Family I. **Oceanospirillaceae** *fam. nov.*

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O.ce.an.o.spi.ril.la' ce.ae. M.L. neut. n. *Oceanospirillum* type genus of the family; *-aceae* ending to denote family; M.L. fem. pl. n. *Oceanospirillaceae* the *Oceanospirillum* family.

The family *Oceanospirillaceae* was circumscribed for this volume on the basis of phylogenetic analysis of 16S rDNA sequences; the family contains the genera *Oceanospirillum* (type genus), *Balneatrix*, *Marinomonas*, *Marinospirillum*, *Neptunomonas*, *Oceanobacter*, *Oleispira*, and *Pseudospirillum*. *Oceanobacter*, *Pseudospirillum* and *Oleispira* were proposed after the cut-off date for inclusion in this volume (June 30, 2001) and are not described here (see Satomi et al. (2002) and Yakimov et al. (2003a), respectively).

Motile by polar flagella. Aerobic; strictly respiratory except for *Neptunomonas*, which gives weak fermentation reactions. Aquatic; *Balneatrix* is found in fresh water, whereas other genera are marine.

Type genus: **Oceanospirillum** Hylemon, Wells, Krieg and Jannasch 1973, 361[AL].

Genus I. **Oceanospirillum** *Hylemon, Wells, Krieg and Jannasch 1973, 361[AL]**

BRUNO POT AND MONIQUE GILLIS

O.ce.an.o.spi.ril' lum. M.L. n. *oceanus* ocean; Gr. n. *spira* a spiral; M.L. dim. neut. n. *spirillum* spirillum a small spiral; *Oceanospirillum* a small spiral (organism) from the ocean (seawater).

Rigid, helical cells 0.4–1.2 μm in diameter. Motile by bipolar tufts of flagella. A polar membrane underlies the cytoplasmic membrane at the cell poles in all species so far examined by electron microscopy. **Intracellular poly-β-hydroxybutyrate is formed. All species form thin-walled coccoid bodies that predominate in old cultures.** Gram negative. **Aerobic**, having a strictly respiratory type of metabolism with oxygen as the terminal electron acceptor. Nitrate respiration does not occur. Nitrate can be reduced to nitrite in all oceanospirilla. Optimum temperature for growth, 25–32°C. **Oxidase positive.** Indole and aryl sulfatase negative. Casein, starch, hippurate, and esculin are not hydrolyzed. **Seawater is required for growth. Carbohydrates are neither oxidized nor fermented.** Amino acids or the salts of organic acids serve as carbon sources. Growth factors are not usually required. Isolated from coastal seawater, decaying seaweed, and putrid infusions of marine mussels.

The mol% G + C of the DNA is: 45–50.

Type species: **Oceanospirillum linum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374 (*Spirillum linum* Williams and Rittenberg 1957, 82.)

FURTHER DESCRIPTIVE INFORMATION

All species of *Oceanospirillum* consist of helical cells; however, variants having less curvature may arise after prolonged transfer. For example, the type strain of *O. japonicum* consisted initially of long, helical cells with several turns (Watanabe, 1959), but now consists of slightly curved or S-shaped cells. The cells have a constant and characteristic type of clockwise (right-handed) helix. Only one phylogenetically unrelated species (*O. pusillum*) has a counterclockwise (left-handed) helix (Terasaki, 1972). Photographs showing the size and shape of various species of oceanospirilla and *O. minutulum* (now *Marinospirillum minutulum*) are presented in Fig. BXII.γ.104.

An unusual elaboration of the plasma membrane, the "polar membrane", occurs in all of the species so far examined (Beveridge and Murray, unpublished results). It is attached to the inside of the plasma membrane by bar-like links and is located, most commonly, in the region surrounding the polar flagella (Murray and Birch-Andersen, 1963). Such a membrane has been found mainly in genera of helical bacteria, such as *Spirillum*, *Campylobacter*, *Aquaspirillum*, *Ectothiorhodospira*, and *Rhodospirillum*.

All species have intracellular poly-β-hydroxybutyrate, but granules may not be evident in cells having a small diameter and chemical analysis may be required to demonstrate the polymer.

All species have bipolar tufts of flagella and all species show extensive formation of coccoid bodies (sometimes termed "microcysts") in old cultures. These bodies have thin walls and resemble spheroplasts; however, they are resistant to lysis in distilled water (Kelly, 1959). Whether coccoid bodies are resistant to desiccation is not known. Three main modes of formation of coccoid bodies were described by Williams and Rittenberg (1957), as follows: (a) two cells may entwine and apparently fuse. The cells become shorter and thicker and a protuberance develops at the point of fusion. This gradually enlarges and absorbs the organisms to form the coccoid body. More than one coccoid body may develop from a pair of entwined spirilla; (b) a spirillum may become shorter and thicker and a protuberance arises from the center of the cell or from each end of the cell. The protuberances enlarge and eventually merge into a single coccoid body as the helical cell is absorbed; (c) a spirillum may undergo a gradual shortening and rounding to form a coccoid body. The majority of coccoid bodies present in old cultures appears to be viable and can "germinate" when placed into a fresh medium (Williams and Rittenberg, 1956). Germination is by unipolar or bipolar growth of a helical cell from the coccoid body, with the latter being absorbed into the developing helical cell.

Seawater is required for the growth of all species. Media prepared with natural seawater or with 2.75% NaCl have been used for enrichment and isolation (Williams and Rittenberg, 1957; Terasaki, 1963, 1970, 1980). Commonly used culture media for

**Editorial Note:* The genus *Oceanospirillum* has recently undergone taxonomic re-evaluation. *O. minutulum* has been transferred to the genus *Marinospirillum* as *Marinospirillum minutulum* (Satomi et al., 1998); see the Taxonomic Comments section in this chapter. *O. commune* and *O. vagum* are homotypic synonyms of *Marinomonas communis* and *Marinomonas vaga*, respectively. After the cut-off date for inclusion of taxonomic changes in this volume of the *Systematics*, Satomi et al. (2002) emended the description of *Oceanospirillum* and proposed the transfer of *O. jannaschii* to the genus *Marinobacterium* as *Marinobacterium jannaschii*, the transfer of *O. japonicum* to the new genus *Pseudospirillum* as *Pseudospirillum japonicum*, the transfer of *O. kriegii* to the new genus *Oceanobacter* as *Oceanobacter kriegii*, and the transfer of *O. pusillum* to the new genus *Terasakiella* as *Terasakiella pusilla*.

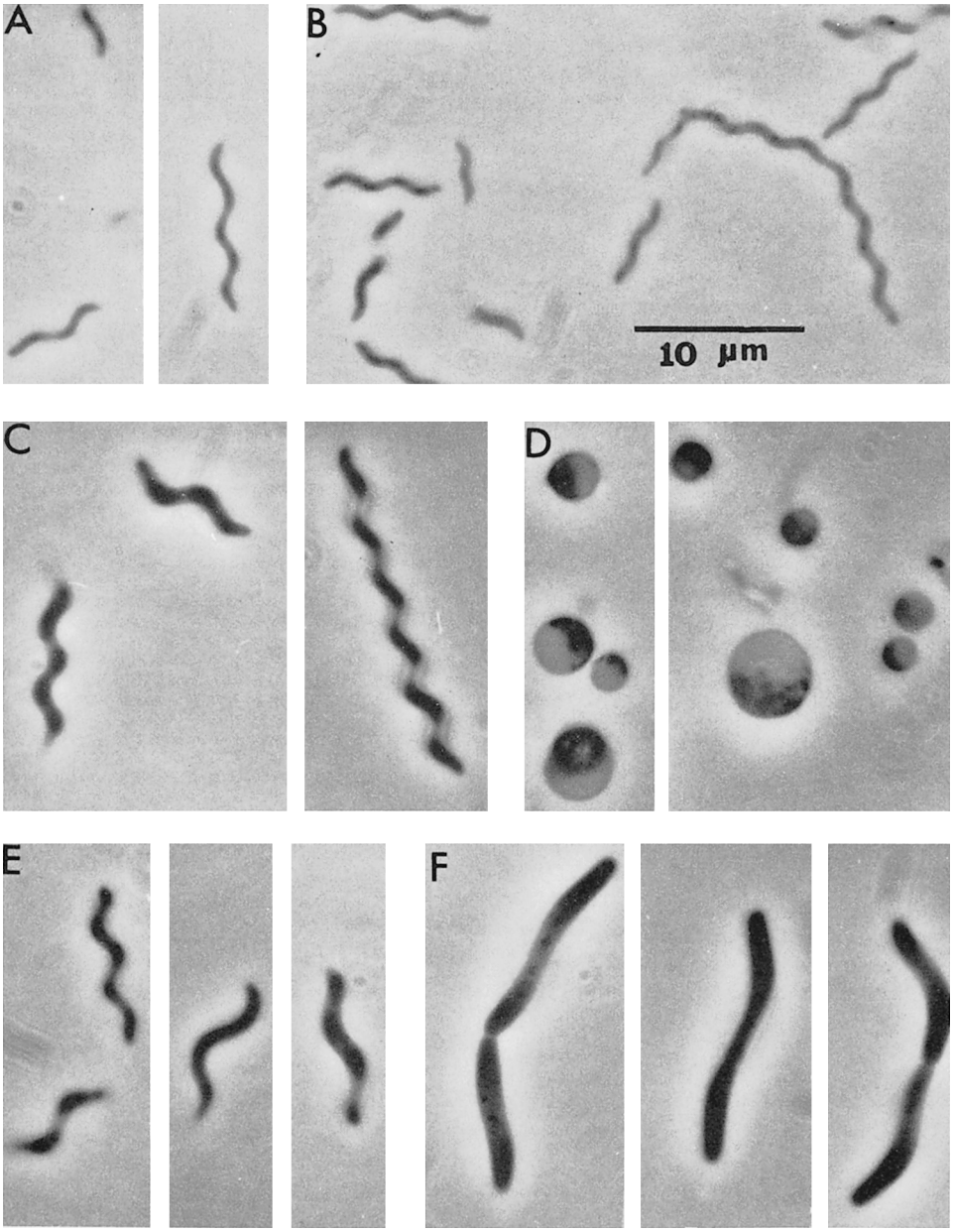

FIGURE BXII.γ.104. Phase contrast photomicrographs of several species of the genus *Oceanospirillum*. All photomicrographs were taken at the same magnification. *A*, *Marinospirillum minutulum* ATCC 19193. *B*, *O. linum* ATCC 11336. *C*, *O. maris* ATCC 27509. *D*, coccoid bodies of *O. maris* formed after 7 d of incubation. *E*, *O. beijerinckii* subsp. *beijerinckii* ATCC 12754. *F*, *O. japonicum* ATCC 19191. Reproduced with permission from N.R. Krieg, Bacteriological Reviews, *40*: 55–115, 1976, ©American Society for Microbiology.)

oceanospirilla are nutrient broth prepared with natural seawater and PSS, or MPSS broth[1] prepared with artificial seawater[2].

Oceanospirilla generally produce moderate to abundant, turbid growth in 2–3 d in PSS seawater broth (Hylemon et al., 1973). In seawater-nutrient broth, membranous masses are often formed at the surface and can be dispersed with shaking to yield turbid cultures (Terasaki, 1972).

Colonies of oceanospirilla generally develop within 2–3 d on PSS seawater agar and are usually white, circular, and convex, ranging from pinpoint to 1.5 mm in diameter (Hylemon et al., 1973). Colonies on seawater-nutrient agar are generally pinpoint in size at 48 h but become larger (up to 2.0 mm in diameter) at 7 d; they are usually convex or umbonate, glistening, opaque, pale yellow, and butyrous (Terasaki, 1972). Rough (R) colonies may arise on prolonged transfer; for example, the colonies of the type strain of *O. japonicum* are presently of the R type. Some species produce a water-soluble, yellow-green fluorescent pigment on PSS seawater agar.

Most species grow best at a temperature of 30–32°C; however, *O. maris* subsp. *hiroshimense* grows best at 25°C (Terasaki, 1972).

1. See the genus *Aquaspirillum* for recipes for these media.

2. Artificial sea water for use in PSS broth, g/l of distilled water: NaCl, 27.5; $MgCl_2$, 50; $MgSO_4$, 2.0; $CaCl_2$, 0.5; KCl, 1.0; and $FeSO_4$, 0.01.

The nutrition of oceanospirilla is generally simple. Most species grow in simple defined media with amino acids or the salts of organic acids as carbon sources and ammonium ions as the nitrogen source. However, *O. linum* is specifically stimulated by methionine in a medium containing succinate and malate as carbon sources, and *O. maris* subsp. *williamsae* has a growth factor requirement that has not yet been identified. A listing of the carbon sources for oceanospirilla is given below in Table BXII.γ.77. Some apparent contradictions occur between the results obtained from different laboratories, although the results within each laboratory are reproducible. These differences are likely attributable to differences in definitions of what constitutes a positive growth response, and in some cases to the use of different strains.

The use of antisera in agglutination tests with a limited number of strains has indicated that the species of *Oceanospirillum* can be distinguished serologically (McElroy and Krieg, 1972). The antisera were prepared against whole cells and adsorbed with heated cells, leaving only antibodies against thermolabile antigens.

Oceanospirilla have been isolated from coastal seawater (Williams and Rittenberg, 1957), decaying seaweed (Jannasch, 1963), and putrid infusions of marine mussels (Terasaki, 1963, 1970, 1980). By direct microscopic counts of the bacteria present in clear and turbid seawaters near Port Aransas, Texas, Oppenheimer and Jannasch (1962) found that spirilla comprised only 0.1–2.5% of the total bacteria present. Whether oceanospirilla occur in the open sea is not known. Based on chemostat experiments, Jannasch (1963) suggested that the growth of oceanospirilla might be restricted to environments of higher nutrient concentration than is found in ordinary seawater, such as in zones surrounding decaying particulate matter. With regard to occurrence of oceanospirilla in putrid infusions of marine mussels, the source is most likely marine mud adherent to the mussels (Terasaki, 1970).

Enrichment and Isolation Procedures

The enrichment and isolation method used by Williams and Rittenberg (1957) is as follows. A seawater sample is mixed with an equal volume of Giesberger's base medium (NH_4Cl, 0.1%; K_2HPO_4, 0.05%; $MgSO_4$, 0.05%) plus 1.0% calcium lactate. After incubation and appearance of spirilla, a portion of the initial culture is sterilized and mixed with an equal volume of sterile Giesberger's medium lacking NH_4Cl. This mixture is then inoculated from the unsterilized portion of the initial culture. One to three subcultures done in this manner are sufficient to establish the spirilla as the predominant type. For isolation, the enrichment is diluted 1:100 to 1:100,000 with sterile seawater. The dilution bottles are shaken vigorously and allowed to stand at room temperature for 20 min to allow migration of spirilla to the surface of the dilution. Isolation is then accomplished by streaking the surface water onto a suitable agar medium such as nutrient agar prepared with seawater and containing 0.3% yeast autolysate. Plates are incubated at 30°C and after 24 h examined for distinctive, granular, umbonate or pulvinate colonies with a ground-glass appearance.

The method of Terasaki (1970) has yielded excellent results for the isolation of oceanospirilla from putrid infusions. Marine mussels are smashed with a hammer and placed in a Petri dish with a teaspoon of marine mud. Sterilized seawater is poured into the dish until the mussels sink completely in the solution. The infusion is incubated at 27–28°C and examined for the development of spirilla after 1, 2, 4, and 7 d. Isolation is accomplished by streaking dilutions onto suitable agar media.

For enrichment by use of continuous cultures, see Jannasch (1967).

Maintenance Procedures

Oceanospirilla may be maintained in semisolid PSS seawater medium (containing 0.15% agar to give a jelly-like consistency) at 30°C with weekly transfer (Hylemon et al., 1973). Cultures may also be maintained as stabs in seawater-nutrient agar at room temperature with monthly transfer (Terasaki, 1972).

Preservation is most easily accomplished by suspending a dense concentration of cells in seawater-nutrient broth containing 10% (v/v) dimethylsulfoxide, with subsequent freezing in liquid nitrogen. A method for freeze-drying oceanospirilla has been reported by Terasaki (1975).

Procedures for Testing Special Characters

Characterization methods for oceanospirilla have been described in detail by Terasaki (1972, 1979) and Hylemon et al. (1973). The comments given in this *Manual* for the genus *Aquaspirillum* also apply to the genus *Oceanospirillum*, except that media containing natural or artificial seawater must be used for all characterization tests.

Differentiation of the genus *Oceanospirillum* from other genera

See the genus *Aquaspirillum*, in Volume 2 Part C in this *Manual*, for characteristics of *Oceanospirillum* that distinguish the genus from other morphologically or physiologically similar genera.

Taxonomic Comments

In the eighth edition of *Bergey's Manual of Determinative Bacteriology* (Krieg, 1974), a single genus, *Spirillum*, contained all of the various aerobic and microaerophilic spirilla, including freshwater and marine species. However, the DNA base composition for the genus ranged from 38 to 65 mol% G + C and appeared to be unusually broad for a bacterial genus. Moreover, three groups were evident within the genus: (a) the aerobic, freshwater spirilla that could not tolerate 3% NaCl (mol% G + C 50–65); (b) the aerobic, marine spirilla that required seawater for growth (mol% G + C = 42–48); and (c) the large, microaerophilic spirilla that belong to the species *S. volutans* (mol% G + C = 38). Accordingly, Hylemon et al. (1973) divided the genus into the three genera *Spirillum*, *Aquaspirillum*, and *Oceanospirillum*, with the marine organisms comprising the latter genus. This subdivision was used in the first edition of *Bergey's Manual of Systematic Bacteriology* (Krieg, 1984a). Although this scheme proved useful for practical purposes, it was only gradually that the phylogenetic aspects of the three subdivisions were revealed.

In an analysis of the 16S rRNA oligonucleotide catalogs of the species *O. minutulum* (now *Marinospirillum minutulum*) and *Oceanospirillum maris*, Woese et al. (1982) found that both organisms belonged to group III of the phototrophic bacteria as defined by Gibson et al. (1979), but they were not closely related to each other. Later, Woese et al. (1985) studied three additional species of *Oceanospirillum*—*O. japonicum*, *O. linum*, and *O. beijerinckii*. These species, together with the families *Enterobacteriaceae* and *Vibrionaceae*, constituted the core of 'subgroup 3' of the *Gammaproteobacteria* (Stackebrandt et al., 1988).

An organism known as *"Spirillum lunatum"* (Williams and Rit-

TABLE BXII.γ.77. Carbon sources used by *Oceanospirillum* species and *Marinospirillum minutulum*

Characteristic	*O. linum*		*O. beijerinckii* subsp. *beijerinckii*		*O. beijerinckii* subsp. *pelagicum*	*O. maris* subsp. *maris*	*O. maris* subsp. *hiroshimense*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*		*O. kriegii*	*O. pusillum*	*M. minutulum*	
Method[a,b]	A[c]	B[d]	A	B	B[d]	A[e]	B	B	A[f]	A	B	A[f]	B	A	B
Carbon source:															
Citrate	−	−	−	−	d	−	−	+	+	−	−	+	+	−	−
Aconitate	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
Isocitrate	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
α-Ketoglutarate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	+	nd	d	nd
Succinate	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+
Fumarate	−	−	−	+	+	−	+	+	+	−	+	+	+	+	+
Malate	−	−	+	−	+	d	+	+	+	+	+	+	+	+	+
Oxaloacetate	−	nd	−	nd	nd	+	nd	nd	nd	+	nd	nd	nd	+	nd
Pyruvate	−	−	+	−	+	−	+	+	+	+	+	+	+	+	+
Lactate	−	−	−	−	+	−	+	+	+	+	+	+	+	+	+
Malonate	−	−	−	−	−	−	−	−	−	−	−	d	+	−	−
Tartrate	nd	−	nd	−	+	nd	+	−	−	nd	nd	−	−	d	−
Acetate	d	−	−	−	+	−	+	+	+	+	+	+	+	−	+
Propionate	−	−	−	−	+	−	+	+	+	−	+	+	+	+	+
Butyrate	nd	−	−	−	nd	nd	nd	+	+	nd	+	d	+	nd	+
Caproate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	d	nd	−	nd
β-Hydroxybutyrate	−	nd	−	nd	nd	−	nd	nd	+	−	nd	+	nd	−	nd
p-Hydroxybenzoate	−	nd	−	nd	d	−	d	nd	−	−	nd	+	nd	−	nd
Ethanol	−	−	−	−	d	−	d	−	+	−	−	+	−	−	−
n-Propanol	−	−	−	−	d	−	d	−	+	−	−	+	−	−	−
n-Butanol	−	−	−	−	−	−	−	−	d	−	−	+	−	−	−
Glycerol	−	−	−	−	nd	−	nd	−	−	−	−	−	+	−	−
L-Histidine	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
L-Tyrosine	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd
L-Phenylalanine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Alanine	−	nd	−	nd	nd	−	nd	nd	+	+	nd	+	nd	−	nd
L-Glutamate	−	nd	−	nd	nd	+	nd	nd	d	+	nd	d	nd	+	nd
L-Aspartate	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Glutamine	−	nd	−	nd	nd	−	nd	nd	nd	+	nd	nd	nd	d	nd
Asparagine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Proline	−	nd	−	nd	nd	d	nd	nd	+	−	nd	+	nd	+	nd
L-Hydroxyproline	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Ornithine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	−	nd	−	nd
L-Citruline	−	nd	−	nd	nd	−	nd	nd	+	−	nd	−	nd	−	nd
L-Arginine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	d	nd	−	nd
L-Lysine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
Putrescine	−	nd	−	nd	nd	−	nd	nd	+	−	nd	+	nd	−	nd
L-Methionine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
L-Serine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Cysteine	−	nd	−	nd	nd	−	nd	nd	nd	−	nd	nd	nd	−	nd
Glycine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Leucine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Isoleucine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Valine	−	nd	−	nd	nd	−	nd	nd	d	−	nd	−	nd	−	nd
L-Tryptophan	−	nd	−	nd	nd	−	nd	nd	−	−	nd	−	nd	−	nd

[a]Method A (Hylemon et al., 1973): A turbidimetrically standardized cell suspension in synthetic seawater was inoculated into a defined, vitamin-free medium containing the carbon sources (0.1%) and ammonium sulfate as the nitrogen source. Growth responses were measured turbidimetrically after one 72-h serial transfer from the initial cultures, using a Klett colorimeter with the blue (420 nm) filter and 16-mm cuvettes. Symbols: +, 10 or more Klett units of turbidity for all strains tested; −, less than 10 Klett units of turbidity; d, differs among strains; nd, not determined.

[b]Method B (Terasaki, 1972, 1979): A cell suspension washed in basal, defined, vitamin-free medium (Williams and Rittenberg, 1957) containing natural seawater and lacking carbon sources. The cells were inoculated into similar media containing the test compounds (0.05%) and ammonium chloride as the nitrogen source. After 7 d, growth was estimated turbidimetrically. Symbols: +, a turbidity of 0.025 absorbance units or greater for all strains tested; −, a turbidity of less than 0.025; d, differs among strains; nd, not determined.

[c]Strain ATCC 12753 failed to grow with any sole carbon source, while strain ATCC 11336 grew only with acetate. Both strains grew abundantly when succinate plus malate were supplied as carbon sources and L-methionine as the nitrogen source.

[d]Strain OF3 (Terasaki, 1972, 1973) differs from the results given in the table in that it grows with a large veriety of sole carbon sources: citrate, succinate, fumarate, malate, pyruvate, lactate, acetate, propionate, and butyrate. Whether this strain should be included in the species *O. linum* is uncertain.

[e]The results are given for *O. maris* subsp. *maris*. *O. maris* subsp. *williamsae* fails to grow with any sole carbon (or sole nitrogen) source and, therefore, appears to have an auxotrophic growth requirement. This requirement has not yet been defined.

[f]As reported by Bowditch et al. (1984a).

tenberg, 1957) was included in the genus *Oceanospirillum* by Hylemon et al. (1973), but this posed taxonomic problems. The characteristics of the type strain (ATCC 11337 or NCMB 54) did not fit the original description of the species, and Linn and Krieg (1978) found that NCMB strain 54 consisted of a mixture of two dissimilar organisms. The first type was a short, vibrioid rod that possessed a single polar flagellum, grew in either the presence or absence of seawater, catabolized sugars, did not form coccoid bodies, and had a mol% G + C of 63–64. The second type was a larger, helical organism that possessed bipolar flagellar tufts, required seawater, failed to attack sugars, formed coccoid bodies, and had a mol% G + C of 45. The smaller organism did not appear to belong to either *Oceanospirillum* or *Aquaspirillum* and it remains unclassified. The larger organism had characteristics more in accord with the original description of *"S. lunatum"* but differed in certain respects; it has been classified as a new subspecies of *O. maris*: *O. maris* subsp. *williamsae*.

Bowditch et al. (1984a, b) added four species to the genus *Oceanospirillum*, mainly based on immunological relationships. These species were *Oceanospirillum commune*, for the organism previously named *Marinomonas communis* (Van Landschoot and De Ley, 1983, 1984), *Oceanospirillum vagum* for *Marinomonas vaga* (Van Landschoot and De Ley, 1983, 1984), and two species *Oceanospirillum jannaschii* and *Oceanospirillum kriegii* for two groups of unnamed marine bacteria I-1 and H-1, respectively. As a result, the genus definition of *Oceanospirillum* needed to be changed drastically, with the unfortunate loss of most of the readily determinable phenotypic features from the genus definition (Krieg, 1984a) and the extension of the upper mol% G + C limit for the genus from 51 to 57. By this extension, a considerable overlap of mol% G + C range was introduced between the genera *Aquaspirillum* (49–65 mol% G + C) and *Oceanospirillum* (42–51 mol% G + C). In this way, one of the most reliable genotypic features discriminating both genera was lost. Phylogenetic data (Pot et al. 1989, Pot, 1996; Satomi et al., 1998), however, have since shown that all four species cannot be regarded as members of the genus *Oceanospirillum*.

On the basis of a polyphasic approach including DNA–DNA and DNA–rRNA hybridizations, Pot et al. (1989) showed that only five species, including the type species, constituted a separate rRNA branch in the *Gammaproteobacteria* and redefined the genus *Oceanospirillum* to contain *O. linum*, *O. maris*, *O. beijerinckii*, *O. multiglobuliferum*, and, more distantly, *O. japonicum*. Based on DNA–DNA hybridizations (as suggested by Krieg, 1984a) and numerical comparison of whole-cell proteins, *O. maris* subsp. *hiroshimense* and *O. beijerinckii* subsp. *pelagicum* were created for the former species *O. hiroshimense* and *O. pelagicum*. *O. pusillum* was shown to belong to the *Alphaproteobacteria*, and *O. commune* and *O. vagum* were relegated to their original generic positions as *Marinomonas communis* and *Marinomonas vaga*, respectively. The two species *O. jannaschii* and *O. kriegii* were shown to be phylogenetically too remote to be considered members of the genus *Oceanospirillum*, and, together with *O. minutulum*, they constituted separate rRNA branches in the *Gammaproteobacteria*.

Subsequently, this phylogenetic heterogeneity was confirmed by studies of fatty acid, quinone, and polyamine compositions (Hamana et al., 1994; Sakane and Yokota, 1994; Bertone et al., 1996). All species, except *O. pusillum*, contained ubiquinone-8 (Q-8) as a major respiratory quinone (Table BXII.γ.78). Like other spirilla from the *Alphaproteobacteria* (see the genus *Aquaspirillum* in this book), *O. pusillum* contained over 90% Q-10. The thirteen strains of *Oceanospirillum* that have been investigated for their fatty acid composition by Sakane and Yokota were divided into three groups (Table BXII.γ.79 and BXII.γ.80). Group I included the 10 strains belonging to *O. linum*, *O. maris* subsp. *hiroshimense*, *O. maris* subsp. *williamsae*, *O. beijerinckii* subsp. *beijerinckii*, *O. beijerinckii* subsp. *pelagicum*, *O. multiglobuliferum*, and *O. japonicum*, all of which have a low mol% G + C (42.5–48.4). Group II included the two type strains of *O. jannaschii* and *O. kriegii* and had a high mol% G + C content (54.8–54.9). Group III included only *O. pusillum* and could be clearly distinguished from other marine spirilla in having $C_{14:0\ 3OH}$ as the major 3-hydroxy fatty acid, besides Q-10 (Table BXII.γ.80). Bertone et al. (1996) confirmed the separate position of *O. japonicum*, *O. jannaschii*, and *O. kriegii*.

All *Oceanospirillum* species including *O. jannaschii* and *O. kriegii* contain both putrescine and spermidine. The relative content (Table BXII.γ.81) of putrescine is very small when compared with the level found in members of the *Alphaproteobacteria*. The relative concentration of putrescine for *O. pusillum* corresponds with that of other members of the *Alphaproteobacteria*. The absence of 2-hydroxy putrescine and homospermidine is a unifying character for the *Gammaproteobacteria*. The polyamine profile of *Oceanospirillum* I and II is not different, nor are their fatty acid profiles.

Later, 16S rDNA sequence analysis of all *Oceanospirillum* spe-

TABLE BXII.γ.78. Cellular quinone systems in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	Group	Quinone system				
			Q-6	Q-7	Q-8	Q-9	Q-10
O. linum	IFO 15448^T	Ic	3	4	91	2	
	IFO 15449	Ic	1	2	96	1	
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	Id	1	12	83	4	
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	Id	2	7	91	1	
O. maris subsp. *hiroshimense*	IFO 13616^T	Ic	1	4	94	1	
O. maris subsp. *williamsae*	IFO 15468^T	Ic	12	7	80	1	
O. multiglobuliferum	IFO 13614^T	Ic	1	4	94	1	
O. jannaschii	IFO 15466^T	IIb	3	7	89	1	
O. japonicum	IFO 15446^T	Ib	1	14	84	1	
	IFO 15447	Ib		9	88	3	
O. kriegii	IFO 15467^T	IIa	2	5	89	4	
O. pusillum	IFO 13613^T	III			1	6	93
Marinospirillum minutulum	IFO 15450^T	Ia	1	1	97	1	

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

TABLE BXII.γ.79. Cellular concentrations of non-polar fatty acids in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	Non-polar fatty acid[c]										
		$C_{12:0}$	$C_{12:1}$	$C_{14:0}$	$C_{14:1}$	$C_{15:0}$	$C_{16:0}$	$C_{16:1}$	$C_{17:0}$	$C_{17:1}$	$C_{18:0}$	$C_{18:1}$	$C_{20:0}$
O. linum	IFO 15448^T	3	2	1			16	48				30	
	IFO 15449	4	4	1			16	45				29	
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	4		4		1	32	50				9	
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	4		2	1		22	46				23	3
O. maris subsp. *hiroshimense*	IFO 13616^T	4	2	1			27	49			1	15	
O. maris subsp. *williamsae*	IFO 15468^T	4	4	2			31	47			1	11	
O. multiglobuliferum	IFO 13614^T	3	2	2			28	44				20	
O. jannaschii	IFO 15447	2		1			19	46			1	31	
O. japonicum	IFO 15466^T	3		1			25	57				14	
	IFO 15446^T	3		1			22	53				21	
O. kriegii	IFO 15467^T	7	4	1	1	2	16	36	2	3	1	27	
O. pusillum	IFO 13613^T	3		1	3		15	18			1	58	
Marinospirillum minutulum	IFO 15450^T	2			4		35	26				32	

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[c]The percentage of the acid relative to the total non-polar acids.

TABLE BXII.γ.80. Cellular concentrations of 2- and 3-hydroxy fatty acids in *Oceanospirillum* species and *Marinospirillum minutulum*[a]

Species[b]	Strain	3-hydroxy fatty acids[c]						2-hydroxy fatty acid[d]
		$C_{10:0}$	$C_{12:0}$	$C_{14:0}$	$C_{14:1}$	$C_{16:0}$	$C_{18:0}$	
O. linum	IFO 15448^T	100						−
	IFO 15449	100						−
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	63		30		6		−
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	60		30		9		−
O. maris subsp. *hiroshimense*	IFO 13616^T	100						−
O. maris subsp. *williamsae*	IFO 15468^T	100						−
O. multiglobuliferum	IFO 13614^T	100						−
O. jannaschii	IFO 15447	100						−
O. japonicum	IFO 15466^T	4	96					−
	IFO 15446^T	3	97					−
O. kriegii	IFO 15467^T	19	54			27		−
O. pusillum	IFO 13613^T			87		2	10	+ ($C_{18:1}$)
Marinospirillum minutulum	IFO 15450^T		61	3	36			−

[a]After Sakane and Yokota (1994).

[b]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[c]The percentage of the acid relative to the total 3-hydroxy acids.

[d]−, absent; +, present

cies confirmed the above findings. Kawasaki et al. (1997), in a phylogenetic study of helically shaped bacteria in the *Alphaproteobacteria*, showed that *O. pusillum* was not related to other taxa of spirilla and constituted a separate branch in the *Alphaproteobacteria*, also confirming previous findings of Woese et al. (1985) and Pot (1996). Therefore, *O. pusillum* cannot belong to the genus *Oceanospirillum* (Kawasaki et al., 1997). Phenotypic characteristics support the removal of *O. pusillum* from the genus: a single flagellum at each pole, a counterclockwise type of helix, and a mol% G + C of 51, which is slightly higher than the range of 42–48 for the rest of the genus. As a formal transfer has not been proposed, *O. pusillum* is therefore listed below as "Species assigned but phylogenetically not belonging in *Oceanospirillum*".

Satomi et al. (1998) determined and compared 16S rDNA sequences of all the *Oceanospirillum* species. They found that *O. linum*, *O. maris*, *O. beijerinckii*, and *O. multiglobuliferum* constituted a single rRNA cluster, separate from the branches formed by *Marinobacter*, *Marinobacterium*, and *Marinomonas*. Based on their findings, they also excluded *O. japonicum* from the genus *Oceanospirillum*. Phenotypically, *O. japonicum* is different from other *Oceanospirillum* species since it does not form coccoid bodies and its flagella appear to be crescent shaped with less than one helical turn, whereas those of other species have one or more helical turns. Moreover, *O. japonicum* grows best at 35–37°C. As a formal new description has not been proposed, *O. japonicum* is therefore listed below as a "Species assigned but phylogenetically not belonging in *Oceanospirillum*".

In the same study, it was shown that *O. minutulum* clustered on a separate branch together with new isolates from kusaya gravy (Satomi et al., 1998). For this branch, a new genus *Marinospirillum* has been proposed, containing the two species *M. minutulum* and *M. megaterium* (Satomi et al., 1998).

Compared to the other *Oceanospirillum* species, *O. jannaschii* and *O. kriegii* both have a higher mol% G + C (54.8–54.9) and occupy a separate phylogenetic position (Satomi et al., 1998). Many phenotypic characteristics discriminate these species from the genus *Oceanospirillum* (Table BXII.γ.82). Although not discussed separately by the authors, *O. jannaschii* occurred on the same branch as *Marinobacterium* (González et al., 1997). Further taxonomic research, including DNA–DNA hybridizations, should be performed to substantiate the exact level of genotypic relationship between *O. jannaschii* and *Marinobacterium georgiense*. *O. kriegii* constituted a separate branch in the 16S rRNA dendrogram (Satomi et al., 1998).

TABLE BXII.γ.81. Cellular concentrations of polyamines in *Oceanospirillum* and *Marinospirillum minutulum*[a,b,c]

Species[d]	Strain	Medium[e]	Dap	H-Put	Put	Cad	Spd	HSpd
O. linum	IFO 15448^T	199SW	–	–	0.01	–	0.65	–
	IFO 15449	199SW	–	–	0.02	–	0.80	–
O. beijerinckii subsp. *beijerinckii*	IFO 15445^T	199SW	–	–	0.01	–	0.64	–
O. beijerinckii subsp. *pelagicum*	IFO 13612^T	199S	–	–	0.06	–	0.48	–
		199SW	–	–	0.01	–	0.65	–
O. maris subsp. *hiroshimense*	IFO 13616^T	199S	–	–	0.02	–	0.86	–
		199SW	–	–	0.03	–	0.90	–
O. maris subsp. *williamsae*	IFO 15468^T	199SW	–	–	0.03	–	0.90	–
O. multiglobuliferum	IFO 13614^T	199S	–	–	0.08	–	0.40	–
		199SW	–	–	0.01	–	0.45	–
O. jannaschii	IFO 15466^T	199SW	–	–	0.02	–	0.80	–
O. japonicum	IFO 15446^T	199SW	–	–	0.01	–	1.11	–
	IFO 15447	199SW	–	–	0.01	–	1.23	–
O. kriegii	IFO 15467^T	199SW	–	–	0.03	–	0.84	–
O. pusillum	IFO 13613^T	199S	–	–	0.15	–	1.40	–
		199SW	–	–	0.24	–	1.19	–
Marinospirillum minutulum	IFO 15450^T	199SW	–	–	0.10	–	0.72	–

[a]Cells were harvested at stationary growth phase.

[b]After Hamana et al. (1994).

[c]Abbreviations: Dap, diaminopropane; H-Put, 2-hydroxyputrescine; Put, putrescine; Cad, cadaverine; Spd, spermidine; HSpd, homospermidine; –, not detectable (<0.005).

[d]*Oceanospirillum maris* subsp. *maris* has not been investigated.

[e]Media:199, polyamine-free growth medium from Flow Lab., Irvine, U.K., pH 7.0.; 199S, medium 199 dissolved in 70% synthetic seawater, pH 7.0; 199SW, medium 199 dissolved in seawater, pH 7.0.

Based on 16S rRNA gene sequence analysis, the genus *Oceanospirillum* should therefore be limited to *O. linum*, *O. maris* subsp. *maris*, *O. maris* subsp. *hiroshimense*, *O. maris* subsp. *williamsae*, *O. beijerinckii* subsp. *beijerinckii*, *O. beijerinckii* subsp. *pelagicum*, and *O. multiglobuliferum*. Consequently, *O. japonicum*, *O. jannaschii*, *O. kriegii*, and *O. pusillum* should be removed from the genus. The genus definition described above has been adapted accordingly. The precise taxonomic affiliation of the last four species needs to be further determined.

Note added in proof: Satomi et al. (2002) have formally revised the taxonomic status of the genus *Oceanospirillum* and proposed the formal transfer of the species assigned to but not belonging to the genus *Oceanospirillum* to new and existing genera. (See Kimura et al., 2002 in Further Reading section below.)

Acknowledgments

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Further Reading

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Satomi, M., B. Kimura, T. Hamada, S, Harayama, and T. Fujii. 2002. 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. Int J Syst Evol Microbiol. *52*: 739–747.

Differentiation of the species of the genus *Oceanospirillum*

Morphological and physiological characteristics of the species of *Oceanospirillum* are indicated in Tables BXII.γ.77 and BXII.γ.82. Chemotaxonomic characteristics of the species are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, and BXII.γ.81.

List of species of the genus Oceanospirillum

1. **Oceanospirillum linum** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374^{AL} (*Spirillum linum* Williams and Rittenberg 1957, 82.)
li' num. L. n. *linum* flax, thread.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Growth in defined media is usually poor; however, abundant growth occurs in defined media containing malate plus succinate as carbon sources and methionine as the nitrogen source. Nitrate is not used.

Strain OF3, isolated by Terasaki (1972, 1973), differs from other strains of *O. linum* in that it can grow well in defined media with a variety of sole carbon sources and

TABLE BXII.γ.82. Morphological and physiological characteristics of the species of *Oceanospirillum* and of *Marinospirillum minutulum*[a]

Characteristics	*O. linum*	*O. beijerinckii*	*O. maris*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*	*O. kriegii*	*O. pusillum*	*Marinospirillum minutulum*
Type of helix[b]	C	C	C	C	SR	C	SR	CC	C
Wavelength of helix, μm	1.8–4.0	6.3–7.2	3.5–7.0	3.5–5.0	nd	7.0–20.0	nd	1.7–2.0	2.0–2.8
Helix diameter, μm	0.8–1.4	1.5–3.0	1.4–2.8	1.0–2.0	nd	2.0–5.0	nd	1.0–1.2	0.6–1.5
Length of helix, μm	4.0–30.0	2.0–15.5	2.5–40.0	2.0–10.0	nd	5.0–75.0	nd	1.2–4.0	3.0–8.0
Cell diameter, μm	0.4–0.6	0.6–1.2	0.6–1.1	0.5–0.9	nd	0.8–1.4	nd	0.3–0.5	0.3–0.4
Flagellar arrangement[c]	BT	BT	BT	BT	M1-2	BT	MS	BS	BT
Polar membrane present	nd	+d	+[d]	nd	nd	+	nd	nd	+
Intracellular poly-β-hydroxybutyrate formed	+	+	+	+	+	+	+	+	+[e]
Coccoid bodies predominant:									
After 3–4 weeks	+	+	+	+	nd	−	nd	+	+
After 24–48 h	−	−	−	+	nd	−	nd	−	−
Range of NaCl (%) for growth in peptone water after 7 d[f]	0.5–8.0	0.5–8.0	nd	0.5–4.0	nd	0.5–8.0	nd	0.5–8.0	0.5–8.0
Growth in PSS-seawater broth containing:[g]									
9.75% total NaCl	+	+	+	nd	nd	nd	nd	nd	+
12.75% total NaCl	−	−	−	nd	nd	nd	nd	nd	d
Temperature range for growth (°C)[h]	11–39	8–41	2–35	6–37		10–43		6–40	11–37
Optimum growth temperature 25°C rather than 30–32°C	−	−	d[i]	−	+	−	+	−	−
Phosphatase	+	+	d[i]	+	nd	w	nd	w	−
Nitrate reduced to nitrite	−	−	−	−	+	−	−	+	+
Oxidase	+	+	+	+	+	+	+	+	+
Catalase	+ or w	+ or w	di	+	nd	w or −	nd	w or −	−
Anaerobic growth with nitrate	−	−	−	−	nd	−	nd	−	−
Denitrification	−	−	−	−	−	−	−	−	−
Acid produced from sugars	−	−	−	−	nd	−	nd	−	−
Hydrolysis of esculin, hippurate, starch, or casein	−	−	−	−	nd	−	nd	−	−
Indole test	−	−	−	−	nd	−	nd	−	−
Sulfatase (0.1% phenolphthalein disulfate)[g]	−	−	−	nd	nd	nd	nd	nd	−
Gelatin liquefaction at 20°C after: [f,j]									
7 d	−	d	−	−	−	d	−	−	−
28 d	+	d	−	−	−	d	−	−	−
42 d	+	d	−	−	−	d	−	−	−

(*continued*)

TABLE BXII.γ.82. *(cont.)*

Characteristics	*O. linum*	*O. beijerinckii*	*O. maris*	*O. multiglobuliferum*	*O. jannaschii*	*O. japonicum*	*O. kriegii*	*O. pusillum*	*Marinospirillum minutulum*
Gelatin hydrolysis at 30°C after:									
4 d[d,g]	−	−	−	nd	−	−	−	nd	−
Reduction of 0.3% H_2SeO_3[g]	+	−	−	nd	nd	nd	nd	nd	+
Water-soluble brown pigment formed in the presence of:[g]									
0.1% tyrosine	+	+	d	nd	nd	nd	nd	nd	−
0.1% phenylalanine	−	−	−	nd	nd	nd	nd	nd	−
0.1% tryptophan	d	−	d	nd	nd	nd	nd	nd	d
Growth in the presence of:[g]									
0.1% oxgall	+	+	+	nd	nd	nd	nd	nd	+
0.1% glycine	+	−	+	nd	nd	nd	nd	nd	+
Growth on:[g]									
Eosin methylene blue agar	−	−	d	nd	nd	nd	nd	nd	−
MacConkey agar	−	−	−	nd	nd	nd	nd	nd	−
Triple-Sugar iron agar	+	−	+	nd	nd	nd	nd	nd	+
Sellers agar	−	−	−	nd	nd	nd	nd	nd	−
Methyl red-Voges–Proskauer broth	+	−	+	nd	nd	nd	nd	nd	−
Water-soluble yellow-green fluorescent pigment formed[g]	+	−	d	nd	−	nd	−	nd	−
Deoxyribonuclease[g]	−	+	−	nd	nd	nd	nd	nd	−
Ribonuclease[g]	d	+	−	nd	nd	nd	nd	nd	−
Urease[g]	−	−	−	nd	nd	nd	nd	nd	−
Auxotrophic growth requirement	+[k]	−	d[k]	−	nd	−	nd	−	−
Mol% (G + C) of DNA	48–50	47–49	45–47	46	56–57	45	54–56	51	42–44

[a]Phenotypic data are from Krieg (1984a), Baumann et al. (1972), and Bowditch et al. (1984a); +, present in all strains; −, lacking in all strains; d, depending on the strain; w, weak reaction; nd, not determined.

[b]C, clockwise helix; CC, counterclockwise helix (determined by focusing on the bottom of the cells.

[c]SR, straight rod; BT, bipolar tufts, BS, bipolar single; M1-2, 1–2 flagella at one pole; MS, monopolar single.

[d]Characteristic has not been tested in *O. maris* subsp. *hiroshimense* and *O. beijerinckii* subsp. *pelagicum*.

[e]No granules are visible microscopically in *Marinospirillum minutulum* but chemical analysis indicates presence of the polymer.

[f]Data from Terasaki (1972, 1979).

[g]Data from Hylemon et al. (1973).

[h]*Oceanospirillum kriegii* grows at 35°C but not at 40°C; *Oceanospirillum jannaschii* does not grow at either 35°C or 40°C.

[i]*Oceanospirillum maris* subsp. *hiroshimense* has an optimal growth temperature of 25°C; catalase reaction can be negative, weak, or positive; phosphatase reaction can be positive or negative. For detailed information: see Hylemon et al. (1973), Terasaki (1972, 1979), and Table BXII.γ.83.

[j]Gelatin liquefaction was not tested in *O. maris* subsp. *maris*. It was negative in *O. maris* subsp. *hiroshimense*, positive in *O. beijerinckii* subsp. *beijerinckii*, and differed depending on the strain in *O. beijerinckii* subsp. *pelagicum*.

[k]*Oceanospirillum linum* grows poorly or not at all in a defined medium with a single carbon source and ammonium ions as the nitrogen source; however, abundant growth ocurs in a defined medium containing succinate plus malate as carbon source and methionine as the nitrogen source. (See also footnote [d] in Table BXII.γ.82.) *Oceanospirillum maris* subsp. *williamsae* ATCC 2954^T does not grow in vitamin-free medium and requires an unidentified growth factor.

ammonium ions as the nitrogen source (see footnote d, Table BXII.γ.77). Other characteristics of this strain are similar to those of the type strain (Terasaki, 1973), but whether it should be included in this species is uncertain.

The species includes organisms previously assigned to the two species *Spirillum linum* and *Spirillum atlanticum* by Williams and Rittenberg (1957). The two species were combined into the single species *O. linum* by Hylemon et al. (1973), based on a high degree of similarity in phenotypic characters and in DNA base composition.

Isolated from coastal seawater.

The mol% G + C of the DNA is: 48–50 (T_m).

Type strain: ATCC 11336, DSM 6292, NCMB 56.

2. **Oceanospirillum beijerinckii** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 374[AL] (*Spirillum beijerinckii* Williams and Rittenberg 1957, 90.)

bei.jer.inck'i.i. M.L. gen. n. *beijerinckii* of Beijerinck; named after Prof M.W. Beijerinck of Delft, Holland.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from coastal seawater.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: ATCC 12754, DSM 7166, NCMB 52.

a. **Oceanospirillum beijerinckii** *subsp.* **beijerinckii** (Williams and Rittenberg 1957) Hylemon, Wells, Krieg and Jannasch 1973, 375[AL] (*Spirillum beijerinckii* Williams and Rittenberg 1957, 90.)

Differs from *O. beijerinckii* subsp. *pelagicum* as indicated in Table BXII.γ.83.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: ATCC 12754 , DSM 7166, NCMB 52.

b. **Oceanospirillum beijerinckii** *subsp.* **pelagicum** (Terasaki 1973) Pot, Gillis, Hoste, Van de Velde, Bekaert, Kersters and De Ley 1989, 32[VP] (*Spirillum pelagicum* Terasaki 1973, 65.)

pe.la'gi.cum. L. neut. adj. *pelagicum* belonging to the sea.

Differs from the *O. beijerinckii* subsp. *beijerinckii* as indicated in Table BXII.γ.83.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 49 (T_m).

Type strain: ATCC 33337, DSM 6288, IFO 13612, NCMB 2228.

GenBank accession number (16S rRNA): AB006761.

3. **Oceanospirillum maris** Hylemon, Wells, Krieg and Jannasch 1973, 376[AL]

ma'ris. L. n. *mare* the sea; L. gen. n. *maris* of the sea.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77.

Isolated from coastal seawater (Jannasch, 1967).

The mol% G + C of the DNA is: 45–46 (T_m).

Type strain: ATCC 27509, DSM 6286, LMG 5213.

GenBank accession number (16S rRNA): AB006771.

TABLE BXII.γ.83. Differentiating characteristics for the subspecies of *Oceanospirillum beijerinckii*[a]

Characteristic	*O. beijerinckii* subsp. *beijerinckii*	*O. beijerinckii* subsp. *pelagicum*
Temperature range for growth (°C)	14–37	8–41
Growth with:		
Lactate, citrate, malate, pyruvate, acetate, propionate, tartrate	−	+
p-Hydroxybenzoate, ethanol, *n*-propanol	−	d
Gelatin liquified at 20°C	+	d
Growth after 7 d in peptone water with:		
0.5–6.0% NaCl	+	−
0.5–8.0% NaCl	−	+
Mol% G + C of DNA	47	49

[a]Phenotypic data from Terasaki (1972, 1979); +, present in all strains; −, lacking in all strains; d, depending on the strain

c. **Oceanospirillum maris** *subsp.* **maris** Hylemon, Wells, Krieg and Jannasch 1973, 376[AL]

Differs from the *O. maris* subsp. *williamsae* and the *O. maris* subsp. *hiroshimense* as indicated in Table BXII.γ.84.

The mol% G + C of the DNA is: 46 (T_m).

Type strain: ATCC 27509, DSM 6286, LMG 5213.

GenBank accession number (16S rRNA): AB006771.

d. **Oceanospirillum maris** *subsp.* **hiroshimense** (Terasaki 1973) Pot, Gillis, Hoste, Van de Velde, Bekaert, Kersters and De Ley 1989, 33[VP] (*Spirillum hiroshimense* Terasaki 1973, 62.)

hi.ro.shi.men'se. M.L. neut. adj. *hiroshimense* pertaining to Hiroshima. Japan.

Characters are as described for the species. Differs from the *O. maris* subsp. *maris* and the *O. maris* subsp. *williamsae* as indicated in Table BXII.γ.84.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 47 (T_m).

Type strain: IFO 13616, DSM 9524.

e. **Oceanospirillum maris** *subsp.* **williamsae** Linn and Krieg 1984, 355[VP]

will'iam.sae. M.L. gen. n. *williamsae* of Williams; named after Marion A. Williams, who was the first to describe species of marine spirilla.

Characters are as described for the species. Differs from the *O. maris* subsp. *maris* and the *O. maris* subsp. *hiroshimense* as indicated in Table BXII.γ.84.

Isolated from a mixture of organisms comprising NCMB strain 54 by Linn and Krieg (1978).

The mol% G + C of the DNA is: 45 (T_m).

Type strain: ATCC 29547, IFO 15468.

GenBank accession number (16S rRNA): AB006763.

4. **Oceanospirillum multiglobuliferum** (Terasaki 1973) Terasaki 1979, 143[AL] (*Spirillum multiglobuliferum* Terasaki 1973, 69.)

mul.ti.glo.bu.li'fe.rum. L. adj. *multus* much, many; L. dim. n. *globulus* a small sphere, globule; L. v. *fero* to bear, carry; M.L. neut. adj. *multiglobuliferum* bearing many globules.

The morphological characters are listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources

TABLE BXII.γ.84. Differentiating characteristics for the subspecies of *Oceanospirillum maris*[a]

Characteristic	*O. maris* subsp. *maris*	*O. maris* subsp. *hiroshimense*	*O. maris* subsp. *williamsae*
Optimal growth temperature (°C)	30–32	25	30–32
Catalase reaction	strongly +	d	weakly +
Phosphatase activity	−	+	−
DNase activity	−		+
RNase activity	−		+
Growth with 1% glycine	+		−
Growth in vitamin-free, defined growth medium	+		−
Growth with:			
L-Glutamate, oxaloacetate	+		−
Succinate, pyruvate, lactate, tartrate, acetate, propionate	−	+	−
Mol% G + C of DNA	46	47	45

[a]Phenotypic data from Hylemon et al. (1973); +, present in all strains; −, lacking in all strains; d, depending on the strain

are listed in Table BXII.γ.77. Differs from other species by forming unusually large numbers of coccoid bodies even in 24- to 48-h-old broth cultures. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 46 (T_m).

Type strain: IFO 13614.

GenBank accession number (16S rRNA): AB006764.

Species assigned but phylogenetically not belonging in *Oceanospirillum*

1. **Oceanospirillum jannaschii** Bowditch, Baumann and Bauman 1984b, 503^{VP} (Effective publication: Bowditch, Baumann and Bauman 1984a, 227.)
jan.nasch′i.i. M.L. gen. n. *jannaschii* of Jannasch; named after H.W. Jannasch.

Straight rods. Some morphological, physiological, and chemotaxonomic characters are listed in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82 (see also Baumann et al., 1972). Sole carbon sources are listed in Table BXII.γ.77. Utilize 39–46 organic compounds including fatty acids, tricarboxylic acid cycle intermediates, alcohols, amino acids, and amines. Utilizes γ-aminovalerate and histamine (Baumann et al., 1972).

Isolated from seawater after enrichment (Baumann et al., 1972).

The mol% G + C of the DNA is: 56–57 (T_m).

Type strain: ATCC 27135, DSM 6295, IFO 15466.

GenBank accession number (16S rRNA): AB006765.

2. **Oceanospirillum japonicum** (Watanabe 1959) Hylemon, Wells, Krieg and Jannasch 1973, 375^{AL} (*Spirillum japonicum* Watanabe 1959, 78.)
ja.pon′i.cum. M.L. neut. adj. *japonicum* pertaining to Japan.

The morphological characters are depicted in Fig. BXII.γ.104 and listed in Table BXII.γ.82; however, the type strain presently has morphological features that differ from the original description, in that the cells are no longer helical with several waves but instead are curved, straight, or S-shaped; moreover, colonies of this strain are presently of the R (rough) type. Therefore, it is likely that the type strain has undergone alteration since its isolation in 1959. Three reference strains isolated by Terasaki (1972, 1973) have morphological features that more nearly correspond to those given in the original description (strains IF4, IF8, and UF3), and also they form colonies of the S (smooth) type.

The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77.

Isolated from putrid infusions of marine mussels.

The mol% G + C of the DNA is: 45 (T_m).

Type strain: ATCC 19191, DSM 7165.

3. **Oceanospirillum kriegii** Bowditch, Baumann and Bauman 1984b, 503^{VP} (Effective publication: Bowditch, Baumann and Bauman 1984a, 227.)
krie′gi.i. M.L. gen. n. *kriegii* of Krieg; named after N.R. Krieg.

Straight rods. Some morphological and physiological characters are listed in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82 (see also Baumann et al., 1972). Sole carbon sources are listed in Table BXII.γ.77. Utilize 29–33 organic compounds including D-glucose and D-fructose but no other pentose, hexose, or disaccharide; also utilize tricarboxylic acid cycle intermediates, alcohols, amino acids, and amines. Produces an extracellular lipase (Baumann et al., 1972).

Isolated from see water after enrichment (Baumann et al., 1972).

The mol% G + C of the DNA is: 54–56 (T_m).

Type strain: ATCC 27133, DSM 6294, IFO 15467, NCMB 2042.

GenBank accession number (16S rRNA): AB006767.

4. **Oceanospirillum pusillum** (Terasaki 1973) Terasaki 1979, 142^{AL} (*Spirillum pusillum* Terasaki 1973, 67.)
pu.sil′lum. L. dim. neut. adj. *pusillum* very small.

The morphological characters are listed in Table BXII.γ.82. The physiological and chemotaxonomic characters are indicated in Tables BXII.γ.78, BXII.γ.79, BXII.γ.80, BXII.γ.81, and BXII.γ.82. Sole carbon sources are listed in Table BXII.γ.77. Ammonium ions can serve as a sole nitrogen source; nitrate is not used.

Isolated from putrid infusions of marine mussels.

Belongs to the *Alphaproteobacteria*.

The mol% G + C of the DNA is: 51 (T_m).

Type strain: ATCC 33338, DSM 6293, IAM 14442, IFO 13613, NCMB 2229.

GenBank accession number (16S rRNA): AB006768.