Phylogenetic study of the genus *Oceanospirillum* based on 16S rRNA and *gyrB* genes: emended description of the genus *Oceanospirillum*, description of *Pseudospirillum* gen. nov., *Oceanobacter* gen. nov. and *Terasakiella* gen. nov. and transfer of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov.

¹ Department of Food Science and Technology, Tokyo University of Fisheries, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan

² Marine Biotechnology Institute, Kamaishi Laboratories, 3-75-1 Heita, Kamaishi City, Iwate 026-0001, Japan

Masataka Satomi,^{1†} Bon Kimura,¹ Tohru Hamada,² Shigeaki Harayama² and Tateo Fujii¹

Author for correspondence: Bon Kimura. Tel: +81 3 5463 0603. Fax: +81 3 5463 0602.
e-mail: kimubo@tokyo-u-fish.ac.jp

The phylogenetic relationships of *Oceanospirillum* strains were analysed by using the nucleotide sequences of 16S rRNA and *gyrB* genes. Results from sequence analysis demonstrated that the *Oceanospirillum* core group consisted of four species, *Oceanospirillum linum*, *Oceanospirillum maris*, *Oceanospirillum beijerinckii* and *Oceanospirillum multiglobuliferum*, with enough distance to separate them as different species. However, four other *Oceanospirillum* species occupied taxonomic positions separate from the *Oceanospirillum* core group: *Oceanospirillum jannaschii*, *Oceanospirillum japonicum* and *Oceanospirillum kriegii* in the γ -Proteobacteria and *Oceanospirillum pusillum* in the α -Proteobacteria. *Oceanospirillum jannaschii* clustered with *Marinobacterium georgiense*, *Pseudomonas iners* and *Pseudomonas stanieri* on the basis of phylogenetic analysis of 16S rRNA and *gyrB* genes. The other three species did not cluster with known genera. Also, the sequence similarity values of the *gyrB* genes between the three subspecies of *Oceanospirillum maris* and those between the two subspecies of *Oceanospirillum beijerinckii* were above 99%. The close relationships between the subspecies of *Oceanospirillum maris* and of *Oceanospirillum beijerinckii* were further supported by similar physiological properties and high DNA–DNA hybridization values, suggesting that these subspecies should not be regarded as valid. From these results, *Oceanospirillum sensu stricto* should be defined to consist of *Oceanospirillum linum*, *Oceanospirillum maris*, *Oceanospirillum beijerinckii* and *Oceanospirillum multiglobuliferum*. We propose to create the following new genera: *Pseudospirillum* gen. nov. for *Oceanospirillum japonicum* as *Pseudospirillum japonicum* comb. nov.; *Oceanobacter* gen. nov. for *Oceanospirillum kriegii* as *Oceanobacter kriegii* comb. nov.; and *Terasakiella* gen. nov. for *Oceanospirillum pusillum* as *Terasakiella pusilla* comb. nov. The transfer is

[†] Present address: National Research Institute of Fisheries Science, 2-12-4 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-8648, Japan.

Abbreviation: PHB, poly- β -hydroxybutyrate.

The DDBJ accession numbers for the *gyrB* sequences reported in this paper are AB014929, AB014932–AB014937, AB032183–AB032190 and AB048519–AB048521, as detailed in Table 1.

proposed of *Oceanospirillum jannaschii* and *Pseudomonas stanieri* to *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. Furthermore, *Pseudomonas iners* should be reclassified as a strain of *Marinobacterium georgiense*. Finally, the subspecies of *Oceanospirillum maris* (*O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense* and *O. maris* subsp. *williamsae*) and *Oceanospirillum beijerinckii* (*O. beijerinckii* subsp. *beijerinckii* and *O. beijerinckii* subsp. *pelagicum*) should be combined as *Oceanospirillum maris* and *Oceanospirillum beijerinckii*, respectively.

Keywords: *Oceanospirillum*, *Oceanobacter*, *Pseudospirillum*, *Marinobacterium*, *Terasakiella*

INTRODUCTION

The genus *Oceanospirillum* was established by the division of the genus *Spirillum* Ehrenberg 1832 by Hylemon *et al.* (1973). Its members were isolated from the ocean, from putrid marine shellfish (Terasaki, 1972, 1973, 1975, 1979; Watanabe, 1959) and from coastal sea water (Baumann *et al.*, 1972; Hylemon *et al.*, 1973; Linn & Krieg, 1978; Williams & Rittenberg, 1957), and are presently known to be distributed ubiquitously in marine environments. According to Holt *et al.* (1994), all helical, halophilic, chemo-organotrophic and aerobic bacteria belong to the genus *Oceanospirillum*, which currently consists of eight species, two of which include a total of five subspecies. However, there is much intrageneric diversity in this group, as revealed by rRNA–DNA hybridization experiments (Pot *et al.*, 1989, 1992), fatty acid analysis (Sakane & Yokota, 1994) and the compositions of isoprenoid quinones (Sakane & Yokota, 1994) and polyamines (Hamana *et al.*, 1994). In a previous study, based on analysis of 16S rRNA gene sequences (16S rDNA), *Oceanospirillum minutulum* was transferred to a new genus, *Marinospirillum* (Satomi *et al.*, 1998). However, the taxonomic positions of four species, *Oceanospirillum jannaschii*, *Oceanospirillum japonicum*, *Oceanospirillum kriegii* and *Oceanospirillum pusillum*, and the validity of the subspecies belonging to *Oceanospirillum maris* and *Oceanospirillum beijerinckii* remained to be clarified. In order to understand better the phylogenetic relationships of these organisms, it is preferable to perform phylogenetic analysis based on multiple gene sequences. Several genes, such as *gyrB* and *rpoD*, and the internal transcribed spacer region of rRNA genes, have been evaluated as alternatives to the 16S rDNA sequence (Takewaki *et al.*, 1994; Viale *et al.*, 1994; Yamamoto & Harayama, 1995, 1996, 1998; Morse *et al.*, 1996; Edgell *et al.*, 1997). Notably, protein-encoding genes are known to evolve much faster than 16S rDNA and may thus provide precise phylogenetic information about closely related species or subspecies. In this study, we determined the *gyrB* nucleotide sequences of strains in the genus *Oceanospirillum* to facilitate the study of their phylogenetic relationships.

METHODS

Bacterial strains and growth conditions. The bacterial strains investigated are listed in Table 1. *Oceanospirillum* and its related strains were obtained from the Institute for Fermentation Osaka (IFO), the ATCC, the Institute of Applied Microbiology (IAM) and Dr Y. Terasaki (formerly of Suzugamine Women's College, Hiroshima, Japan). These strains were maintained by stab culture in medium 325 (IFO, 1996) semi-solid agar, which contained the following ingredients: 1.0% (w/v) polypeptone (Difco), 0.2% yeast extract (Difco), 0.05% MgSO₄·7H₂O, 75% (w/v) sea water, 25% (w/v) distilled water and 0.2% agar. The pH was adjusted to 7.2 with 1.0 M NaOH. The cultures were incubated for 3 days at 20 °C. In order to obtain large amounts of cells, cultures were incubated in medium 325 broth at 90 r.p.m. on a rotary shaker.

Phenotypic characteristics. Tolerance of NaCl and growth at various temperatures were tested according to Terasaki (1972). Glycine tolerance and production of catalase, DNase and RNase were determined according to Hylemon *et al.* (1973). Other physiological and biochemical tests were performed by using API 20E, API 20NE and API ZYM test kits (bioMérieux), which were prepared according to the manufacturer's specifications, except that bacterial strains were suspended in 3% NaCl solution.

DNA preparation and DNA–DNA homology. Late exponential phase cultures were centrifuged and then cell pellets were suspended in TE buffer (pH 8.0) to extract chromosomal DNA. Cell suspensions were treated with 0.5% SDS for lysis. Chromosomal DNA was purified by standard methods (Sambrook *et al.*, 1989). DNA–DNA homology was studied by microplate hybridization methods (Ezaki *et al.*, 1989) with photobiotin labelling and colorimetric detection as described previously (Satomi *et al.*, 1997).

***gyrB* nucleotide sequencing and phylogenetic analysis.** The 1.2 kbp nucleotide sequences of the *gyrB* genes of *Oceanospirillum* species and related organisms (covering base positions 274–1525, *Escherichia coli* numbering) were amplified by using PCR with universal primer sets (Yamamoto & Harayama, 1995) and sequenced. The PCR conditions were as described by Yamamoto & Harayama (1995). The PCR products were visualized by electrophoresis with 1.5% (w/v) agarose gel (Nippon Gene) stained with ethidium bromide.

Table 1. Strains investigated in this study

The *gyrB* genes were sequenced in this study.

Strain	Accession number	
	16S rRNA	<i>gyrB</i>
<i>Oceanospirillum linum</i> IFO 15448 ^T (= ATCC 11336 ^T)	M22365	AB014935
<i>Oceanospirillum linum</i> IFO 15449 (= ATCC 12753)	—	—
<i>Oceanospirillum maris</i> subsp. <i>maris</i> ATCC 27509 ^T	AB006771	AB014936
<i>Oceanospirillum maris</i> subsp. <i>hirosimense</i> IFO 13616 ^T	AB006762	AB032183
<i>Oceanospirillum maris</i> subsp. <i>williamsae</i> IFO 15468 ^T (= ATCC 29547 ^T)	AB006763	AB032184
<i>Oceanospirillum beijerinckii</i> subsp. <i>beijerinckii</i> IFO 15445 ^T (= ATCC 12754 ^T)	AB006760	AB032185
<i>Oceanospirillum beijerinckii</i> subsp. <i>pelagicum</i> IFO 13612 ^T (= ATCC 33337 ^T)	AB006761	AB014933
<i>Oceanospirillum multiglobuliferum</i> IFO 13614 ^T (= ATCC 33336 ^T)	AB006764	AB032186
<i>Oceanospirillum japonicum</i> ATCC 19191 ^T (= IFO 15446 ^T)	AB006766	AB032187
<i>Oceanospirillum kriegii</i> IFO 15467 ^T (= ATCC 27133 ^T)	AB006767	AB032188
<i>Oceanospirillum jannaschii</i> IFO 15466 ^T (= ATCC 27135 ^T)	AB006765	AB014934
<i>Oceanospirillum pusillum</i> IFO 13613 ^T (= ATCC 33338 ^T)	AB006768	AB014937
<i>Marinobacterium georgiense</i> ATCC 700074 ^T	U58339	AB048519
<i>Pseudomonas iners</i> IAM 1419 ^T	AB021408	AB048520
<i>Pseudomonas stanieri</i> ATCC 27130 ^T	AB021367	AB048521
<i>Marinospirillum minutulum</i> ATCC 19193 ^T (= IFO 15450 ^T)	AB006769	AB032189
<i>Marinospirillum megaterium</i> JCM 10129 ^T	AB006770	AB032190
<i>Marinomonas communis</i> ATCC 27118 ^T	AF173967	AB014929
<i>Marinomonas vaga</i> ATCC 27119 ^T	X67025	AB014932

Direct sequencing of the amplified DNA fragments was performed as described previously (Satomi *et al.*, 1997). 16S rDNA sequences used for phylogenetic studies were obtained from the DDBJ database. The accession numbers of the sequences used this study are listed in Table 1.

The 1.4 kbp nucleotide sequences of the 16S rRNA gene (covering base positions 66–1448, *E. coli* numbering) and 1.1 kbp nucleotide sequences of the *gyrB* gene (covering base positions 316–1472, *E. coli* numbering) were used for phylogenetic analysis. Sequence data were edited with the DNASIS software (Takara). In order to search for organisms with phylogenetic relationships to *Oceanospirillum* species, their 16S rDNA sequences were compared to 16S rDNA sequence information in the GenBank, EMBL and DDBJ databases by using the BLAST algorithm (Altschul *et al.*, 1990). Sequences that exhibited close relatedness in a BLAST search were used for phylogenetic analysis. Multiple alignment, calculation of nucleotide substitution rates (K_{nuc} values) as described by Kimura (1980) and construction of phylogenetic trees by the neighbour-joining method (Saitou & Nei, 1987) were performed by using the CLUSTAL W computer program (Thompson *et al.*, 1994). Alignment gaps, primer regions for PCR amplification and unidentified base positions were not taken into consideration for the calculations. The robustness of the topology on phylogenetic trees was evaluated by a bootstrap analysis with 1000 replications.

RESULTS AND DISCUSSION

Phylogenetic trees of *Oceanospirillum* strains based on the nucleotide sequences of 16S rDNA and *gyrB* nucleotide sequences are shown in Fig. 1. The topo-

logies of the 16S rDNA- and *gyrB*-based trees were similar. Four species, *Oceanospirillum linum*, *Oceanospirillum maris* (including three subspecies), *Oceanospirillum beijerinckii* (including two subspecies) and *Oceanospirillum multiglobuliferum*, formed a strongly supported cluster (bootstrap value 100%) within the γ -*Proteobacteria* based on 16S rDNA analysis (referred to as the *Oceanospirillum* core group in this study), with enough distance to separate each other at the species level. The sequence similarity values among the *Oceanospirillum* core group were more than 95 and 78%, respectively, for 16S rDNA and *gyrB* nucleotide sequences. However, the sequence similarity values between the *Oceanospirillum* core group and the remaining *Oceanospirillum* species were less than 90 and 72%, respectively, for 16S rDNA and *gyrB* nucleotide sequences. *Oceanospirillum jannaschii*, *Oceanospirillum japonicum* and *Oceanospirillum kriegii* were assigned within the γ -*Proteobacteria*, while *Oceanospirillum pusillum* was assigned within the α -*Proteobacteria*. These data are in agreement with the earlier finding of Pot *et al.* (1989), who proposed the removal of *Oceanospirillum jannaschii*, *Oceanospirillum kriegii* and *Oceanospirillum pusillum* from the genus *Oceanospirillum* on the basis of a polyphasic study in which DNA–rRNA hybridizations played an important role. In that study, *Oceanospirillum japonicum* was assigned to the genus *Oceanospirillum* (Pot *et al.*, 1989). However, the present results indicate that its 16S rDNA and *gyrB* sequences are not sufficiently similar to those of strains of the *Oceanospirillum* core group to be so assigned.

Fig. 1. Phylogenetic trees of the genus *Oceanospirillum* based on the nucleotide sequences of the 16S rRNA (a) and *gyrB* (b) genes. The trees were constructed by using the neighbour-joining method and nucleotide substitution rates (K_{nuc} values) were computed by Kimura's 2-parameter model. Bars, genetic distance (K_{nuc}). Numbers at nodes indicate the percentages of occurrence in 1000 bootstrapped trees. Only values greater than 40% are shown. *O.*, *Oceanospirillum*. *Terasakiella pusilla* was included as an outgroup.

Oceanospirillum jannaschii exhibited close relationships to *Marinobacterium georgiense*, *Pseudomonas iners* and *Pseudomonas stanieri*, with respective 16S rDNA similarity values of 93, 92 and 95%. These species formed clusters supported by bootstrapping resampling values of more than 99% in phylogenetic trees based on 16S rDNA and *gyrB* nucleotide sequences (Fig. 1). Although the phylogenetic distance between *Oceanospirillum jannaschii* and *Marinobacterium georgiense* was much larger than that between *Pseudomonas stanieri* and *Marinobacterium georgiense*, their DNA base compositions were the same (55 mol% G+C). This suggested that the four species were related as closely as members of the same genus. *Pseudomonas iners* and *Pseudomonas stanieri* were obviously misnamed, since these two species exhibited large phylogenetic distances from the type species of the genus *Pseudomonas* (*Pseudomonas aeruginosa*) and its related organisms based on 16S rDNA sequence analysis (Anzai *et al.*, 2000). According to the original descriptions of *Oceanospirillum jannaschii* (Bowditch *et al.*, 1984), *Marinobacterium georgiense* (González *et al.*, 1997), *Pseudomonas iners* (Iizuka & Komagata, 1964) and *Pseudomonas stanieri* (Baumann *et al.*, 1983), they are all Gram-negative, rod-shaped, motile and strictly aerobic bacteria and they also have

similar biochemical characteristics. Therefore, we propose that *Oceanospirillum jannaschii* and *Pseudomonas stanieri* be transferred to the genus *Marinobacterium* as *Marinobacterium jannaschii* comb. nov. and *Marinobacterium stanieri* comb. nov. The results of the present study also suggest that there is no significant genotypic or phenotypic difference to justify the separation of *Marinobacterium georgiense* and *Pseudomonas iners*. The sequence similarity values between *Marinobacterium georgiense* and *Pseudomonas iners* for 16S rDNA and *gyrB* were 99.7 and 98.5%, and the amino acid sequences of GyrB of the two species were identical. The DNA–DNA hybridization value between *Marinobacterium georgiense* and *Pseudomonas iners* was 80% (Table 2), which exceeds the value of 70% required to consider two strains to be members of the same species (Wayne *et al.*, 1987). Also, their physiological properties as tested by API 20E, API 20NE and API ZYM were confirmed to be identical except for citrate utilization (negative for *Pseudomonas iners*) and acid production from rhamnose (negative for *Marinobacterium georgiense*). According to the Rules of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), *Marinobacterium georgiense* and *Pseudomonas iners* should be unified as *Marinobacterium georgiense*.

Table 2. Levels of DNA–DNA reassociation among members of *Oceanospirillum* and related species

Source of unlabelled DNA	Relatedness (%) to labelled DNA from:				
	1	3	4	6	9
1. <i>Oceanospirillum linum</i> IFO 15448 ^T	100	24	20	25	NT
2. <i>Oceanospirillum linum</i> IFO 15449	94	NT	NT	25	NT
3. <i>Oceanospirillum maris</i> subsp. <i>maris</i> ATCC 27509 ^T	24	100	91	25	NT
4. <i>Oceanospirillum maris</i> subsp. <i>hiroshimense</i> IFO 13616 ^T	20	91	100	20	NT
5. <i>Oceanospirillum maris</i> subsp. <i>williamsae</i> IFO 15468 ^T	26	89	91	26	NT
6. <i>Oceanospirillum beijerinckii</i> subsp. <i>beijerinckii</i> IFO 15445 ^T	20	20	18	100	NT
7. <i>Oceanospirillum beijerinckii</i> subsp. <i>pelagicum</i> IFO 13612 ^T	16	19	18	89	NT
8. <i>Oceanospirillum multiglobuliferum</i> IFO 13614 ^T	22	17	13	15	NT
9. <i>Marinobacterium georgiense</i> ATCC 700074 ^T	NT	NT	NT	NT	100
10. <i>Pseudomonas iners</i> IAM 1419 ^T	NT	NT	NT	NT	80
11. <i>Pseudomonas stanieri</i> ATCC 27130 ^T	NT	NT	NT	NT	23
12. <i>Oceanospirillum jannaschii</i> IFO 15466 ^T	9	NT	NT	NT	13

NT, Not tested.

The 16S rDNA sequences of *Oceanospirillum japonicum*, *Oceanospirillum pusillum* and *Oceanospirillum kriegii* were compared with all the sequences available in the DDBJ. No sequence showed identity higher than 90% to these sequences. The phylogenetic tree based on the nucleotide sequences of 16S rDNA showed that *Oceanospirillum japonicum* did not cluster with any other known bacterial species (Fig. 1). Although the phylogenetic tree based on *gyrB* nucleotide sequences showed that *Oceanospirillum japonicum* and the species of *Marinospirillum* formed a cluster supported by a high bootstrapping value (98.9%) (Fig. 1), that based on amino acid sequences of GyrB translated from *gyrB* nucleotide sequence showed that this branch was only weakly supported, with a low bootstrapping value (44.6%; data not shown). Moreover, the phylogenetic distance between *Oceanospirillum japonicum* and the species of *Marinospirillum* was too large to combine them in the same genus (88 and 68% sequence similarity values for 16S rDNA and *gyrB* nucleotide sequences). These results indicate that the taxonomic position of *Oceanospirillum japonicum* is separate from that of the genus *Marinospirillum*. Likewise, *Oceanospirillum pusillum* and *Oceanospirillum kriegii* did not cluster with other known organisms within the α -Proteobacteria and γ -Proteobacteria, respectively. Kawasaki *et al.* (1997) also reported that *Oceanospirillum pusillum* did not have a suitable taxonomic position within the α -Proteobacteria. These results suggest that *Oceanospirillum japonicum*, *Oceanospirillum pusillum* and *Oceanospirillum kriegii* are clearly distinct from any previously described species at the genus level. Therefore, we propose to create new genera for them.

The present results, indicating the close genetic relationships between the subspecies of *Oceanospirillum maris* and those of *Oceanospirillum beijerinckii*, question the validity of the subspecies within these species.

The similarity values between the subspecies of each species for both 16S rDNA and *gyrB* nucleotide sequences were greater than 99%. Moreover, the deduced amino acid sequences of GyrB translated from *gyrB* nucleotide sequences were identical for the subspecies of each species. Yamamoto & Harayama (1995, 1996, 1998) reported that the resolution of the *gyrB* nucleotide sequence was much higher than that of 16S rDNA because of fast nucleotide substitution caused by the mutation of third codon positions. Although a unified genetic criterion for creating subspecies needs to be established, it is likely that the significant sequence similarity (more than 99%) of the *gyrB* nucleotide sequences corresponds to a genetic distance much closer than that of subspecies. This notion was supported by the results of DNA–DNA hybridization (Table 2). The hybridization value between *Oceanospirillum beijerinckii* subsp. *beijerinckii* and *Oceanospirillum beijerinckii* subsp. *pelagicum* was 89% while, between the *Oceanospirillum maris* subspecies, it was also more than 89%. Pot *et al.* (1989) also reported the close relationships of these subspecies based on whole-cell protein electrophoresis patterns. Pot *et al.* (1989, 1992), however, retained these subspecies, citing some differences between these subspecies in the phenotypic data determined previously. These included the optimum temperature for growth, nutrient requirements, the utilization of organic acids, growth in the presence of 1% glycine and some enzymic activities in *Oceanospirillum maris* and the range of temperature and NaCl concentration for growth and the utilization of organic compounds in *Oceanospirillum beijerinckii*. In this study, we have performed a fresh comparative analysis of the phenotypes of these species, including the ranges of temperature and NaCl concentration for growth and tests using API 20E, 20NE and API ZYM. The subspecies of *Oceanospirillum maris* could be differentiated by the optimum temperature for growth and tolerance of 1%

Table 3. Taxonomic characteristics of marine bacteria related to the genus *Oceanospirillum*

Data other than DNA G+C contents and isoprenoid quinone types were taken from this study unless otherwise indicated. Taxa are listed as: 1, *Marinobacterium georgiense*; 2, *Marinobacterium jamaaschii*; 3, *Marinobacterium stantieri*; 4, *Marinomonas* spp. (data from Holt *et al.*, 1994); 5, *Marinospirillum minutulum* (Satomi *et al.*, 1998); 6, *Marinospirillum megaterium* (Satomi *et al.*, 1998); 7, *Oceanobacter kriegii* (Bowditch *et al.*, 1984); 8, *Oceanospirillum linum* (Williams & Rittenberg, 1957); 9, *Oceanospirillum beijeinckii*; 10, *Oceanospirillum maris*; 11, *Oceanospirillum multiglobuliferum* (Terasaki, 1973); 12, *Pseudospirillum japonicum* (Watanabe, 1959); 13, *Terasakiella pusilla* (Terasaki, 1973). +, Positive reaction; –, negative reaction; v, variable; ND, no data. All taxa are motile.

Characteristic	1	2	3	4	5	6	7	8	9	10	11	12	13
Morphology*	Rods	Rods	Rods	Rods	Spirilla/c	Spirilla/c	Rods	Spirilla/c	Spirilla/c	Spirilla/c	Spirilla/c	Rods-Spirilla/c	Spirilla/c
Flagella	Single polar	Single polar	Single polar	Single polar	Polar tuft	Polar tuft	Single polar	Bipolar tufts	Bipolar tufts	Bipolar tufts	Bipolar tufts	Bipolar tufts	Polar tuft
O ₂ requirement	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Microaerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic	Aerobic
Catalase	–	–	–	–	+	+	–	+	+	+	+	–	+
Oxidase	+	+	+	+	+	+	+	+	+	+	+	v	v
Reduction of nitrate	v	+	+	v	+	+	+	+	+	+	+	+	+
Gelatin hydrolysis	–	–	–	v	–	–	–	–	–	–	–	–	–
PHB accumulation	–	–	–	–	–	–	–	–	–	–	–	–	–
DNA G+C content (mol %) [†]	55 ^a	55	55 ^b	44–48	43	45 ^c	55	48	47	46	45	44–45	48
Isoprenoid quinone type [†]	Q-8 ^d	Q-8	Q-8 ^d	Q-8 ^e	Q-8	Q-8 ^e	Q-8	Q-8	Q-8	Q-8	Q-8	Q-8	Q-10
Subclass on 16S rRNA	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	γ	α

*C, Clockwise helix; AC, anticlockwise helix.

[†]Data were taken from Sakane & Yokota (1994) unless indicated as follows: a, González *et al.* (1997); b, Baumann *et al.* (1983); c, Satomi *et al.* (1998); d, this study; e, Akagawa-Matsushita *et al.* (1992).

glycine, in accordance with the previous data (Pot *et al.*, 1989). Likewise, the subspecies of *Oceanospirillum beijeinckii* could be differentiated by their ranges of temperature and NaCl for growth, in accordance with the previous data (Pot *et al.*, 1989). Other physiological traits tested were identical for the subspecies of each species. Various identical characters of the subspecies of *Oceanospirillum maris* revealed in this study, such as the production of catalase (negative in all strains), DNase (negative in all strains) and phosphatase (positive in all strains), were originally shown to be different and were used to differentiate them (Pot *et al.*, 1989). Likewise, characters identical for the subspecies of *Oceanospirillum beijeinckii* such as the utilization of DL-malate and citrate (positive in all strains) were originally shown to differ and were used to differentiate them (Pot *et al.*, 1989). These differences from the previous data are probably due to differences in researchers and methods. At the level of the physiological differences described above, the basis for retaining the subspecies is questioned, since a considerable difference in phenotypes among strains of a species could occur with the current definition of a species (Wayne *et al.*, 1987). Since strains with approximately 70% DNA–DNA relatedness will logically have at least 2–4% DNA sequence difference (10⁵ nucleotides) (Stackebrandt & Goebel, 1994), population surveys using more strains should be carried out in future to demonstrate the presence of phenotypically distinct groups within *Oceanospirillum maris* and *Oceanospirillum beijeinckii*. Therefore, at present, we propose that the subspecies of *Oceanospirillum maris* (*O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense* and *O. maris* subsp. *williamsae*) and *Oceanospirillum beijeinckii* (*O. beijeinckii* subsp. *beijeinckii* and *O. beijeinckii* subsp. *pelagicum*) should be rejected.

Differential characteristics of marine bacteria related to the genus *Oceanospirillum* are shown in Table 3.

Definition of *Oceanospirillum sensu stricto*

From our phylogenetic studies, we conclude that it is logical to eliminate four misplaced *Oceanospirillum* species, *Oceanospirillum pusillum*, *Oceanospirillum jamaaschii*, *Oceanospirillum japonicum* and *Oceanospirillum kriegii*, from the genus *Oceanospirillum* and to eliminate the subspecies within each of the species *Oceanospirillum maris* and *Oceanospirillum beijeinckii*. Thus, we propose to define the genus *Oceanospirillum sensu stricto* as including four species, *Oceanospirillum linum*, *Oceanospirillum maris*, *Oceanospirillum beijeinckii* and *Oceanospirillum multiglobuliferum*. The subspecies of *Oceanospirillum maris* (*O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense* and *O. maris* subsp. *williamsae*) and *Oceanospirillum beijeinckii* (*O. beijeinckii* subsp. *beijeinckii* and *O. beijeinckii* subsp. *pelagicum*) should be rejected.

The definitions of the species *Oceanospirillum beijeinckii* and *Oceanospirillum maris* remain as their original descriptions (Williams & Rittenberg, 1957; Hylemon

et al., 1973) and the original descriptions of *Oceanospirillum linum* (Williams & Rittenberg, 1957) and *Oceanospirillum multiglobuliferum* (Terasaki, 1979) are also unchanged.

Emended description of *Oceanospirillum* Hylemon *et al.* 1973^{AL}

Oceanospirillum [O.ce.an.o.spi.ril'lum. Gr. n. *okeanos* the ocean; N.L. dim. neut. n. *spirillum* a small spiral from Gr. n. *spira* spiral; N.L. *Oceanospirillum* a small spiral organism from the ocean (sea water)].

Rigid, helical cells with clockwise helix. Cells 0.4–1.2 µm in diameter; length of the helix, 2.0–40 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 (PHB) is formed. All species form thin-walled coccoid bodies, which predominate in old cultures. Gram-negative. Motile by bipolar tufts of flagella. Chemo-organotrophic, 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, 25–32 °C. Oxidase-positive. Indole-negative. Casein, starch, hippurate and aesculin are not hydrolysed. Sea water 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 sea water, from decaying seaweed and from putrid infusions of marine mussels. The G+C content of the genomic 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 *et al.* 1973^{AL}.

Description of *Pseudospirillum* gen. nov.

Pseudospirillum (Pseu.do.spi.ril'lum. Gr. adj. *pseudes* false; N.L. n. *Spirillum* genus of spiral-shaped bacteria; N.L. n. *Pseudospirillum* false *Spirillum*).

Gram-negative, curved, straight or S-shaped, non-spore-forming, halophilic, aerobic, chemo-heterotrophic and PHB-accumulating bacteria. Motile by means of flagella. Oxidase-positive. Catalase-negative or -positive reaction. Carbohydrates are not catabolized. Genomic DNA G+C content of 45 mol% (as determined by HPLC). The isoprenoid quinone type is Q-8. The type species is *Pseudospirillum japonicum* comb. nov. (basonym *Oceanospirillum japonicum*).

Description of *Pseudospirillum japonicum* comb. nov.

Basonym: *Oceanospirillum japonicum* (Watanabe 1959).

The type strain is ATCC 19191^T. The original description of the species given by Watanabe (1959) is unchanged.

Description of *Oceanobacter* gen. nov.

Oceanobacter (O.ce.a.no.bac'ter. Gr. n. *okeanos* the ocean; L. masc. n. *bacter* rod; N.L. masc. n. *Oceanobacter* rod of the sea).

Gram-negative, straight rod-shaped, non-spore-forming, halophilic, aerobic, chemo-heterotrophic and PHB-accumulating bacteria. Motile by means of flagella. Oxidase-positive. Catalase-positive reaction. Carbohydrates are not catabolized. Nitrate is reduced to nitrite. Genomic DNA G+C content of 55 mol% (as determined by HPLC). The isoprenoid quinone type is Q-8. The type species is *Oceanobacter kriegii* comb. nov. (basonym *Oceanospirillum kriegii*).

Description of *Oceanobacter kriegii* comb. nov.

Basonym: *Oceanospirillum kriegii* (Bowditch *et al.* 1984).

The type strain is ATCC 27133^T. The original description of the species given by Bowditch *et al.* (1984) is unchanged.

Description of *Terasakiella* gen. nov.

Terasakiella (Te.ra.sa.ki.el'la. L. dim. ending -ella; N.L. fem. n. *Terasakiella* named to honour Y. Terasaki, the Japanese microbiologist, who has made many contributions to our understanding of the classification and identification of spiral-shaped bacteria).

Gram-negative, rigid, helical cells with anticlockwise helix. Non-spore-forming, coccoid body-forming, halophilic, aerobic, chemo-heterotrophic and PHB-accumulating bacteria. Motile by means of flagella. Oxidase-positive. Catalase-negative or -positive reaction. Carbohydrates are not catabolized. Nitrate is reduced to nitrite. Genomic DNA G+C content of 48 mol% (as determined by HPLC). The isoprenoid quinone type is Q-10. The type species is *Terasakiella pusilla* comb. nov. (basonym *Oceanospirillum pusillum*).

Description of *Terasakiella pusilla* comb. nov.

Basonym: *Oceanospirillum pusillum* (Terasaki 1979).

The type strain is IFO 13613^T. The original description of the species given by Terasaki (1973) is unchanged.

Description of *Marinobacterium jannaschii* comb. nov.

Basonym: *Oceanospirillum jannaschii* (Bowditch *et al.* 1984).

The type strain is ATCC 27135^T. Physiological, chemotaxonomic and phylogenetic data indicate that *Oceanospirillum jannaschii* is more closely related to *Marinobacterium georgiense* than to *Oceanospirillum linum*, the type species of the genus *Oceanospirillum*. The original description of the species given by Bowditch *et al.* (1984) is unchanged.

Description of *Marinobacterium stanieri* comb. nov.

Basonym: *Pseudomonas stanieri* (Baumann *et al.* 1983).

The type strain is ATCC 27130^T. Physiological, chemotaxonomic and phylogenetic data indicate that *Pseudomonas stanieri* is more closely related to *Marinobacterium georgiense* than to *Pseudomonas aeruginosa*, the type species of the genus *Pseudomonas*. The original description of the species given by Baumann *et al.* (1983) is unchanged.

Emended description of *Marinobacterium georgiense*

Physiological, chemotaxonomic and phylogenetic data indicate that *Marinobacterium georgiense* should include strains formerly classified as *Pseudomonas iners* Iizuka and Komagata 1964. The original description of *Marinobacterium georgiense* given by González *et al.* (1997) is unchanged. The type strain is ATCC 700074^T.

ACKNOWLEDGEMENTS

The authors thank Dr K. Venkateswaran for helpful discussions and Dr Y. Terasaki for providing us with bacterial strains and for his useful advice. This study was partly supported by a Grant-in-Aid from the Ministry of Education, Science, Sports and Culture of Japan (B 09460094).

REFERENCES

- Akagawa-Matsushita, M., Ito, T., Katayama, Y., Kuraishi, H. & Yamasato, K. (1992). Isoprenoid quinone composition of some marine *Alteromonas*, *Marinomonas*, *Deleya*, *Pseudomonas* and *Shewanella* species. *J Gen Microbiol* **138**, 2275–2281.
- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990). Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Anzai, Y., Kim, H., Park, J.-Y., Wakabayashi, H. & Oyaizu, H. (2000). Phylogenetic affiliation of the pseudomonads based on 16S rRNA sequence. *Int J Syst Evol Microbiol* **50**, 1563–1589.
- Baumann, L., Baumann, P., Mandel, M. & Allen, R. D. (1972). Taxonomy of aerobic marine eubacteria. *J Bacteriol* **110**, 402–429.
- Baumann, P., Bowditch, R. D., Baumann, L. & Beaman, B. (1983). Taxonomy of marine *Pseudomonas* species: *P. stanieri* sp. nov.; *P. perfectomarina* sp. nov., nom. rev.; *P. nautica*; and *P. doudoroffii*. *Int J Syst Bacteriol* **33**, 857–865.
- Bowditch, R. D., Baumann, L. & Baumann, P. (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.
- Edgell, D. R., Klenk, H.-P. & Doolittle, W. F. (1997). Gene duplications in evolution of archaeal family B DNA polymerases. *J Bacteriol* **179**, 2632–2640.
- Ezaki, T., Hashimoto, Y. & Yabuuchi, E. (1989). Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int J Syst Bacteriol* **39**, 224–229.
- González, J. M., Mayer, F., Moran, M. A., Hodson, R. E. & Whitman, W. B. (1997). *Microbulbifer hydrolyticus* gen. nov., sp. nov., and *Marinobacterium georgiense* gen. nov., sp. nov., two marine bacteria from a lignin-rich pulp mill waste enrichment community. *Int J Syst Bacteriol* **47**, 369–376.

- Hamana, K., Sakane, T. & Yokota, A. (1994). Polyamine analysis of the genera *Aquaspirillum*, *Magnetospirillum*, *Oceanospirillum*, and *Spirillum*. *J Gen Appl Microbiol* **40**, 75–82.
- Holt, J. G., Krieg, N. R., Sneath, P. H. A., Staley, J. T. & Williams, S. T. (editors). (1994). *Bergey's Manual of Determinative Bacteriology*, 9th edn. Baltimore: Williams & Wilkins.
- Hylemon, P. B., Wells, J. S., Jr, Krieg, N. R. & Jannasch, H. W. (1973). The genus *Spirillum*: a taxonomic study. *Int J Syst Bacteriol* **23**, 340–380.
- IFO (1996). List of media. In *List of Cultures. Microorganisms*, 10th edn, p. 515. Edited by Institute for Fermentation, Osaka (IFO). Osaka: Institute for Fermentation.
- Iizuka, H. & Komagata, K. (1964). Microbiological studies on petroleum and natural gas. II. Determination of pseudomonads isolated from oil-brines and related materials. *J Gen Appl Microbiol* **10**, 223–231.
- Kawasaki, H., Yamasato, K. & Sugiyama, J. (1997). Phylogenetic relationships of the helical-shaped bacteria in the alpha Proteobacteria inferred from 16S rDNA sequences. *J Gen Appl Microbiol* **43**, 89–95.
- Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* **16**, 111–120.
- Lapage, S. P., Sneath, P. H. A., Lessel, E. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria (1990 Revision). Bacteriological Code*. Washington, DC: American Society for Microbiology.
- Linn, D. M. & Krieg, N. R. (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. Request for an Opinion. *Int J Syst Bacteriol* **28**, 132–138.
- Morse, R., Collins, M. D., O'Hanlon, K., Wallbanks, S. & Richardson, P. T. (1996). Analysis of the β' subunit of DNA-dependent RNA polymerase does not support the hypothesis inferred from 16S rRNA analysis that *Oenococcus oeni* (formerly *Leuconostoc oenos*) is a tachytelic (fast-evolving) bacterium. *Int J Syst Bacteriol* **46**, 1004–1009.
- Pot, B., Gillis, M., Hoste, B., Van De Velde, A., Bekaert, F., Kersters, K. & De Ley, J. (1989). Intra- and intergeneric relationships of the genus *Oceanospirillum*. *Int J Syst Bacteriol* **39**, 23–34.
- Pot, B., Gillis, M. & De Ley, J. (1992). The genus *Oceanospirillum*. In *The Prokaryotes: a Handbook on the Biology of Bacteria – Ecophysiology, Isolation, Identification, Applications*, 2nd edn, vol. 4, pp. 3230–3236. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. New York: Springer.
- Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Sakane, T. & Yokota, A. (1994). Chemotaxonomic investigation of heterotrophic, aerobic and microaerophilic spirilla, the genera *Aquaspirillum*, *Magnetospirillum*, and *Oceanospirillum*. *Syst Appl Microbiol* **17**, 128–134.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Satomi, M., Kimura, B., Mizoi, M., Sato, T. & Fujii, T. (1997). *Tetragenococcus muriaticus* sp. nov., a new moderately halophilic lactic acid bacterium isolated from fermented squid liver sauce. *Int J Syst Bacteriol* **47**, 832–836.
- Satomi, M., Kimura, B., Hayashi, M., Shouzen, Y., Okuzumi, M. & Fujii, T. (1998). *Marinospirillum* gen. nov., with descriptions of *Marinospirillum megaterium* sp. nov., isolated from kusaya gravy, and transfer of *Oceanospirillum minutulum* to *Marinospirillum minutulum* comb. nov. *Int J Syst Bacteriol* **48**, 1341–1348.
- Stackebrandt, E. & Goebel, B. M. (1994). Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. *Int J Syst Bacteriol* **44**, 846–849.
- Takewaki, S., Okuzumi, K., Manabe, I., Tanimura, M., Miyamura, K., Nakahara, K., Yazaki, Y., Ohkubo, A. & Nagai, R. (1994). Nucleotide sequence comparison of the mycobacterial *dnaJ* gene and

PCR-restriction fragment length polymorphism analysis for identification of mycobacterial species. *Int J Syst Bacteriol* **44**, 159–166.

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.

Terasaki, Y. (1973). Studies on the genus *Spirillum* Ehrenberg. II. Comments on type and reference strains of *Spirillum* and description of new species and subspecies. *Bull Suzugamine Women's Coll Nat Sci* **17**, 1–71.

Terasaki, Y. (1975). Freeze-dried cultures of water spirilla made on an experimental basis. *Bull Suzugamine Women's Coll Nat Sci* **19**, 1–10.

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.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Viale, A. M., Arakaki, A. K., Soncini, F. C. & Ferreyra, R. G. (1994). Evolutionary relationships among eubacterial groups as inferred from

GroEL (chaperonin) sequence comparisons. *Int J Syst Bacteriol* **44**, 527–533.

Watanabe, N. (1959). On four new halophilic species of *Spirillum*. *Bot Mag (Tokyo)* **72**, 77–86.

Wayne, L. G., Brenner, D. J., Colwell, R. R. & 9 other authors (1987). Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Williams, M. A. & Rittenberg, S. C. (1957). A taxonomic study of the genus *Spirillum* Ehrenberg. *Int Bull Bacteriol Nomencl Taxon* **7**, 49–111.

Yamamoto, S. & Harayama, S. (1995). PCR amplification and direct sequencing of *gyrB* genes with universal primers and their application to the detection and taxonomic analysis of *Pseudomonas putida* strains. *Appl Environ Microbiol* **61**, 1104–1109.

Yamamoto, S. & Harayama, S. (1996). Phylogenetic analysis of *Acinetobacter* strains based on the nucleotide sequences of *gyrB* genes and on the amino acid sequences of their products. *Int J Syst Bacteriol* **46**, 506–511.

Yamamoto, S. & Harayama, S. (1998). Phylogenetic relationships of *Pseudomonas putida* strains deduced from the nucleotide sequences of *gyrB*, *rpoD* and 16S rRNA genes. *Int J Syst Bacteriol* **48**, 813–819.