Intra- and Intergeneric Relationships of the Genus *Oceanospirillum*

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A comprehensive study of the relationships of *Oceanospirillum* species by a polyphasic approach is described. Hybridizations were performed between labeled ribosomal ribonucleic acids (rRNAs) from *Oceanospirillum linum*, *Oceanospirillum jannaschii*, *Oceanospirillum kriegii*, and several other organisms and deoxyribonucleic acids (DNAs) from representative *Oceanospirillum* strains and many other gram-negative organisms. Five *Oceanospirillum* species, including the type species, constitute a separate rRNA branch in rRNA superfamily II, to which the genus *Oceanospirillum* should be restricted. The finer relationships among the different members of the *Oceanospirillum* rRNA branch were further defined by DNA:DNA hybridizations and comparative gel electrophoresis of whole-cell proteins. After comparison of our results with the previously available phenotypic data, we redefine the genus *Oceanospirillum* to contain *Oceanospirillum linum*, *Oceanospirillum maris*, *Oceanospirillum beijerinckii*, *Oceanospirillum multiglobuliferum*, and *Oceanospirillum japonicum*; we also create *Oceanospirillum maris* subsp. *hirosimense* comb. nov. and *Oceanospirillum beijerinckii* subsp. *pelagicum* comb. nov. for the former *Oceanospirillum hirosimense* and *Oceanospirillum pelagicum*, respectively. The following taxa are generically misnamed: *Oceanospirillum jannaschii*, *Oceanospirillum kriegii*, and *Oceanospirillum minutulum*. Together with *Cellvibrio mixtus* and some misnamed *Pseudomonas* strains, these three taxa constitute at least three separate rRNA branches in rRNA superfamily II. *Oceanospirillum pusillum* is also misnamed as it belongs in rRNA superfamily IV. *Oceanospirillum vagum* and *Oceanospirillum commune* should be relegated to their real generic positions, *Marinomonas vaga* and *Marinomonas communis*, respectively.

During the last 15 years the classification of the spirilla has changed considerably. In *Bergey's Manual of Determinative Bacteriology*, 8th ed. (28), all of the various aerobic and microaerophilic spirilla, including freshwater and marine species, constituted the genus *Spirillum* Ehrenberg 1832. Later, Hylemon et al. (22) and Krieg (29) divided this genus into three genera, *Spirillum*, *Oceanospirillum*, and *Aquaspirillum*, on the basis of deoxyribonucleic acid (DNA) base composition and some physiological properties. *Oceanospirillum* was created for all of the marine aerobic spirilla requiring seawater for growth and possessing a mean guanine-plus-cytosine (G+C) content range of 42 to 51 mol%. A pattern of phenotypic features was considered to be typical for the genus. Hylemon et al. (22) originally described the following five species: *Oceanospirillum linum*, *Oceanospirillum minutulum*, *Oceanospirillum beijerinckii*, *Oceanospirillum maris*, and *Oceanospirillum japonicum*. Four other species, *Oceanospirillum hirosimense*, *Oceanospirillum pelagicum*, *Oceanospirillum pusillum*, and *Oceanospirillum multiglobuliferum*, were described later by Terasaki (40, 42). All of the species contain helical cells with bipolar flagella; they have a strictly respiratory type of metabolism, fail to oxidize or ferment carbohydrates, fail to grow anaerobically in the presence of nitrates, fail to reduce nitrate beyond the nitrite stage, and have a positive oxidase reaction. The cell diameter ranges from 0.3 to 1.4 μm , and the length of the helix ranges from 1.2 to 75 μm . The type species is *O. linum* (48). Some species have unusual characteristics, indicating a possible heterogeneity in the genus (29).

Based on DNA:ribosomal ribonucleic acid (rRNA) hybridization data, Van Landschoot and De Ley (43) created a new genus, *Marinomonas*, with two species, *Marinomonas vaga* and *Marinomonas communis*, for the former species *Alteromonas vaga* and *Alteromonas communis*, respectively. *Marinomonas* constitutes a separate rRNA branch in rRNA

superfamily II. Bowditch et al. (5) reported close immunological relationships among *O. linum*, *O. beijerinckii*, *Alteromonas vaga*, *Alteromonas communis*, and two unnamed groups (groups H-1 and I-1) of marine bacteria. Although these relationships were not confirmed by other methods, Bowditch et al. concluded that *Alteromonas communis* and *Alteromonas vaga* should be assigned to the genus *Oceanospirillum* as *Oceanospirillum vagum* and *Oceanospirillum commune*, and they created two new species for groups I-1 and H-1, *Oceanospirillum jannaschii* and *Oceanospirillum kriegii*, respectively. The new combinations *Marinomonas vaga* and *Marinomonas communis* were validated (23), as were *O. vagum*, *O. commune*, *O. jannaschii*, and *O. kriegii* (24). Because of the inclusion of these new species, the genus description of *Oceanospirillum* had to be changed drastically. The unfortunate consequences of this extension (5) were the loss of most of the readily determinable phenotypic features from the previous definition of the genus (29) and the extension of the upper G+C content limit for the genus from 51 to 57 mol%.

Until now, no comprehensive studies have been undertaken to elucidate the inter- and intrageneric relationships of the species of this genus, and extensive comparisons of rRNA and rRNA cistrons were called for. The 16S rRNAs from five strains, each representing a species, were compared by using the oligonucleotide cataloging method (50, 51). According to this study *O. maris*, *O. japonicum*, *O. linum*, and *O. beijerinckii* constitute, together with the *Enterobacteriaceae* and the *Vibrionaceae*, the core of subgroup 3 of the gamma subclass of the *Proteobacteria* (38, 51). The DNA:rRNA hybridization method has proven to be a powerful tool in the study of the relationships among bacteria at the generic and suprageneric levels. Since many strains per species or group can easily and quickly be compared by this technique, its results lead to decisive taxonomic conclusions (14, 18, 20, 35, 44).

In this paper we report on an extensive reexamination of

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TABLE 1. List of strains investigated

Sequence no.	Name as received	Strain no. as received ^a	Other strain designations ^a	Source, place and year of isolation	Reference(s)
1	<i>Oceanospirillum linum</i>	ATCC 11336 ^T	VPI 32 ^T , ^b NCMB 56 ^T , LMG 5214 ^T	Coastal water, United States, 1957	22, 48
2	<i>Oceanospirillum linum</i>	ATCC 12753	VPI 33, ^b NCMB 55, LMG 5332	Coastal water, United States, 1957	22, 48
3	<i>Oceanospirillum maris</i> subsp. <i>maris</i>	ATCC 27509 ^T	Jannasch 101 ^T , ^c VPI 35 ^T , ^b LMG 5213 ^T	Seawater, 1973	22
4	<i>Oceanospirillum maris</i> subsp. <i>maris</i>	ATCC 27648	Jannasch 206, ^c VPI 37, ^b LMG 5212	Seawater, 1973	22
5	<i>Oceanospirillum maris</i> subsp. <i>maris</i>	ATCC 27649	Jannasch 102, ^c VPI 36, ^b LMG 5333	Seawater, 1973	22
6	<i>Oceanospirillum maris</i> subsp. <i>williamsae</i>	ATCC 29547 ^T	Linn 2b from ATCC 11337, ^d NCMB 54 ^T , LMG 5210 ^T	From mixed culture, 1978	32
7	<i>Oceanospirillum multiglobuliferum</i>	IFO 13614 ^T	SWC OF1 ^T , ^e ATCC 33336 ^T , LMG 5306 ^T	Marine shellfish, 1960	42
8	<i>Oceanospirillum beijerinckii</i>	NCMB 52 ^T	VPI 34 ^T , ^b ATCC 12754 ^T , LMG 5405 ^T	Coastal water, United States, 1957	22, 48
9	<i>Oceanospirillum hiroshimense</i>	IFO 13616 ^T	SWC OF2 ^T , ^e LMG 7371 ^T	Marine shellfish, 1963	42
10	<i>Oceanospirillum pelagicum</i>	IFO 13612 ^T	SWC UF1 ^T , ^e ATCC 33337 ^T , LMG 5307 ^T	Marine shellfish, 1966	42
11	<i>Oceanospirillum japonicum</i>	ATCC 19191 ^T	VPI 38 ^T , ^b NCMB 1346 ^T , LMG 5215 ^T	Marine shellfish, 1959	22, 46
12	[<i>Oceanospirillum</i>] <i>kriegii</i>	ATCC 27133 ^T	Baumann 197 ^T (group H-1), ^f LMG 6238 ^T	Coastal surface water, Oahu, Hawaii, 1972	2
13	[<i>Oceanospirillum</i>] <i>kriegii</i>	CCUG 16056	Baumann 196 (group H-1), ^f LMG 7639	Coastal surface water, Oahu, Hawaii, 1972	2
14	[<i>Oceanospirillum</i>] <i>jannaschii</i>	ATCC 27135 ^T	Baumann 207 ^T (group I-1), ^f LMG 6239 ^T	Coastal surface water, Oahu, Hawaii, 1972	2
15	[<i>Oceanospirillum</i>] <i>minutulum</i>	ATCC 19192	VPI 39, ^b NCMB 1348, LMG 5211	Marine shellfish, 1959	22, 46
16	[<i>Oceanospirillum</i>] <i>minutulum</i>	ATCC 19193 ^T	VPI 40 ^T , ^b NCMB 1347 ^T , LMG 5334 ^T	Marine shellfish, 1959	22, 46
17	[<i>Oceanospirillum</i>] <i>pusillum</i>	IFO 13613 ^T	SWC IF6 ^T , ^e ATCC 33338 ^T , LMG 5308 ^T , LMG 7372 ^T	Marine shellfish, 1961	42
18	<i>Marinomonas vaga</i>	ATCC 27119 ^T	Baumann 40 ^T (group A-2), ^f LMG 2845 ^T	Coastal surface water, Oahu, Hawaii, 1972	43
19	<i>Marinomonas communis</i>	ATCC 27118 ^T	Baumann 1 ^T (group A-1), ^f LMG 2864 ^T	Coastal surface water, Oahu, Hawaii, 1972	43
20	<i>Pseudomonas fluorescens</i>	MMCA 40 ^T	ATCC e13525 ^T , NCTC 10038 ^T , NCIB 9046 ^T , LMG 1794 ^T		
21	[<i>Pseudomonas</i>] <i>doudoroffii</i>	ATCC 27123 ^T	Baumann 70 ^T (group C-3), ^f CCUG 12373 ^T , CIP 74.09 ^T , CCUG 1600 ^T , LMG 2180 ^T		
22	[<i>Pseudomonas</i>] <i>nautica</i>	ATCC 27132 ^T	Baumann 179 ^T (group G-3), ^f CCUG 16032 ^T , LMG 2226 ^T		
23	[<i>Pseudomonas</i>] <i>elongata</i>	ATCC 10144 ^T	LMG 2182 ^T		
24	[<i>Pseudomonas</i>] <i>atlantica</i>	CIP 59.31	LMG 2139		
25	" <i>Deleya aquamarina</i> "	NCMB 557t ₁ ^T	ATCC 14400 ^T , DSM 30161 ^T , LMG 2853t ₁ ^T		
26	<i>Deleya halophila</i>	CCM 3662	LMG 6456		
27	<i>Deleya faecalis</i> subsp. <i>homari</i>	L-1 ^T	ATCC 33127 ^T , NCMB 2116 ^T , LMG 3339 ^T		
28	<i>Acinetobacter calcoaceticus</i>	ATCC 23055t ₁ ^T	CCUG 12804 ^T , DSM 30006 ^T , IFO 13718 ^T , LMG 1046 ^T		
29	<i>Moraxella lacunata</i> biotype <i>liquefaciens</i>	ATCC 17952	Lautrop A169, ^g NCTC 7911, CIP 70.41, LMG 1009		
30	<i>Xanthomonas campestris</i>	NCPPB 528 ^T			
31	<i>Alteromonas macleodii</i>	ATCC 27126 ^T	Baumann 107 ^T (group E-1), ^f CCUG 16128 ^T , LMG 2843 ^T		
32	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i>	NCIB 8634 ^T	LMG 2847 ^T		
33	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i>	NCIB 8633	LMG 2848		
34	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i>	NCIB 8975	LMG 2849		
35	<i>Psychrobacter immobilis</i>	CCUG 9708 ^T	Lautrop 351 ^T , ^g Juni 14 ^T , ^h LMG 7091 ^T		
36	<i>Psychrobacter immobilis</i>	LMG 7091			
37	<i>Cardiobacterium hominis</i>	SSIAB 2106			

^a Abbreviations: ATCC, American Type Culture Collection, Rockville, Md.; CIP, Collection de l'Institut Pasteur, Paris, France; CCM, Czechoslovak Collection of Micro-organisms, Brno, Czechoslovakia; CCUG, Culture Collection of the Department of Clinical Bacteriology, University of Göteborg, Göteborg, Sweden; DSM, Deutsche Sammlung von Mikroorganismen, Braunschweig, Federal Republic of Germany; IFO, Institute for Fermentation-Osaka, Osaka, Japan; LMG, Culture Collection, Laboratorium Microbiologie, Gent, Belgium; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland; NCMB, National

Oceanospirillum species and related organisms by a polyphasic approach. Because of the heterogeneity of the group, DNA:rRNA hybridization data constituted the main part of this study. DNAs from representative strains of all species of *Oceanospirillum* and from possibly related bacteria were hybridized with radioactively labeled rRNAs from the type strains of *O. linum*, *O. kriegii*, and *O. jannaschii* and with labeled reference rRNAs from other organisms. In addition, DNA:DNA hybridizations and polyacrylamide gel electrophoresis of proteins were performed in order to elucidate the finer inter- and intraspecific relationships of *Oceanospirillum* sensu stricto.

Below, we use brackets to indicate generically misnamed taxa.

MATERIALS AND METHODS

Bacterial strains and growth media. Table 1 lists the bacterial strains which we used. For each *Oceanospirillum* species we included all of the strains available to us. For some species only one strain has been described. The compositions of the growth media (see Table 2) have been described previously (16, 36, 43). Medium 959 (American Type Culture Collection Catalog, 1986; American Type Culture Collection, Rockville, Md.) contains 0.5% (wt/vol) peptone, 0.1% (wt/vol) sodium succinate, 0.2% (wt/vol) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1% (wt/vol) $(\text{NH}_4)_2\text{SO}_4$, 0.0002% (wt/vol) $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, and 0.0002% (wt/vol) $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ (pH 7) in artificial seawater (2.75% NaCl, 1.02% $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 0.2% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.05% CaCl_2 , 0.1% KCl, 0.001% FeSO_4 , pH 6.8). The purity of the strains was checked by plating and microscopic examination of living and gram-stained cells. Mass cultures for extraction of DNA were grown as described by De Smedt and De Ley (16). The cells were harvested in 0.01 M phosphate buffer (pH 7) containing the same percentage of NaCl as the corresponding growth medium.

Preparation of high-molecular-weight DNA and fixation of single-stranded high-molecular-weight DNA on membrane filters. The procedures of Gillis and De Ley (21) were used for all organisms except *O. beijerinckii*, *O. japonicum*, *O. maris* subsp. *williamsae*, and *O. [F.] minutulum*, for which the NaClO_4 step was replaced by a proteinase K treatment (50 $\mu\text{g}/\text{ml}$) for 2 h at 60°C. Whenever possible, the procedures were carried out at 4°C.

Chemical determination of DNA. For DNA:DNA hybridizations the concentration of DNA was determined chemically by the method of Burton (6). For DNA:rRNA hybridizations the amount of filter-fixed DNA was determined by the method of Meys and Schilperoort, as reported by Van Landschoot and De Ley (43).

Preparation of labeled rRNA. [^3H]rRNAs were prepared from *O. linum* ATCC 11336^T (T = type strain), [*O. jannaschii* ATCC 27135^T, and [*O. kriegii* ATCC 27133^T. The cells were grown at 28°C in a shaking culture containing 200 ml of medium 959 supplemented with 2.5 mCi of [5,6- ^3H]uracil.

The methods used for extraction of the crude rRNA and isolation of the 23S and 16S rRNA fractions have been described previously (16). The specific activities of the 23S and 16S rRNA fractions from *O. linum* ATCC 11336^T, [*O. jannaschii* ATCC 27135^T, and [*O. kriegii* ATCC 27133^T were 26,500, 80,500, and 36,900 cpm/ μg , respectively. The following labeled rRNAs, already available in our research group, were also used (specific activities in parentheses): *Pseudomonas fluorescens* MMCA 40^T 16S [^{14}C]rRNA (6,000 cpm/ μg), *Marinomonas vaga* ATCC 27119^T 16S [^3H]rRNA (7,000 cpm/ μg), "*Deleya aquamarina*" NCMB 5571^T 23S [^{14}C]rRNA (7,000 cpm/ μg), *Xanthomonas campestris* NCPPB 528^T 16S [^{14}C]rRNA (5,000 cpm/ μg), *Acinetobacter calcoaceticus* ATCC 23055t¹ 23S [^3H]rRNA (21,000 cpm/ μg), *Moraxella lacunata* biotype *liquefaciens* ATCC 17952 23S [^3H]rRNA (24,000 cpm/ μg), and *Alteromonas macleodii* ATCC 27126^T 16S [^{14}C]rRNA (6,000 cpm/ μg).

Hybridizations between labeled rRNA and filter-fixed DNA. The hybridization method of De Ley and De Smedt (12) was used. Each DNA:rRNA hybrid was characterized by the following two parameters: the midpoint temperature of the thermal denaturation curve [$T_{m(e)}$] and the percentage of rRNA binding (i.e., the amount [in micrograms] of labeled rRNA duplexed to 100 μg of filter-fixed DNA after ribonuclease treatment under standard conditions).

DNA base composition. The average G+C content was determined by thermal denaturation and was calculated by using the equation of Marmur and Doty (34), as modified by De Ley (9).

DNA:DNA hybridization. The degree of binding (D value), a measure of DNA homology, was determined spectrophotometrically from the initial renaturation rates by the method of De Ley et al. (11). D values of 30% and lower are less reliable and were disregarded. The total DNA concentration was ca. 50 $\mu\text{g}/\text{ml}$, and the optimal renaturation temperature in 2 $\times$ SSC (1 $\times$ SSC is 0.15 M NaCl plus 0.015 M sodium citrate, pH 7) was 71.5°C. A model 2600 spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) equipped with a thermostatically controlled cuvette chamber and a model 7225A plotter (Hewlett-Packard Co., Palo Alto, Calif.) was used.

Polyacrylamide gel electrophoresis of proteins. The *Oceanospirillum* strains were grown in Roux flasks at 28°C for 48 h on medium 959. Whole-cell protein extracts were prepared, and sodium dodecyl sulfate-polyacrylamide gel electrophoresis was performed by using small modifications of the procedure of Laemmli (30), as described previously (27).

Numerical analysis of the protein electrophoretic patterns. Protein electrophoretic patterns were scanned by using a model 2202 Ultrosan laser densitometer (LKB, Bromma, Sweden) and a modified LKB Apple Pascal program (GEL-SCAN) run on an Apple IIe microcomputer equipped with a Transwarp accelerator card (Applied Engineering, Carrollton, Tex.). Raw digitized data were normalized and interpolated by using the Pascal INTFILE program (B. Pot and P.

Collection of Marine Bacteria, Aberdeen, Scotland; SWC, Suzugamine Women's College, Hiroshima, Japan; VPI, Anaerobe Culture Collection, Virginia Polytechnic Institute and State University, Blacksburg.

^b Original strain isolated by N. R. Krieg and H. W. Jannasch, Virginia Polytechnic Institute and State University, Blacksburg.

^c Original strain isolated by H. W. Jannasch, Woods Hole Oceanographic Institution, Woods Hole, Mass.

^d Original strain isolated by D. M. Linn and N. R. Krieg, Virginia Polytechnic Institute and State University, Blacksburg.

^e Original strain isolated by Y. Terasaki, Biological Laboratory, Suzugamine Women's College, Hiroshima, Japan.

^f Original strain isolated by P. Baumann, Department of Bacteriology, University of California, Davis.

^g Original strain isolated by H. Lautrop, Statens Serum Institut, Copenhagen, Denmark.

^h Original strain isolated by E. Juni, Department of Microbiology and Immunology, University of Michigan, Ann Arbor.

TABLE 2. $T_{m(e)}$ and rRNA binding values of the hybrids formed between DNAs from many organisms and different labeled reference rRNAs from organisms belonging to rRNA superfamilies I and II, as well as the average G+C contents of the organisms

Sequence no.	Source of DNA Strain	Growth medium	G+C content (mol%)	<i>O. linum</i> ATCC 11336 ^T		[<i>O.</i>] <i>jannaschii</i> ATCC 27135 ^T		[<i>O.</i>] <i>kriegii</i> ATCC 27133 ^T	
				$T_{m(e)}$ (°C)	% rRNA binding	$T_{m(e)}$ (°C)	% rRNA binding	$T_{m(e)}$ (°C)	% rRNA binding
1	<i>O. linum</i> ATCC 11336 ^T	959		78.8	0.27				
2	<i>O. linum</i> ATCC 12753	959	49 ^a	79.8	0.22	68.0	0.14	68.3	0.16
3	<i>O. maris</i> subsp. <i>maris</i> ATCC 27509 ^T	959		78.8	0.22				
4	<i>O. maris</i> subsp. <i>maris</i> ATCC 27648	959		78.1	0.22				
5	<i>O. maris</i> subsp. <i>maris</i> ATCC 27649	959		78.2	0.21				
6	<i>O. maris</i> subsp. <i>williamsae</i> ATCC 29547 ^T	959		78.8	0.21				
7	<i>O. multiglobuliferum</i> IFO 13614 ^T	959	46 ^b	76.6	0.17				
8	<i>O. beijerinckii</i> NCMB 52 ^T	959	47 ^b	76.5	0.13				
9	<i>O. hiroshimense</i> IFO 13616 ^T	959	47 ^b	76.4	0.11	68.9	0.09	69.2	0.10
10	<i>O. pelagicum</i> IFO 13612 ^T	959	49 ^b	74.7	0.14				
11	<i>O. japonicum</i> ATCC 19191 ^T	959	45 ^b	70.6	0.16	68.4	0.17	66.6	0.16
12	[<i>O.</i>] <i>kriegii</i> ATCC 27133 ^T	959		69.1	0.11	68.9	0.17		
13	[<i>O.</i>] <i>kriegii</i> CCUG 16056	959		70.5	0.12	67.7	0.18	78.8	0.62
14	[<i>O.</i>] <i>jannaschii</i> ATCC 27135 ^T	959		68.4	0.16	79.8	0.29	68.5	0.18
15	[<i>O.</i>] <i>minutulum</i> ATCC 19192	959		68.6	0.08				
16	[<i>O.</i>] <i>minutulum</i> ATCC 19193 ^T	959	44 ^a	67.0	0.01	66.4	0.10	65.6	0.40
17	[<i>O.</i>] <i>pusillum</i> IFO 13613 ^T	959	51 ^c	61.8	0.04				
18	<i>Marinomonas vaga</i> ATCC 27119 ^T	T	48 ^c	70.6	0.12	70.6	0.20	69.7	0.13
19	<i>Marinomonas communis</i> ATCC 27118 ^T	T	47 ^c	70.1	0.19				
20	<i>Pseudomonas fluorescens</i> MMCA 40 ^T	B	60 ^c	69.6	0.12	68.0	0.13	67.2	0.08
21	[<i>Pseudomonas</i>] <i>doudoroffii</i> ATCC 27123	B	59 ^f	68.8	0.06	63.9	0.13		
22	[<i>Pseudomonas</i>] <i>nautica</i> ATCC 27132	B	59 ^f	70.4	0.03	64.8	0.04	65.3	0.03
23	[<i>Pseudomonas</i>] <i>elongata</i> ATCC 10144	B	58 ^f	70.3	0.08				
24	[<i>Pseudomonas</i>] <i>atlantica</i> CIP 59.31	T	45 ^f	68.2	0.15	63.2	0.10	63.3	0.07
25	" <i>Deleya aquamarina</i> " NCMB 557t ₁ ^T	Z11	58 ^h						
26	<i>Deleya halophila</i> CCM 3662 ^T	Z11	68 ^h			68.3	0.12	66.7	0.19
27	<i>Deleya faecalis</i> subsp. <i>homari</i> L-1	Z11		71.0	0.18				
28	<i>Acinetobacter calcoaceticus</i> ATCC 23055t ₁ ^T	Z5	41 ^c	65.9	0.07	64.4	0.11	65.2	0.10
29	<i>Moraxella lacunata</i> biotype <i>liquefaciens</i> ATCC 17952	H1A	46 ^e	66.2	0.08				
30	<i>Xanthomonas campestris</i> NCPPB 528 ^T	T	65 ^c	67.5	0.06	64.8	0.05	63.9	0.03
31	<i>Alteromonas macleodii</i> ATCC 27126 ^T	Z11	46 ^c						
32	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i> NCIB 8634	Z17	49 ^h	67.7	0.03	66.6	0.16	66.4	0.03
33	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i> NCIB 8633	Z17	52 ^c	69.5	0.03	66.7	0.08	67.3	0.06
34	<i>Cellvibrio mixtus</i> subsp. <i>mixtus</i> NCIB 8975	Z18	48 ^c	66.4	0.02	65.4	0.17	64.6	0.03
35	<i>Psychrobacter immobilis</i> CCUG 9708 ^T	H1A				65.7	0.43		
36	<i>Psychrobacter immobilis</i> LMG 7091	H1A				63.6	0.04	64.9	0.03
37	<i>Cardiobacterium hominis</i> SSIAB 2106	H1A				63.9	0.07	64.4	0.03

^a Data from this study.^b Data from reference 29.^c Data from reference 43.^d Data from reference 45.^e Data from reference 37.^f Data from reference 17.^g Data from reference 18a.^h Data from P. Segers and J. De Ley (manuscript in preparation).

Casteleyn, unpublished data). Objective comparison and normalization of lanes on different slab gels were achieved by including the protein extract of *Psychrobacter* sp. strain LMG 1125 as a reference lane five times in each gel to be analyzed. In addition to the starting point and the endpoint of the electrophoretic trace, four sharp, well-separated protein bands of the reference extract from strain LMG 1125 were localized by the INTFILE program. The mean positions of these bands were determined from more than 100 electrophoretic runs of several preparations of the extract of strain LMG 1125. These mean positions were used to correct for misalignments of raw digitized data collected from different gels or different lanes within one gel or both. The

INTFILE program corrected for small displacements and reduced the 1,000 points of the raw digitized curve to a 400-point curve by using the Lagrange interpolation algorithm. The resulting 400-point traces, with all necessary descriptive information, were transferred to the Siemens model 7570-C mainframe computer of the Centraal Digitaal Rekencentrum of the Rijksuniversiteit, Gent, Belgium. Numerical analysis was performed as described previously (26) on the first 300 points of each interpolated trace. The similarity between all possible pairs of traces was expressed by the Pearson product moment correlation coefficient (r). Strains were then clustered by using the unweighted pair group average linkage method.

TABLE 2—Continued

<i>Marinomonas</i> <i>vaga</i> ATCC 27119 ^T		"Deleya <i>aquamarina</i> " NCMB 557 ^t ₁ ^T		<i>Xanthomonas</i> <i>campestris</i> NCPBP 528 ^T		<i>Acinetobacter</i> <i>calcoaceticus</i> ATCC 23055 ^t ₁ ^T		<i>Moraxella</i> <i>lacunata</i> ATCC 17952		<i>Alteromonas</i> <i>macleodii</i> ATCC 27126 ^T		<i>Pseudomonas</i> <i>fluorescens</i> MMCA 40 ^T	
<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding	<i>T</i> _{m(e)} (°C)	% rRNA binding
69.8	0.27	69.3	0.13			65.6	0.20	66.2	0.15			68.8	0.13
68.2	0.29	69.5	0.14									68.7	0.19
		68.5	0.14	65.8	0.16	66.1	0.14					67.2	0.14
67.2	0.14												
67.5	0.27											68.0	0.20
68.3	0.25												
67.9	0.09												
68.5	0.23	67.6	0.13									68.0	0.18
69.1	0.18	68.3	0.08										
69.2	0.25	69.4	0.12	62.9	0.04	66.2	0.09	67.5	0.04				
66.5	0.11	68.5	0.12	63.3	0.03	63.1	0.10			65.2	0.07		
		70.1	0.07	63.2	0.10			65.7	0.10				
61.7	0.03												
79.5	0.23 ^c	70.0	0.09 ^c			67.0	0.07 ^d			67.5	0.10	71.5	0.16 ^c
78.0	0.24 ^c	70.0	0.10 ^c					66.4	0.13 ^e	67.5	0.11	72.0	0.19 ^c
70.5	0.15					68.0	0.09 ^d			65.0	0.10	81.0	0.14 ^f
69.5	0.20	71.0	0.17 ^g	65.0	0.13 ^g					70.5	0.19 ^g	67.5	0.16 ^g
69.5	0.12	71.5	0.07 ^g	65.0	0.05 ^g					67.5	0.08 ^g	70.5	0.07 ^g
		68.0	0.09 ^g									69.5	0.10 ^g
65.0	0.22												
		81.5	0.17 ^h			67.5	0.10 ^d					70.0	0.14 ^f
		75.2	0.14 ^h					66.2	0.12 ^e				
						79.5	0.24 ^d						
				64.4	0.12 ^e	71.0	0.21 ^e	77.5	0.17 ^e	65.0	0.09 ^e	68.4	0.14 ^e
65.5	0.07 ^c	66.5	0.05 ^h	81.0	0.09 ^f	64.0	0.09 ^d			65.0	0.06 ^c		
67.0	0.12 ^c	68.5	0.06 ^c			64.5	0.09 ^d			79.5	0.14 ^c	67.0	0.09 ^c
69.5	0.10 ^c	65.0	0.09 ^h							65.5	0.08 ^c		
						67.5	0.23 ^d			64.0	0.06 ^c		
68.5	0.12 ^c	65.0	0.07 ^h							65.0	0.07 ^c		

RESULTS

DNA base composition. The average G+C values of the strains which we studied are shown in Table 2. The G+C values of the originally described *Oceanospirillum* species (29) ranged from 45 to 51 mol%; for [*O.*] *kriegii* and [*O.*] *jannaschii* the values were significantly higher (54 to 57 mol%).

DNA:rRNA hybridizations. The results of the DNA:rRNA hybridizations are summarized in Table 2. The two parameters for each DNA:rRNA hybrid formed with 23S [³H]rRNA from *O. linum* ATCC 11336^T are expressed versus each other on the rRNA similarity map in Fig. 1. The *T*_{m(e)} values, the most valuable taxonomic parameters of DNA:rRNA hybrids, are clustered in a *T*_{m(e)} dendrogram in Fig. 2.

Protein electrophoresis. Protein electropherograms were prepared for all of the *Oceanospirillum* strains. Because of the heterogeneity among the named *Oceanospirillum* strains, we restricted the numerical analysis of the protein electropherograms to the strains belonging to the authentic *Oceanospirillum* rRNA branch (Fig. 3).

At the 0.75-*r* level we distinguished the following three groups of strains (Fig. 3): (i) *O. maris* subsp. *maris* ATCC

27509^T, ATCC 27648, and ATCC 27649; (ii) *O. maris* subsp. *williamsae* ATCC 29547^T and *O. hiroshimense* IFO 13616^T; (iii) *O. linum* ATCC 11336^T and ATCC 12753. *O. japonicum* ATCC 19191^T, *O. pelagicum* IFO 13612^T, *O. multiglobuliferum* IFO 13614^T, and *O. beijerinckii* NCMB 52^T each occupy a separate position on the dendrogram.

DNA:DNA hybridizations. Nine strains, representing the different *Oceanospirillum* species which were most closely related to *O. linum* ATCC 11336^T based on the DNA:rRNA hybridization data, were used for DNA:DNA hybridizations. The D values are represented in a matrix in Fig. 4. We found four DNA homology groups (Fig. 4). Within groups the D values varied from 77 to 100%. Between the groups no significant D value was found.

DISCUSSION

In previous papers from our research group the impact and significance of *T*_{m(e)} for detecting intra- and intergeneric relationships in the gram-negative bacteria have been proven extensively (16, 18, 36, 45, 47). On this basis our research group divided the gram-negative bacteria into at least six groups with reciprocal *T*_{m(e)} values of ca. 60°C or lower. These groups are called rRNA superfamilies, indicating a

FIG. 1. rRNA similarity map of hybrids formed between ^3H -labeled 23S rRNA of *O. linum* ATCC 11336^T and a variety of bacterial DNAs. The sequence numbers are given in Tables 1 and 2.

relationship beyond the family level (10, 17). Our rRNA superfamilies I + II, III, and IV as described by De Ley (10) correspond to the gamma, beta, and alpha subclasses, respectively, of the *Proteobacteria* as defined by Stackebrandt et al. (38). Within most rRNA superfamilies we distinguish different rRNA branches [reciprocal $T_{m(e)}$ levels of 67 to 70°C]. One rRNA branch can contain one genus (e.g., *Marinomonas*, *Chromobacterium* [13, 43], etc.) or a group of genera which frequently constitute an official bacterial family (e.g., the *Enterobacteriaceae*, the *Vibrionaceae* [7], etc.). Organisms belonging to one rRNA superfamily and having $T_{m(e)}$ values at the branching level of the different rRNA branches are not closely related to any of the genera on these rRNA branches.

Preliminary DNA:rRNA hybridizations with reference rRNAs representing the different rRNA superfamilies showed that in *Oceanospirillum* (29) all of the species except [*O.*] *pusillum* belong in rRNA superfamily II [approximately at 68.8°C $T_{m(e)}$]. This implies that they are related neither to *Marinomonas* nor to any rRNA branch from rRNA superfamily II (*Deleya* rRNA branch, *Pseudomonas fluorescens* rRNA branch, *Moraxella-Acinetobacter* rRNA branch, etc.). Hence, we showed definitely that the former species [*Alter-*

omonas] *vaga* and [*Alteromonas*] *communis* do not belong in *Oceanospirillum* but in *Marinomonas* (43) (see below). Hybridizations with labeled rRNA from the type strain of the type species, *O. linum* ATCC 11336^T, allowed us to determine the intra- and intergeneric relationships of *Oceanospirillum* species.

***Oceanospirillum* rRNA branch.** DNAs from *Oceanospirillum* species, from possibly related bacteria (Table 2), and from strains representing the different rRNA branches in rRNA superfamily II were hybridized with rRNA from *O. linum* ATCC 11336^T. The similarity map in Fig. 1 and Table 2 show the following three distinct clusters: (i) *O. linum* and *O. maris* constitute one group [$T_{m(e)}$ values from 78.1 to 79.8°C and rRNA binding from 0.21 to 0.27%] (group i); (ii) *O. beijerinckii*, *O. multiglobuliferum*, *O. hiroshimense*, and *O. pelagicum* form a second group with $T_{m(e)}$ values from 74.7 to 76.6°C and rRNA binding from 0.11 to 0.17% (group ii); and (iii) a heterogeneous group of strains with $T_{m(e)}$ values from 65.9 to 71°C and rRNA binding of 0.03 to 0.19% (group iii). Group iii consists of *O. japonicum*, [*O.*] *minutulum*, [*O.*] *kriegii*, [*O.*] *jannaschii*, [*Pseudomonas*] *nautica*, [*Pseudomonas*] *atlantica*, [*Pseudomonas*] *elongata*, *Cellvibrio mixtus* subsp. *mixtus*, and various representative

FIG. 2. Simplified rRNA cistron similarity dendrogram of rRNA superfamily II and part of rRNA superfamily I. Data from references 7, 12, 17, 43, and 45 and unpublished data of P. Segers, K. Kersters, and J. De Ley. The solid bars indicate the ranges observed within a genus, species, or small group.

organisms from the other rRNA branches of rRNA superfamily II.

Groups i and ii together constitute a new rRNA branch in rRNA superfamily II. These findings are in agreement with the cataloging results reported by Woese et al. (50, 51), who

found that four species (*O. maris*, *O. beijerinckii*, *O. linum*, and *O. japonicum*) constitute a separate branch in the gamma subclass of the *Proteobacteria* (38). Reciprocal hybridizations (Table 2) with other reference rRNAs from this rRNA superfamily showed that the rRNA branch with our

FIG. 3. Polyacrylamide gel electrophoretic protein profiles of the authentic *Oceanospirillum* strains located on the *Oceanospirillum* rRNA branch. The correlation coefficient (r) is represented as a dendrogram, calculated by the unweighted average pair grouping method. Each branch of the dendrogram is pointing to the respective electrophoretic trace on the gel.

range of IMD values (83 to 92 IMD units) for their glutamine synthetases, a range differing from the value of 49 to 68 IMD units characteristic for the genus *Alteromonas*. Although the immunological technique which they used is valuable (1, 3, 15) in the elucidation of evolutionary relationships, we cannot agree with the conclusions of Bowditch et al. (5) because recent data (15, 33) invalidate their conclusions (5). (i) The former genus *Vibrio* (1) is very heterogeneous and has recently been split into *Vibrio sensu stricto*, *Listonella* (containing the former species *V. pelagius* and *V. anguillarum*), and another unnamed taxon (25, 33); *Vibrio logei* has been reclassified in *Photobacterium*. Consequently, *Vibrio* (1) was not a suitable example to be used as yardstick to calibrate the IMD range for different species within one genus. (ii) Only *Marinomonas vaga* has a small IMD (Fe-containing superoxide dismutase) (22 IMD units) versus *Marinomonas communis*; the IMDs of *O. linum*, *O. beijerinckii*, [*O.*] *jannaschii*, and [*O.*] *kriegii* are all more than 35 IMD units, with a maximum of 79 IMD units between *Marinomonas communis* and [*O.*] *kriegii* (15). (iii) Because *Alteromonas* belongs in rRNA superfamily I, as described by Van Landschoot and De Ley (43), it is obvious that members of this genus have smaller but diverse IMD values versus *E. coli* than members of rRNA superfamily II (e.g., *Oceanospirillum* and *Marinomonas*). We conclude that none of the arguments used by Bowditch et al. (5) provides evidence about an intrageneric relationship among *Oceanospirillum*, *Marinomonas*, and groups H-1 and I-1. On the contrary, these arguments support the intergeneric relationship found by our DNA:rRNA hybridization studies. The relationships among [*O.*] *kriegii* (group H-1), [*O.*] *jannaschii* (group I-1), and other members of rRNA superfamily II were further studied by DNA:rRNA hybridizations with ³H-labeled rRNAs from [*O.*] *kriegii* ATCC 27133^T and [*O.*] *jannaschii* ATCC 27135^T. Each of these organisms constitutes a separate rRNA branch equidistantly removed from all other rRNA branches of rRNA superfamily II. As both group H-1 and group I-1 are phenotypically well described and can be differentiated, it seems to us that both rRNA branches deserve separate generic rank. Names may be given when more data are available.

[*O.*] *minutulum* is not a member of the *Oceanospirillum* rRNA branch, although it definitely belongs in rRNA superfamily II [mean $T_{m(e)}$, 67.8°C]. This is in agreement with the results of Woese et al. (51), as the [*O.*] *minutulum* rRNA catalog seemed to be slightly more closely related to the rRNA catalogs of *O. linum*, *O. beijerinckii*, *O. maris*, and *O. japonicum* than to the rRNA catalogs in the enteric bacteria-vibrio cluster. According to our results [*O.*] *minutulum* is also only remotely related to [*O.*] *kriegii* and [*O.*] *jannaschii*. It probably represents another separate rRNA branch. We included two [*O.*] *minutulum* strains (Table 1), which displayed very similar protein electrophoretic patterns (data not shown).

Only one strain of [*O.*] *pusillum* is known. Terasaki (40) placed this organism in the former genus *Spirillum* and assigned it later to *Oceanospirillum* (42). However, [*O.*] *pusillum* possesses bipolar single flagella, rather than bipolar tufts, and, in contrast to all other marine spirilla examined so far, it exhibits a counterclockwise helix. A numerical taxonomic study (39) revealed that [*O.*] *pusillum* is only distantly related to the other *Oceanospirillum* species. With mean $T_{m(e)}$ values of 62.5 and 60.0°C versus all rRNA branches from rRNA superfamilies I and II, respectively, [*O.*] *pusillum* does not belong in these rRNA superfamilies; it is generically misnamed. DNA:rRNA hybridizations showed

that [*O.*] *pusillum* belongs in rRNA superfamily IV; it is closest to and equidistantly removed from *Azospirillum*, *Rhodospirillum rubrum*, and some *Aquaspirillum* species (35).

The generically misnamed organisms [*Pseudomonas*] *nautica*, [*Pseudomonas*] *elongata*, and [*Pseudomonas*] *atlantica* were located at the base of the *Oceanospirillum*, [*O.*] *kriegii*, and [*O.*] *jannaschii* rRNA branches.

The generic name *Cellvibrio* was revived by Blackall et al. (4) after they isolated several strains that were similar to the original generic description of Winogradsky (49) and that had a cellular morphology, flagellation, DNA base composition and physiology similar to those of existing strains of "*Cellvibrio vulgaris*." These strains can be clearly differentiated from the genus *Pseudomonas*, in which "*Cellvibrio vulgaris*" and "*Cellvibrio fulvus*" were placed as species incertae sedis (19). Since the original type species, "*Cellvibrio ochraceus*," is not available, Blackall et al. (4) proposed a new type species, *Cellvibrio mixtus*, with two subspecies, *Cellvibrio mixtus* subsp. *mixtus* and *Cellvibrio mixtus* subsp. *dextranolyticus*. The former "*Cellvibrio vulgaris*" and "*Cellvibrio fulvus*" are now members of *Cellvibrio mixtus* subsp. *mixtus*; both of these organisms (Table 2) are not more closely related to the [*O.*] *kriegii* and [*O.*] *jannaschii* rRNA branches than to any other rRNA branch of rRNA superfamily II.

***Oceanospirillum sensu stricto*.** As a result of our genotypic and protein electrophoretic studies, we conclude that the genus *Oceanospirillum* should be restricted to the organisms of the *Oceanospirillum* rRNA branch and that some species have to be redefined. The removal of several species from the genus *Oceanospirillum* requires a new genus definition, which is only slightly different from the description of Krieg (29).

Genus *Oceanospirillum* Hylemon, Wells, Krieg and Jannasch 1973^{AL}. *Oceanospirillum* (O.ce.an.o.spi.ril'lum. M.L. n. *oceanus*, ocean; Gr. n. *spira*, spiral; M.L. dim. neut. n. *spirillum*, a small spiral; *Oceanospirillum*, a small spiral organism from the ocean [seawater]). Rigid, helical cells with clockwise helix. Cells 0.4 to 1.4 µm in diameter; length of the helix, 1.2 to 75 µm. A polar membrane underlies the cytoplasmic membrane at the cell poles in all species examined so far by electron microscopy. Intracellular poly-β-hydroxybutyrate is formed. The strains of most species form thin-walled coccoid bodies which predominate in old cultures. Gram negative. Motile by bipolar tufts of flagella. Chemoorganotrophic, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate respiration does not occur; nitrate is not reduced to nitrite or beyond the nitrite stage. Optimum temperature, 25 to 32°C. Oxidase positive. Indole negative. Casein, starch, hippurate, and esculin are not hydrolyzed. Seawater is required for growth. Carbohydrates are neither fermented nor oxidized. Amino acids or the salts of organic acids serve as carbon sources. Growth factors are not usually required. Isolated from coastal seawater, from decaying seaweed, and from putrid infusions of marine mussels. The G+C content of the DNA ranges from 45 to 50 mol% (as determined by the thermal denaturation method). The type species is *Oceanospirillum linum* (Williams and Rittenberg, 1957) Hylemon, Wells, Krieg and Jannasch 1973^{AL}.

We propose that *O. pelagicum* should be united with *O. beijerinckii* and also that *O. hiroshimense* should be united with *O. maris*, because of their genotypic and protein gel electrophoretic similarities and because enough phenotypic differences (Table 3) remain to differentiate the newly defined species (*O. beijerinckii* and *O. maris* according to Rule

TABLE 3. Differential characteristics of the species in the redefined genus *Oceanospirillum*^a

Species	Length of helix (µm)	Cell diam (µm)	Coccoid bodies predominant at:		Maximum salt tolerance not higher than 4% NaCl	Optimum temp 25°C rather than 30–32°C	Phosphatase	Catalase	Auxotrophic growth requirement	G+C content of DNA (mol%)
			3–4 weeks	24–48 h						
<i>O. linum</i>	4.0–30.0	0.4–0.6	+	–	–	–	+	+ or w	+	48–50
<i>O. maris</i>	2.5–40.0	0.6–1.1	+	–	–	d ^d	d ^d	d ^d	d ^e	45–47
<i>O. beijerinckii</i>	2.0–15.5	0.6–1.2	+	–	–	–	+	+ or w	–	47–49
<i>O. multiglobuliferum</i>	2.0–10.0	0.5–0.9	+	+	+	–	+	+	–	46
<i>O. japonicum</i>	5.0–75.0	0.8–1.4	–	–	–	–	w	w or –	–	45

^a The phenotypic data are from reference 29.

^b +, Present in all strains; –, absent in all strains; d, differs among strains; w, weak reactions.

^c *O. linum* grows poorly or not at all in defined media containing single carbon sources and ammonium ions as the nitrogen source; however, abundant growth occurs in a defined medium containing succinate and malate as the carbon sources and methionine as the nitrogen source.

^d *O. maris* strains can have an optimum temperature of 25°C; the catalase reaction can be negative, weak, or positive; and the phosphatase reaction may be positive or negative. For detailed information see references 22, 39, and 42.

^e *O. maris* ATCC 29547 does not grow in vitamin-free defined media and has a growth factor requirement that has not been identified yet.

43 of the *International Code of Nomenclature of Bacteria* [31]). On phenotypic grounds (29) we retain the two subspecies of *O. maris* (29) and create a new subspecies, *O. maris* subsp. *hiroshimense* (Rule 50b [31]), for the former species *O. hiroshimense*. Similarly, we create the new subspecies *O. beijerinckii* subsp. *pelagicum* for the former species *O. pelagicum*. According to Rules 45 and 46 (31) *O. beijerinckii* subsp. *beijerinckii* should also be described.

***Oceanospirillum beijerinckii* (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973^{AL}.** *Oceanospirillum beijerinckii* (beij.er.inck'i.i. M.L. gen. n. *beijerinckii*, of Beijerinck, named for M. W. Beijerinck from Delft, The Netherlands). Cell diameter, 0.6 to 1.2 µm; wavelength of helix, 3.0 to 7.2 µm; length of helix, 2 to 15.5 µm. Coccoid bodies are predominant in old cultures. Cells have phosphatase activity. Catalase reaction can be weak. Some strains liquefy gelatin at 20°C. Ammonium ions can serve as a sole nitrogen source. No acid is produced from sugars. The temperature range for growth varies between 14 and 37°C. No auxotrophic growth requirements. Grows in the presence of 6% (wt/vol) NaCl. Succinate, fumarate, malate, and pyruvate can be used as sole carbon sources. Some strains can use citrate, lactate, tartrate, acetate, propionate, *p*-hydroxybenzoate, ethanol, and *n*-propanol. Isolated from coastal seawater or from putrid infusions of marine mussels. The G+C content is 47 to 49 mol%. The type strain is strain NCMB 52.

***Oceanospirillum beijerinckii* subsp. *beijerinckii* (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973 subsp. nov.** *Oceanospirillum beijerinckii* subsp. *beijerinckii* (beij.er.inck'i.i. M.L. gen. n. *beijerinckii*, of Beijerinck, named for M. W. Beijerinck from Delft, The Netherlands). Features are as described above for the species. Differs from *O. beijerinckii* subsp. *pelagicum* in failing to use citrate, malate, pyruvate, lactate, tartrate, acetate, propionate, *p*-hydroxybenzoate, ethanol, and *n*-propanol, as tested by Terasaki (39, 42). However, malate and pyruvate are used when cells are tested by the method of Hylemon et al. (22). The temperature range for growth is 14 to 37°C. Gelatin is liquefied at 20°C. The range of NaCl concentrations for growth in peptone water (7 days) is 0.5 to 6.0%. Isolated from coastal seawater. The type strain is strain NCMB 52. The G+C content of the type strain is 47 mol%.

***Oceanospirillum beijerinckii* subsp. *pelagicum* (Terasaki 1973) subsp. nov.** *Oceanospirillum beijerinckii* subsp. *pelagicum* (pe.la'gi.cum. L. neut. adj. *pelagicum*, belonging to the sea). Features are as described above for the species. Differs from *O. beijerinckii* subsp. *beijerinckii* in the use of

citrate, malate, pyruvate, lactate, tartrate, acetate, and propionate (all strains). The use of *p*-hydroxybenzoate, ethanol, and *n*-propanol as carbon sources and the liquefaction of gelatin at 20°C differ from strain to strain. The temperature range for growth is 8 to 41°C. The range of NaCl concentrations for growth in peptone water (7 days) is 0.5 to 8.0%. Isolated from putrid infusions of marine mussels. The type strain is strain IFO 13612 with a G+C content of 49 mol%.

***Oceanospirillum maris* Hylemon, Wells, Krieg and Jannasch 1973^{AL}.** *Oceanospirillum maris* (ma'ris. L. n. *mare* the sea; L. gen. n. *maris*, of the sea). Cell diameter, 0.6 to 1.1 µm; wavelength of helix, 3.0 to 7.0 µm; length of helix, 2.5 to 40 µm. Coccoid bodies are predominant in old cultures. Catalase and phosphatase reactions differ from strain to strain. Gelatin can be liquefied at 20°C. Ammonium ions can serve as a sole nitrogen source. No acid is produced from sugars. Temperature range for growth, 14 to 35°C. Cannot use citrate, malonate, or *n*-butanol. The use of succinate, fumarate, malate, pyruvate, lactate, acetate, tartrate, propionate, *p*-hydroxybenzoate, ethanol, and *n*-propanol differs among the subspecies.

Isolated from coastal seawater or from putrid infusions of marine mussels. The G+C content is 45 to 47 mol%. The type strain is strain ATCC 27509.

***Oceanospirillum maris* subsp. *maris* Hylemon, Wells, Krieg and Jannasch 1973^{AL}.** *Oceanospirillum maris* subsp. *maris* (ma'ris. L. n. *mare* the sea; L. gen. n. *maris*, of the sea). Features are as described above for the species. Differs from *O. maris* subsp. *williamsae* and *O. maris* subsp. *hiroshimense* in having a strong catalase reaction. Can grow in the presence of 1% glycine; deoxyribonuclease and ribonuclease activities are lacking. Can grow on oxaloacetate and L-glutamate when cells are tested in a defined vitamin-free medium. The type strain is strain ATCC 27509; the G+C content is 46 mol%.

***Oceanospirillum maris* subsp. *williamsae* Linn and Krieg 1978^{AL}.** *Oceanospirillum maris* subsp. *williamsae* (wil'iam.sae. M. L. gen. n. *williamsae*, of Williams, named for Marion A. Williams, who was the first to describe species of marine spirilla). Features are as described above for the species. Differs from *O. maris* subsp. *maris* and *O. maris* subsp. *hiroshimense* in failing to grow in defined vitamin-free medium. Cannot grow in the presence of 1% glycine. Possesses deoxyribonuclease and ribonuclease activities. Very weak catalase activity. The auxotrophic growth requirement has not been identified yet.

Isolated from a mixture of organisms comprising strain

NCMB 54 by Linn and Krieg (32). The type strain is strain ATCC 29547. The G+C content of the type strain is 45 mol%.

Oceanospirillum maris subsp. hiroshimense (Terasaki 1973) **comb. nov.** *Oceanospirillum maris subsp. hiroshimense* (hi.ro.shi.men'se. M.L. neut. adj. *hiroshimense*, pertaining to Hiroshima, Japan). Features are as described above for the species. Differs from *O. maris subsp. maris* and *O. maris subsp. williamsae* in having phosphatase activity and in the optimum temperature for growth (25°C rather than 30 to 32°C). Differs from *O. maris subsp. maris* in the following C sources used in a defined vitamin-free medium: succinate, fumarate, pyruvate, lactate, tartrate, acetate, and propionate. Catalase reaction is weak or is lacking.

Isolated from putrid infusions of marine mussels. The type strain is strain IFO 13616; the G+C content of the type strain is 47 mol%.

The definitions of *O. multiglobuliferum*, *O. japonicum*, and *O. linum* remain unchanged.

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LITERATURE CITED

- Bang, S. S., L. Baumann, M. J. Woolkalis, and P. Baumann. 1981. Evolutionary relationships in *Vibrio* and *Photobacterium* as determined by immunological studies of superoxide dismutase. *Arch. Microbiol.* **130**:111-120.
- Baumann, L., P. Baumann, M. Mandel, and R. D. Allen. 1972. Taxonomy of aerobic marine eubacteria. *J. Bacteriol.* **110**:402-429.
- Baumann, P., L. Baumann, S. S. Bang, and M. J. Woolkalis. 1980. Reevaluation of the taxonomy of *Vibrio*, *Beneckea*, and *Photobacterium*: abolition of the genus *Beneckea*. *Curr. Microbiol.* **4**:127-132.
- Blackall, L. L., A. C. Hayward, and L. I. Sly. 1985. Cellulolytic and dextranolytic gram-negative bacteria: revival of the genus *Cellvibrio*. *J. Appl. Bacteriol.* **59**:81-97.
- Bowditch, R. D., L. Baumann, and P. Baumann. 1984. Description of *Oceanospirillum kriegii* sp. nov. and *O. jannaschii* sp. nov. and assignment of two species of *Alteromonas* to this genus as *O. commune* comb. nov. and *O. vagum* comb. nov. *Curr. Microbiol.* **10**:221-230.
- Burton, K. 1956. A study of the conditions and mechanisms of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid. *Biochem. J.* **62**:315-323.
- Colwell, R. R., M. T. MacDonell, and J. De Ley. 1986. Proposal to recognize the family *Aeromonadaceae* fam. nov. *Int. J. Syst. Bacteriol.* **36**:473-477.
- Costas, M., and R. J. Owen. 1987. Numerical analysis of electrophoretic protein patterns of *Streptobacillus moniliformis* strains from human, murine and avian infections. *J. Med. Microbiol.* **23**:303-311.
- De Ley, J. 1970. Reexamination of the association between melting point, buoyant density, and chemical base composition of deoxyribonucleic acid. *J. Bacteriol.* **101**:738-754.
- De Ley, J. 1978. Modern molecular methods in bacterial taxonomy: evaluation, application, prospects, p. 347-357. In *Proceedings of the 4th International Conference on Plant Pathogenic Bacteria*, vol. 1. Gibert-Clarey, Tours, France.
- De Ley, J., H. Cattoir, and A. Reynaerts. 1970. The quantitative measurement of DNA hybridization from renaturation rates. *Eur. J. Biochem.* **12**:133-142.
- De Ley, J., and J. De Smedt. 1975. Improvements of the membrane filter method for DNA:RNA hybridization. *Antonie van Leeuwenhoek J. Microbiol. Serol.* **41**:287-307.
- De Ley, J., P. Segers, and M. Gillis. 1978. Intra- and intergeneric similarities of *Chromobacterium* and *Janthinobacterium* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* **28**:154-168.
- De Ley, J., P. Segers, K. Kersters, W. Mannheim, and A. Lievens. 1986. Intra- and intergeneric similarities of the *Bordetella* ribosomal ribonucleic acid cistrons: proposal for a new family, *Alcaligenaceae*. *Int. J. Syst. Bacteriol.* **36**:405-414.
- Delong, E. F., L. Baumann, R. D. Bowditch, and P. Baumann. 1984. Evolutionary relationships of superoxide dismutases and glutamine synthetases from marine species of *Alteromonas*, *Oceanospirillum*, *Pseudomonas* and *Deleya*. *Arch. Microbiol.* **138**:170-178.
- De Smedt, J., and J. De Ley. 1977. Intra- and intergeneric similarities of *Agrobacterium* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* **27**:222-240.
- De Vos, P., and J. De Ley. 1983. Intra- and intergeneric similarities of *Pseudomonas* and *Xanthomonas* ribosomal ribonucleic acid cistrons. *Int. J. Syst. Bacteriol.* **33**:487-509.
- De Vos, P., K. Kersters, E. Falsen, B. Pot, M. Gillis, P. Segers, and J. De Ley. 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.
- De Vos, P., A. Van Landschoot, P. Segers, R. Tytgat, M. Gillis, M. Bauwens, R. Rossau, M. Goor, B. Pot, K. Kersters, P. Lizzaraga, and J. De Ley. 1989. Genotypic relationships and taxonomic localization of unclassified *Pseudomonas* and *Pseudomonas*-like strains by deoxyribonucleic acid:ribosomal ribonucleic acid hybridizations. *Int. J. Syst. Bacteriol.* **39**:35-49.
- Doudoroff, M., and N. J. Palleroni. 1974. *Pseudomonas* Migula 1894, p. 217-243. In R. E. Buchanan and N. F. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Dreyfus, B., J. L. Garcia, and M. Gillis. 1988. Characterization of *Azorhizobium caulinodans* gen. nov., sp. nov., a stem-nodulating nitrogen-fixing bacterium isolated from *Sesbania rostrata*. *Int. J. Syst. Bacteriol.* **38**:89-98.
- Gillis, M., and J. De Ley. 1980. Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acetobacter* and *Gluconobacter*. *Int. J. Syst. Bacteriol.* **30**:7-27.
- Hylemon, P. B., J. S. Wells, Jr., N. R. Krieg, and H. W. Jannasch. 1973. The genus *Spirillum*: a taxonomic study. *Int. J. Syst. Bacteriol.* **23**:340-380.
- International Journal of Systematic Bacteriology. 1984. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 13. *Int. J. Syst. Bacteriol.* **34**:91-92.
- International Journal of Systematic Bacteriology. 1984. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 16. *Int. J. Syst. Bacteriol.* **34**:503-504.
- International Journal of Systematic Bacteriology. 1986. Validation of the publication of new names and new combinations previously effectively published outside the IJSB. List no. 20. *Int. J. Syst. Bacteriol.* **36**:354-356.
- Kersters, K., and J. De Ley. 1975. Identification and grouping of bacteria by numerical analysis of their electrophoretic protein patterns. *J. Gen. Microbiol.* **87**:333-342.
- Kiredjian, M., B. Holmes, K. Kersters, I. Guilvout, and J. De Ley. 1986. *Alcaligenes piechaudii*, a new species from human clinical specimens and the environment. *Int. J. Syst. Bacteriol.* **36**:282-287.
- Krieg, N. R. 1974. *Spirillum* Ehrenberg 1832, p. 196-207. In R. E. Buchanan and N. F. Gibbons (ed.), *Bergey's manual of determinative bacteriology*, 8th ed. The Williams & Wilkins Co., Baltimore.
- Krieg, N. R. 1984. Aerobic/microaerophilic, motile, helical/vibroid gram-negative bacteria, p. 71-95. In N. R. Krieg and J. G. Holt (ed.), *Bergey's manual of systematic bacteriology*,

- vol. 1. The Williams & Wilkins Co., Baltimore.
30. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of the bacteriophage T4. *Nature (London)* **227**:680-685.
 31. Lapage, S. P., P. H. A. Sneath, E. F. Lessel, V. B. D. Skerman, H. P. R. Seelinger, and W. A. Clark (ed.). 1975. International code of nomenclature of bacteria, 1975 revision. American Society for Microbiology, Washington, D. C.
 32. Linn, D. M., and N. R. Krieg. 1978. Occurrence of two organisms in cultures of the type strain of *Spirillum lunatum*: proposal for rejection of the name *Spirillum lunatum* and characterization of *Oceanospirillum maris subsp. williamsae* and an unclassified vibrioid bacterium. *Int. J. Syst. Bacteriol.* **28**:132-138.
 33. MacDonell, M. T., and R. R. Colwell. 1985. Phylogeny of the *Vibrionaceae*, and recommendation for two genera, *Listonella* and *Shewanella*. *Syst. Appl. Microbiol.* **6**:171-182.
 34. Marmur, J., and P. Doty. 1962. Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J. Mol. Biol.* **5**:109-118.
 35. Reinhold, B., T. Hurek, I. Fendrik, B. Pot, M. Gillis, K. Kersters, S. Thielemans, and J. De Ley. 1987. *Azospirillum halopraeferens* sp. nov., a nitrogen-fixing organism associated with roots of Kallar grass (*Leptochloa fusca* (L.) Kunth). *Int. J. Syst. Bacteriol.* **37**:43-51.
 36. Rossau, R., K. Kersters, E. Falsen, E. Jantzen, P. Segers, A. Union, L. Nehls, and J. De Ley. 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.
 37. Rossau, R., A. Van Landschoot, W. Mannheim, and J. De Ley. 1986. Inter- and intrageneric similarities of ribosomal ribonucleic acid cistrons of the *Neisseriaceae*. *Int. J. Syst. Bacteriol.* **36**:323-332.
 38. Stackebrandt, R., C. R. Woese, and R. G. E. Murray. 1988. *Proteobacteria* classis nov., a name for the phylogenetic taxon that includes the "purple bacteria and their relatives." *Int. J. Syst. Bacteriol.* **38**:321-325.
 39. Terasaki, Y. 1972. Studies on the genus *Spirillum* Ehrenberg. I. Morphological, physiological, and biochemical characteristics of water spirilla. *Bull. Suzugamine Women's Coll. Nat. Sci.* **16**:1-146.
 40. Terasaki, Y. 1973. Studies on the genus *Spirillum* Ehrenberg. II. Comments on type and reference strains of *Spirillum* and description of new species and new subspecies. *Bull. Suzugamine Women's Coll. Nat. Sci.* **17**:1-71.
 41. Terasaki, Y. 1975. Freeze-dried cultures of water spirilla made on experimental basis. *Bull. Suzugamine Women's Coll. Nat. Sci.* **19**:1-10.
 42. Terasaki, Y. 1979. Transfer of five species and two subspecies of *Spirillum* to other genera (*Aquaspirillum* and *Oceanospirillum*), with emended descriptions of the species and subspecies. *Int. J. Syst. Bacteriol.* **29**:130-144.
 43. Van Landschoot, A., and J. De Ley. 1983. Intra- and intergeneric similarities of the rRNA cistrons of *Alteromonas*, *Marinomonas* (gen. nov.) and some other gram-negative bacteria. *J. Gen. Microbiol.* **129**:3057-3074.
 44. Van Landschoot, A., P. De Vos, and J. De Ley. 1984. An alternative parameter for Tm(e) in DNA:rRNA hybridization studies. *Syst. Appl. Microbiol.* **5**:143-156.
 45. Van Landschoot, A., R. Rossau, and J. De Ley. 1986. Intra- and intergeneric similarities of the ribosomal ribonucleic acid cistrons of *Acinetobacter*. *Int. J. Syst. Bacteriol.* **36**:150-160.
 46. Watanabe, N. 1959. On four new halophilic species of *Spirillum*. *Bot. Mag. (Tokyo)* **72**:77-86.
 47. Willems, A., M. Gillis, K. Kersters, L. Van Den Broecke, and J. De Ley. 1987. Transfer of *Xanthomonas ampelina* Panagopoulos 1969 to a new genus, *Xylophilus* gen. nov., as *Xylophilus ampelinus* (Panagopoulos 1969) comb. nov. *Int. J. Syst. Bacteriol.* **37**:422-430.
 48. Williams, M. A., and S. C. Rittenberg. 1957. A taxonomic study of the genus *Spirillum* Ehrenberg. *Int. Bull. Bacteriol. Nomencl. Taxon.* **7**:49-111.
 49. Winogradsky, S. 1929. Études sur la microbiologie du sol. Sur la dégradation de la cellulose dans le sol. *Ann. Inst. Pasteur (Paris)* **43**:549-633.
 50. Woese, C. R., P. Blanz, R. B. Hespell, and C. M. Hahn. 1982. Phylogenetic relationships among various helical bacteria. *Curr. Microbiol.* **7**:119-124.
 51. Woese, C. R., W. G. Weisburg, C. M. Hahn, B. J. Paster, L. B. Zablen, B. J. Lewis, T. J. Macke, W. Ludwig, and E. Stackebrandt. 1985. The phylogeny of purple bacteria: the gamma subdivision. *Syst. Appl. Microbiol.* **6**:25-33.