucose, xylose, and maltose, the failure to produce a pellicule in broth cultures, and the production of an intracellular carotenoid pigment. The levels of DNA relatedness range from 50 to 100%. On tryptic soy agar, spermidine is the major polyamine component. The type strain is LMG 2350 (= ATCC 11426 = CCUG 2032). The G+C content is 65 to 66 mol%.

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