The Genus *Spirillum*: a Taxonomic Study¹

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The morphological, physiological, and nutritional characteristics and the deoxyribonucleic acid (DNA) base compositions of 39 strains of *Spirillum* (including the available type strains) were determined. A uniform methodology provided a basis for comparison of the strains. The results, together with previous serological data and with previous results for two strains of *Spirillum volutans*, indicate that the present genus *Spirillum* should be divided into three genera, with the original name being restricted to obligately microaerophilic freshwater forms having a large cell diameter and a DNA base composition of 36 to 38 mol % guanine + cytosine (G + C). This genus would contain at present only one species, the type species, *S. volutans*. The generic name *Aquaspirillum* is proposed for the aerobic, freshwater forms having a DNA base composition of 49 to 65 mol % G + C. Thirteen species are included in this genus: the type species *A. serpens* (Müller) comb. nov.; *A. metamorphum* (Terasaki) comb. nov.; *A. putridiconchylum* (Terasaki) comb. nov.; *A. sinuosum* (Williams and Rittenberg) comb. nov.; *A. gracile* (Canale-Parola et al.) comb. nov.; *A. itersonii* (Giesberger) comb. nov.; *A. dispar* sp. nov.; *A. polymorphum* (Williams and Rittenberg) comb. nov.; *A. aquaticum* sp. nov.; *A. delicatum* (Leifson) comb. nov.; *A. peregrinum* (Pretorius) comb. nov.; *A. anulus* (Williams and Rittenberg) comb. nov.; and *A. giesbergeri* (Williams and Rittenberg) comb. nov.; and *A. giesbergeri* (Williams and Rittenberg) comb. nov. Strains of the latter two species, plus an additional strain (ATCC 12289), appear to be related to one another but should not be included together in a single species without additional supporting evidence (e.g., DNA homology studies). The following are proposed as type or neotype strains: *A. serpens* ATCC 12638 (neotype); *A. metamorphum* ATCC 15280 (type); *A. putridiconchylum* ATCC 15279 (type); *A. gracile* ATCC 19624 (type); *A. itersonii* ATCC 12639 (neotype); *A. dispar* ATCC 27510 (type); and *A. aquaticum* ATCC 11330 (type). The generic name *Oceanospirillum* is proposed for marine forms which do not attack carbohydrates and which possess a DNA base composition of 42 to 48 mol % G + C; five species are included. These are as follows: the type species *O. linum* (Williams and Rittenberg) comb. nov.; *O. minutum* (Watanabe) comb. nov.; *O. beijerinckii* (Williams and Rittenberg) comb. nov.; *O. japonicum* (Watanabe) comb. nov.; and *O. maris* sp. nov. The following are proposed as type or neotype strains: *O. minutum* ATCC 19192 (neotype); *O. japonicum* ATCC 19191 (neotype); and *O. maris* ATCC 27509 (type).

Although *Spirillum* Ehrenberg 1832 is one of the oldest bacterial generic names and although spirilla are widespread in freshwater and marine environments, characterization of this group has not been extensive. This may be attributed to the following factors: (i) lack of medical

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significance, except for *Spirillum minor*, the causative agent of one of the forms of rat-bite fever in man (3, 8, 29); (ii) difficulty of isolation (7, 28, 45, 49); and (iii) past difficulties in preservation of cultures, with only continual transfer, the recently developed use of L-drying (17), or liquid nitrogen being effective.

Several morphological taxonomic schemes for *Spirillum* were devised prior to 1936, as reviewed by Williams (46), but Giesberger (Ph.D. thesis, Delft Univ., Netherlands, 1936) was the first investigator to consider nutrition

as well. He indicated that, by either morphological or nutritional criteria, four distinct groups could be recognized with the available isolates. Williams and Rittenberg (49) similarly found that all the strains of a single morphological group had a very similar nutrition, but they also reported that isolates belonging to distinctly different morphological groups did, in certain cases, exhibit the same nutritional pattern. Moreover, they suggested that if strains within the same morphological group were to be tested with a larger variety of carbon and nitrogen sources, differences in their nutritional patterns would likely be found. Williams and Rittenberg used morphology primarily and nutrition secondarily to divide the genus into 19 species and provided many supplementary physiological data. Since the work of Williams and Rittenberg, a number of additional species have been described (7, 19, 27, 40, 41, 44). The serological relationships among spirilla, based on thermolabile antigens, have recently been investigated by McElroy and Krieg (24). Nineteen serological groups were established, and high antigenic specificity permitted delineation of most of the species from one another and from members of related genera.

The type species of the genus *Spirillum* has long been considered to be *Spirillum undula* (Müller) Ehrenberg 1832 (see reference 5). However, Hylemon et al. (16) have recently requested the Judicial Commission to issue an Opinion establishing *Spirillum volutans* Ehrenberg 1832 (14) as the type species of the genus and placing *S. undula* on the list of rejected names. Wells' strain (ATCC 19554) was designated as the neotype strain of *S. volutans*.

To further develop the characterization and classification of *Spirillum*, we began in 1965 a comprehensive study of the type and other available strains of spirilla. It was apparent from the literature that a number of different methods, many of which were not well defined, had been employed for the characterization of these organisms. We compared all strains on the same basis, using defined procedures. The results reported here are based to a great extent on the Ph.D. dissertations of two of the authors (P.B.H. and J.S.W., Virginia Polytechnic Institute and State University) completed in 1970 and 1971, respectively. A portion of the work was presented at the 69th Annual Meeting of the American Society for Microbiology in 1969 (P. B. Hylemon and N. R. Krieg, and J. S. Wells, Jr., and N. R. Krieg, Bacteriol. Proc., p. 20, 1969). The results of the investigation reported here formed the basis for the descriptions of the genus *Spirillum* and its species in the forthcoming eighth edition of Bergey's *Manual*

of *Determinative Bacteriology*. After completion of our studies, we found that a very comprehensive morphological, physiological, and biochemical characterization of 49 strains of spirilla, including 23 new isolates, had been published in 1972 by Terasaki in Japan (43). Our investigation differed from that of Terasaki with respect to our use of a different methodology, of certain additional species, of electron microscopy, and of deoxyribonucleic acid (DNA) base compositions. In general, however, the two investigations appear to support one another, although a few contradictions occur. Our results indicate that, at present, the genus *Spirillum* consists of species too diverse to be contained within a single genus, and we propose a new classification scheme and also designate type and neotype strains for certain species.

MATERIALS AND METHODS

Strains studied. Table 1 lists the various *Spirillum* strains investigated.

Morphology. The medium used in the determination of cell dimensions was a peptone-succinate-salts (PSS) broth of the following composition (%): peptone (Difco), 1.0; succinic acid, 0.1; $(\text{NH}_4)_2\text{SO}_4$, 0.1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.0002; and $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 0.0002; pH 6.8. Because spirilla have been reported to be sensitive to low concentrations of Na^+ (33), KOH was used to adjust the pH. For marine organisms, a synthetic sea water (35) of the following composition (%) was substituted for distilled water: NaCl, 2.75; MgCl_2 , 0.50; MgSO_4 , 0.20; CaCl_2 , 0.05; KCl, 0.10; and FeSO_4 , a trace. Media were sterilized at 121 C (15 lb/in²) for 15 min. Spirilla were cultured without shaking for several transfers in cotton-stoppered, 250-ml Erlenmeyer flasks containing 70 ml of broth prior to measurement of cell dimensions, phase-contrast photomicrography, or electron microscopy. A Leitz Ortholux microscope equipped with a Heine phase-contrast condenser, Pv Apo 90/1.15 objective, and ocular micrometer was used for measurement of the ranges of cell diameter, helix diameter, helix length, and wavelength for 18-h-old cultures. Phase-contrast photomicrographs were also prepared of various strains to provide the reader with a rapid visual comparison of cell types. Most strains were also examined by electron microscopy to determine types of flagellation; an RCA EMU-3 electron microscope and shadowing by tungsten oxide were used. In the case of *Spirillum delicatum*, a negative staining method previously described (39) was also employed. The presence of coccoid bodies in 4-wk-old PSS cultures was determined by phase-contrast microscopy. Such coccoid bodies have been termed "microcysts" by Williams and Rittenberg (48), but because resistance to desiccation and the presence of thickened walls have not been demonstrated, we prefer to use a term lacking these connotations.

Maintenance of stock cultures. Stocks were maintained in PSS semisolid (0.15% agar) medium at 30 C

TABLE 1. *Summary of Spirillum strains studied^a*

VPI and SU no.	National culture collection no.	Designation	Source	Reference
1	ATCC 27510	Strain 104 of H. W. Jannasch ^b	Freshwater	7
2	ATCC 19625	<i>S. gracile</i> strain D5	Pond water	7
3	ATCC 19624	<i>S. gracile</i> strain D4	Pond water	7
4	ATCC 19626	<i>S. gracile</i> strain D6	Pond water	7
5	ATCC 14667	<i>S. delicatum</i> type strain	Distilled water	19
6	ATCC 27650	Strain 103 of H. W. Jannasch	Freshwater	
7	ATCC 15280	<i>S. metamorphum</i>	Purid infusion of freshwater	41
9	NCIB 9072	<i>S. polymorphum</i> type strain	Pond water	49
10	ATCC 15387	<i>S. peregrinum</i> type strain	Primary oxidation pond	27
11	ATCC 11331	<i>S. tiersonii</i> subsp. <i>vulgatum</i> type strain	Pond water	49
12	ATCC 12639	<i>S. tiersonii</i> strain of S. C. Rittenberg ^c	Pond water	11
13	ATCC 12639	<i>S. tiersonii</i> subsp. <i>tiersonii</i>	Freshwater	
15	ATCC 11334	<i>S. giesbergeri</i> type strain	Pond water	49
16	ATCC 19259	<i>S. anulus</i>	Freshwater	
43	NCIB 9012	<i>S. anulus</i> type strain	Pond water	49
17	NCIB 8230	<i>S. graniferum</i> type strain	Freshwater	20, 49
18	ATCC 9786	<i>S. sinuosum</i> type strain	Freshwater	20, 49
19	ATCC 15279	<i>S. puridiconchylum</i>	Purid infusion of freshwater	40
20	ATCC 11330	<i>S. serpens</i> subsp. <i>serpens</i> ^d	shellfish	
21	ATCC 12289	<i>S. serpens</i>	Freshwater	49
22	ATCC 11335	<i>S. serpens</i> subsp. <i>azotum</i> type strain	Freshwater	
23		<i>S. serpens</i> St. Rhodes strain of R. G. E. Murray ^{e, f}	Pond water	49
24		<i>S. serpens</i> VHL strain of R. G. E. Murray ^f	See strain 25	6
25		<i>S. serpens</i> VH strain of R. G. E. Murray ^f	From sewer at Victoria Hospital, London, Canada	
26	ATCC 15278	<i>S. serpens</i> subsp. <i>serpens</i>	Purid infusion of freshwater	42
27		<i>S. serpens</i> VHA strain of R. G. E. Murray ^f	shellfish	
28		<i>S. serpens</i> Victoria strain of R. G. E. Murray ^f	See strain 25	6
29	ATCC 12638	<i>S. serpens</i> subsp. <i>serpens</i>	Freshwater	
30		<i>S. serpens</i> VHS strain of R. G. E. Murray ^f	See strain 25	
31		<i>S. serpens</i> strain of S. C. Rittenberg	Freshwater	11
32	ATCC 11336	<i>S. linum</i> type strain	Coastal seawater	49
33	ATCC 12753	<i>S. atlanticum</i> type strain	Coastal seawater	49
34	ATCC 12754	<i>S. bejerinckii</i> type strain	Coastal seawater	49
35	ATCC 27509	Strain 101 of H. W. Jannasch	Seawater	
36	ATCC 27649	Strain 102 of H. W. Jannasch	Seawater	
37	ATCC 27648	Strain 206 of H. W. Jannasch	Seawater	
38	ATCC 19191	<i>S. japonicum</i>	Marine shellfish	44
39	ATCC 19192	<i>S. halophilum</i>	Marine shellfish	44
40	ATCC 19193	<i>S. minutum</i>	Marine shellfish	44

^a Abbreviations: VPI and SU, Virginia Polytechnic Institute and State University, Blacksburg, Va.; ATCC, American Type Culture Collection, Rockville, Md.; NCIB, National Collection of Industrial Bacteria, Aberdeen, Scotland.

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^d This strain was designated as the neotype strain of *S. serpens* subsp. *serpens* (49), but in a personal communication to the ATCC, M. A. Williams stated that she intended ATCC 12638 to be the neotype strain instead (1, 32).

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^f Strains 23 to 25, 27, 28, and 30 are said to be different derivatives of strain 25. These derivatives differ in minor ways; e.g., VHL has lost the hexagonal protein layer on the surface of the cells while the St. Rhodes derivative is less helical (personal communication from R. G. E. Murray).

with weekly transfers. In 1969, liquid-nitrogen facilities became available to our laboratory, and stock cultures were preserved at this time by the following procedure. PSS broth cultures were centrifuged and resuspended to a dense concentration in PSS broth containing 15% glycerol. The suspensions were frozen in glass or plastic ampoules in 0.2-ml quantities by a mixture of dry ice and ethanol, and then were placed directly in liquid nitrogen. All of our strains of spirilla have retained viability to date by this method of preservation.

Colony characteristics. The diameter and color of colonies were determined from streaked PSS plates (1.5% agar) after 72 h of incubation at 30 C.

Optimal temperature. The optimal temperature for each strain was determined by incubating PSS broth cultures at 10, 15, 20, 25, 30, 37, 42, and 45 C, using a Fisher Low Temperature Incubator (Fisher Scientific Co., Fairlawn, N.J.) for 10, 20, and 25 C, a Psychrotherm Incubator (New Brunswick Scientific Co., New Brunswick, N.J.) for 15 C, and Fisher IsoTemp box incubators (Fisher Scientific Co., Fairlawn, N.J.) for the remaining temperatures. Media were conditioned to the various temperatures for 2 h prior to inoculation. A visual estimation of growth responses was made after 1 wk of incubation.

Biochemical characteristics. All strains were cultured in screw-capped tubes (20 by 125 mm) containing 10 ml of PSS broth; 0.04 ml from the second 24-h serial transfer was used as the inoculum for test media. All cultures were incubated at 30 C. Tests were performed and interpreted by the methods of Cowan and Steel (12) except where noted. PSS broth supplemented with appropriate substrates was employed for determination of: (i) H_2S production from 0.2% cysteine within 7 days by the use of lead acetate strips suspended above the medium; (ii) aerobic reduction of 0.3% H_2SeO_3 within 1 wk, based on development of a pink color; (iii) aerobic reduction of 0.1% KNO_3 within 48 h by production of a red color with sulfanilic acid and α -naphthylamine; to tubes not showing a red color, powdered zinc was added to detect possible reduction beyond the nitrate stage; for testing nitrate reduction, $(NH_4)_2SO_4$ was omitted from the PSS broth; (iv) indole production within 48 h from 0.1% tryptophan, by ether extraction followed by acidic *p*-dimethylaminobenzaldehyde; (v) visible growth within 2 wk in the presence of 1% oxgall bile (Difco), or 1% glycine, or in NaCl concentrations ranging from 3% to 10% added salt; and (vi) hydrolysis within 4 days of 1% sodium hippurate or of 0.1% esculin plus 0.05% ferric citrate. The ability to grow anaerobically within 1 wk in the presence of 0.1% KNO_3 was determined by the use of freshly autoclaved semisolid PSS medium (0.15% agar) with $(NH_4)_2SO_4$ being omitted; tubes were immediately sealed with sterile petrolatum-paraffin wax after inoculation of the molten (45 C) medium. Solidified PSS medium (1.5% agar) supplemented with appropriate substrates was used for determination of: (i) hydrolysis of 0.01% phenolphthalein diphosphate or disulfate (sodium salts) within 4 days; (ii) production within 1 wk of a yellow or brown water-soluble

pigment in the presence of 0.2% L-tyrosine, L-phenylalanine, or L-tryptophan; (iii) hydrolysis within 2 wk of 10% soluble starch or of casein, single-strength milk being incorporated in the medium for the latter; and (iv) hydrolysis within 1 wk of 0.1% DNA or ribonucleic acid (RNA) with a clear zone around the streak after flooding with 0.1 N HCl as an indicator of activity. The oxidase test was performed by the addition of *N,N*-dimethyl-*p*-phenylenediamine monohydrochloride (1%) directly to 48-h-old PSS broth cultures or by smearing a loopful of culture on a moistened test disk (Difco). PSS broth containing 12% gelatin was chilled at 4 days for detection of rapid liquefaction. For catalase, 4 to 5 drops of 3% H_2O_2 were added to 24- to 48-h-old semisolid cultures; the appearance of bubbles within 30 min, compared to controls of sterile medium, indicated activity. Fluorescent pigment production on PSS agar plates was tested at 48 to 72 h with a 253.7-nm ultraviolet source. Acid reactions from 32 carbohydrates (1.0%) were detected by use of a modified PSS broth: succinate was omitted and 0.0018% phenol red indicator was incorporated. The pH was adjusted to 7.2. Moreover, it was essential to decrease the peptone concentration to 0.2% for acid reactions to occur, as reactions were uniformly negative with the use of 1% peptone. Carbohydrate media were tubed in 10-ml quantities using screw-capped tubes (20 by 125 mm). The ability of acid producers to ferment carbohydrates was subsequently tested by the use of a similar, but semisolid (0.15% agar), medium: after inoculation of the molten medium (45 C), tubes were immediately sealed with sterile petrolatum-paraffin wax. Control tubes without a wax seal were prepared simultaneously. Urease activity was tested by micro diffusion techniques and, more conveniently, by use of a synthetic medium of the following composition (%): BES buffer (*N,N*-bis-(2-hydroxyethyl)-2-aminoethane sulfonic acid), 0.1065; urea, 2.0; and phenol red, 0.001; pH 7.0. (Commercial urea broth was not suitable for use with spirilla.) Cells were cultured in PSS broth for 24 h and suspended in sterile distilled water to a dense concentration; 0.5 ml of the latter was added to 2.0-ml quantities of the urease test medium and incubated at 30 C for 24 h. Controls lacking urea were employed.

Nutritional characteristics. Compounds tested as sole carbon or nitrogen sources were obtained in the highest commercially available purity but were not assayed or further purified in our laboratory. For sole carbon sources, the defined basal medium (DBM) to which the sources were added (0.1%) had the following composition (%): $(NH_4)_2SO_4$, 0.1; $MgSO_4 \cdot 7H_2O$, 0.1; K_2HPO_4 , 0.05; $FeCl_3 \cdot 6H_2O$, 0.00047; $MnSO_4 \cdot H_2O$, 0.00025; $CaCO_3$, 0.0001; $ZnSO_4 \cdot 7H_2O$, 0.000072; $CuSO_4 \cdot 5H_2O$, 0.0000125; $CoSO_4 \cdot 7H_2O$, 0.000014; H_3BO_3 , 0.0000031; $Na_2MoO_4 \cdot 2H_2O$, 0.0000245; and distilled water passed through a Bantam standard multi-bed resin cartridge (Barnstead Co., Boston, Mass.). The pH was adjusted to 7.0 with KOH. Media were sterilized at 112 C for 10 min. Thermolabile substrates were sterilized separately by filtration and added asep-

tically. For marine spirilla, a synthetic sea water (51) of the following composition (%) was substituted for demineralized water and the media were sterilized by filtration: NH_4NO_3 , 0.0002; H_3BO_3 , 0.0027; CaCl_2 , 0.114; FePO_4 , 0.0001; MgCl_2 , 0.5143; KBr , 0.010; KCl , 0.069; NaHCO_3 , 0.020; NaCl , 2.432; NaF , 0.0003; Na_2SiO_3 , 0.0002; Na_2SO_4 , 0.406; and SrCl_2 , 0.0026. Neither freshwater- nor seawater-defined media contained precipitate that required removal as in other defined media employed for spirilla (49). The medium used for determination of sole nitrogen sources (0.1%) was DBM with $(\text{NH}_4)_2\text{SO}_4$ omitted; moreover, a combination of succinate plus malate (0.1% each) was used as the carbon source. The medium used for determination of the tolerance of certain freshwater spirilla to low concentrations of NaCl consisted of DBM plus succinate, pyruvate, and L-glutamate (0.1% each); for strains failing to grow in this medium, DBM plus 0.1% succinate and 0.1% Casamino Acids (Difco) was used. Media for determination of the effect of Ca^{++} on growth consisted of DBM plus 0.05% CaCl_2 . A positive growth response with sole carbon or sole nitrogen sources was defined as the production of at least 10 Klett units of turbidity for the second serial transfer at 72 h, using a Klett colorimeter (Klett Manufacturing Co., New York), a blue (420 nm) filter, and 16-mm cuvettes. The standard inoculum for 10 ml of defined medium (contained in screw-capped tubes [16 by 150 mm]) was 0.1 ml of a 100-Klett unit saline suspension of freshwater spirilla, and, for marine spirilla, a synthetic seawater (51) suspension, previously cultured for 24 to 36 h in PSS broth and washed once in saline (or artificial seawater). The inoculum for the second serial transfer was 0.1 ml of the 72-h growth from the initial culture.

DNA base composition. For determination of the DNA base compositions, cells were suspended in a medium containing 0.15 M NaCl and 0.01 M ethylene-diaminetetraacetic acid (pH 8.0). The cells were lysed with sodium dodecyl sulfate, and the protein was extracted twice with liquefied phenol. The DNA was purified by the method of Marmur and Doty (22) and adjusted to 50 $\mu\text{g}/\text{ml}$ with 0.5 X SSC (SSC = 0.15 M NaCl , 0.015 M sodium citrate, pH 7.0). Melting point (T_m) determinations were made by a variation of the method of Marmur and Doty as previously described (16).

Detection of PHB. All of the type and neotype strains, as well as many additional strains, were examined for the presence of poly- β -hydroxybutyrate (PHB). Cells were cultured for 24 and 48 h in PSS broth in a water-bath shaker (30 C). For about half of the strains studied, centrifuged cells were dried at 90 C and extracted with hot chloroform, and the PHB was precipitated with 2 vol of cold diethyl ether. After a second precipitation, the PHB was dried at 60 C. For the remaining strains, centrifuged cells were extracted with alkaline hypochlorite (50) with subsequent preparation of chloroform extracts (18). For both types of PHB preparations, either a crotonic acid method (18) or infrared spectrophotometry (30) was used to identify the PHB.

RESULTS

Ranges of cell dimensions for each strain are listed in Table 2. There was a tendency of certain strains to become straighter with prolonged transfer (compare Fig. 15 with Fig. 16, and Fig. 18 with Fig. 19). With the exception of strain 5 (*S. delicatum*), all of the strains examined by electron microscopy possessed bipolar tufts of flagella. The latter was reported by Leifson (19) to possess predominantly a single, polar flagellum. Terasaki (43) also reported that, on the basis of flagella stains, this strain possessed a single, polar flagellum. Williams (47) has shown that the flagella of spirilla are frequently tightly agglutinated to form a fascicle which, in stained preparations, would appear as a single flagellum. She revised the descriptions of six species previously thought to possess only a single flagellum at each pole after her electron microscope studies indicated the occurrence of such fascicles instead. Our electron microscope studies of strain 5 indicated that about half of the cells exhibited only a single, polar flagellum and that most of the remaining cells possessed two flagella (see Fig. 48). However, occasionally a cell with up to six flagella could be observed (see Fig. 47). Bipolar flagellation was not observed except for a few dividing cells having a distinct central invagination of the wall. In the case of strain 9 (*S. polymorphum*), Williams and Rittenberg (49) reported bipolar tufts of flagella, but Terasaki (43) found this strain to possess bipolar, single flagella. Our electron microscope studies of this strain indicated that, although most of the cells did possess bipolar, single flagella, some did have up to three flagella at their poles (see Fig. 46). Terasaki (43) reported that strain 20 (*S. serpens*) possessed bipolar, single flagella, but our studies indicated the occurrence of tufts of flagella (see Fig. 33).

Figures 1 to 30 are phase-contrast photomicrographs of various strains of *Spirillum* arranged in order of increasing cell diameter for rapid visual comparison of cell types.

The optimum temperature for growth was 30 C for all strains. Moderate growth occurred at 20 and 37 C in all cases. All marine strains yielded scant to moderate growth at 15 C, except strain 34 (*S. bejerinckii*), which failed to grow. No freshwater strain grew at 15 C. At 42 C, only strains 7, 19, 23 to 25, 27, 28, 30, 31, and 38 exhibited growth, and this was scant. No growth occurred at 10 C with any strain. Strain 31 (*S. serpens*) gave scant growth at 45 C.

PHB was detected in all type or neotype strains (including those designated in Discussion), except for strain 3 (*S. gracile*). Despite several attempts to detect PHB in this strain, and even with the use of large numbers of cells harvested at various times of incubation, not even a trace of PHB could be found.

Only a few *Sphillum* strains were capable of producing acidic reactions from carbohydrates;

these strains are indicated in Table 3. Tests of the ability of these strains to ferment carbohydrates by the use of aerobic and anaerobic tubes of semisolid carbohydrate media indicated that the acidic reactions were produced only under aerobic conditions, except in the case of fructose; here, although strains 10 to 13 produced an acidic reaction (yellow color) quickly under anaerobic conditions and in

TABLE 2. *Dimensions of Sphillum strains*^a

Strain no.	Designation	Range (μm)			
		Diameter	Wavelength	Helix diameter	Length
2	<i>S. gracile</i>	0.2-0.3	2.8-3.5	0.5-2.1	3.5-14.0
3	<i>S. gracile</i>	0.2-0.3	2.8-3.5	0.6-2.1	4.0-14.0
4	<i>S. gracile</i>	0.2-0.3	2.8-3.5	0.6-2.1	4.0-14.0
39	<i>S. halophilum</i>	0.3-0.4	2.0-2.8	1.4-1.5	3.5-8.0
40	<i>S. minutulum</i>	0.3-0.4	2.0-2.3	0.6-0.8	3.0-8.0
5	<i>S. delicatum</i>	0.3-0.4	— ^b	0.4-0.7	3.0-5.0
9	<i>S. polymorphum</i>	0.3-0.5	4.0-5.0	1.0-1.5	3.5-8.4
11	<i>S. tiersonii</i>	0.4-0.6	3.0-3.5	1.2-1.6	2.8-5.6
12	<i>S. tiersonii</i>	0.4-0.5	3.0-3.5	1.2-1.6	3.6-7.0
13	<i>S. tiersonii</i>	0.5-0.6	3.0-3.5	1.2-1.6	2.0-5.6
32	<i>S. linum</i>	0.4-0.6	1.8-3.5	0.8-1.0	7.0-30.0
33	<i>S. atlanticum</i>	0.4-0.6	2.0-4.0	1.2-1.4	4.0-14.0
20	<i>S. serpens</i>	0.5-0.6	2.0-5.0	0.8-1.0	2.5-13.5
1	104	0.5-0.7	2.0-2.5	1.0-1.4	3.0-3.8
6	103	0.5-0.7	2.0-3.5	1.0-2.1	2.1-6.5
10	<i>S. peregrinum</i>	0.5-0.7	3.5-4.0	1.4-1.6	5.0-22.0
18	<i>S. sinuosum</i>	0.6-0.9	8.6-10.5	1.4-3.5	5.0-42.0
34	<i>S. bejerinckii</i>	0.7-1.0	6.3-7.2	1.5-3.0	7.0-15.5
35	101	0.8-0.9	3.5-5.0	1.4-2.0	7.0-15.0
37	206	0.7-0.9	4.0-7.0	2.0-2.8	7.0-21.0
36	102	0.8-1.0	5.0-7.0	2.0-2.8	8.5-18.0
30	<i>S. serpens</i>	0.6-0.7	7.0-10.0	1.2-2.0	5.0-10.0
23	<i>S. serpens</i>	0.7-0.8	6.3-7.0	1.4-2.1	10.0-42.0
26	<i>S. serpens</i>	0.7-0.8	6.3-7.0	1.4-2.1	7.0-17.0
28	<i>S. serpens</i>	0.7-0.8	8.4-10.5	1.4-2.1	7.0-35.0
22	<i>S. serpens</i>	0.8-0.9	5.0-10.5	1.6-2.4	5.6-19.0
24	<i>S. serpens</i>	0.8-0.9	7.0-10.0	2.4-3.0	8.0-21.0
25	<i>S. serpens</i>	0.8-0.9	5.0-10.0	1.3-3.0	6.0-14.0
27	<i>S. serpens</i>	0.8-0.9	7.0-10.0	2.1-3.0	7.0-14.0
29	<i>S. serpens</i>	0.8-1.1	3.5-10.0	2.1-2.5	3.5-16.0
21	<i>S. serpens</i>	0.9-1.0	4.2-6.5	2.8-4.2	4.0-22.0
31	<i>S. serpens</i>	0.9-1.0	10.8-12.0	1.8-2.0	8.4-15.4
38	<i>S. japonicum</i>	1.0-1.2	8.0-20.0	2.0-3.0	11.0-75.0
17	<i>S. graniferum</i>	1.0-1.2	7.0-8.4	3.5-4.2	7.0-25.0
19	<i>S. puridiconchylum</i>	0.9-1.1	6.0-7.0	1.8-2.0	4.0-22.0
7	<i>S. metamorphum</i>	1.1-1.3	— ^b	2.8-2.0	4.0-11.0
15	<i>S. giesbergeri</i>	1.1-1.4	— ^b	2.8-3.0	4.0-40.0
43	<i>S. anulus</i>	1.1-1.4	5.0-13.0	1.7-4.0	4.0-52.0
16	<i>S. anulus</i>	1.4-1.5	4.9-7.0	2.8-3.0	7.0-17.0

^a From phase-contrast microscopy of 18-h PSS broth cultures, by calibrated ocular micrometer.
^b —, Cells generally have less than one turn.

FIG. 1 to 30. Phase-contrast photomicrographs of various strains of spirilla arranged in order of increasing cell diameter. The same magnification was used throughout for a basis of comparison. (Note: small white circles present on some photographs are an artifact of the photomicroscope prism system and have no biological significance.) FIG. 1 to 21. Freshwater spirilla of the genera *Aquaspirillum* and *Spirillum*. FIG. 22 to 30. Marine spirilla of the genus *Oceanospirillum*.

FIG. 1. Strain 3 (ATCC 19624), *A. gracile*.

FIG. 2. Strain 5 (ATCC 14667), *A. delicatum*.

FIG. 3. Strain 9 (NCIB 9072), *A. polymorphum*.

- FIG. 16. Strain 7 (ATCC 15280), *A. metamorphum* (showing straighter forms after prolonged transfer).
 FIG. 17. Strain 17 (NCIB 8230), *A. giesbergi* (previously *S. graniferum*).
 FIG. 18. Strain 15 (ATCC 11334), *A. giesbergi*.
 FIG. 19. Strain 15 (ATCC 11334), *A. giesbergi* (showing straighter forms after prolonged transfer).
 FIG. 20. Strain 43 (NCIB 9012), *A. anulus*.
 FIG. 21. ATCC 19554, *S. volutans*.

- FIG. 4. Strain 13 (ATCC 12639), *A. tiersonii*.
 FIG. 5. Strain 20 (ATCC 11330), *A. aquaticum*.
 FIG. 6. Strain 6 (ATCC 27650), *A. dispar*.
 FIG. 7. Strain 1 (ATCC 27510), *A. dispar*.
 FIG. 8. Strain 10 (ATCC 15387), *A. peregrinum*.
 FIG. 9. Strain 18 (ATCC 9786), *A. sinuosum*.
 FIG. 10. Strain 25 (VH), *A. serpens*.
 FIG. 11. Strain 22 (ATCC 11335), *A. serpens*.
 FIG. 12. Strain 21 (ATCC 12289), unclassified.
 FIG. 13. Strain 29 (ATCC 12638), *A. serpens*.
 FIG. 14. Strain 19 (ATCC 15279), *A. putridiconchylum*.
 FIG. 15. Strain 7 (ATCC 15280), *A. metamorphum*.

FIG. 22. Strain 40 (ATCC 19193), *O. minutulum*.
FIG. 23. Strain 39 (ATCC 19192), *O. minutulum* (previously *S. halophilum*).
FIG. 24. Strain 32 (ATCC 11336), *O. linum*.
FIG. 25. Strain 33 (ATCC 12753), *O. linum* (previously *S. atlanticum*).
FIG. 26. Strain 34 (ATCC 12754), *O. betternickii*.
FIG. 27. Strain 37 (ATCC 27648), *O. maris*.
FIG. 28. Strain 35 (ATCC 27509), *O. maris*.
FIG. 29. Strain 36 (ATCC 27649), *O. maris*.
FIG. 30. Strain 38 (ATCC 19191), *O. japonicum*.

TABLE 3. *Spiroplasma* strains producing acid reactions from carbohydrates

Strain no.	Designation	Carbohydrates attacked
11, 12, 13	<i>S. tiersonii</i>	D-fructose ^a , glycerol
2, 3, 4	<i>S. gracile</i>	D-glucose, L-arabinose, D-galactose
10	<i>S. peregrinum</i>	D-fructose ^a

^a With fructose only, acid reactions occurred anaerobically as well as aerobically; however, no growth response occurred anaerobically. With all other carbohydrates listed, neither acid reactions nor growth responses occurred anaerobically.

many cases before a color change occurred in the corresponding aerobic tubes, no growth was evident. In the aerobic tubes, growth was heavy but was restricted to the surface of the semisolid medium. The failure to grow anaerobically with carbohydrates indicates that energy for growth cannot be derived from carbohydrates under anaerobic conditions. On the basis of color change, Terasaki (43) considered strains 10, 11, and 13 to be capable of fermentation; however, our results indicate that such spirilla are essentially oxidative rather than fermentative.

The following carbohydrates failed to yield

acidic reactions with any *Spirillum* strain: D-mannose, D-mannitol, D-ribose, D-x-lylose, L-sorbose, L-rihamnose, lactose, melibiose, sucrose, maltose, cellobiose, D-trehalose, D-raffinose, melzitose, cellulose, glycogen, dextrin, pectin, inulin, erythritol, sorbitol, dulcitol, adonitol, inositol, amygdalin, and salicin.

Certain *Spirillum* strains formed yellow or brown water-soluble pigments on PSS agar containing aromatic amino acids. These strains are listed in Table 4.

All *Spirillum* strains were oxidase positive. The catalase test was positive for all strains but was very weak with strains 19 (*S. putridiconchylum*), 33 (*S. atlanticum*), 34 (*S. bejerinckii*), and 38 (*S. japonicum*). With weak reactions, a definite accumulation of bubbles appeared within 30 min after addition of hydrogen peroxide, whereas no such accumulation ever occurred with sterile medium controls. The results for catalase were in contrast to the negative reactions previously reported for *S. japonicum*, *S. halophilum*, and *S. minutulum* (44), *S. metamorphum* (41), and *S. putridiconchylum* (40), although Terasaki (43) has recently reported positive reactions for all of these except *S. putridiconchylum*. We believe that the use of a semisolid agar medium increases the sensitivity of the test by aiding the entrapment and accumulation of bubbles formed by the cells, whereas other workers have employed broth or slant cultures. For phosphatase activity, only strains 9 (*S. polymorphum*), 35 (101), 36 (102), 37 (206), 39 (*S. halophilum*), and 40 (*S. minutulum*) failed to yield positive reactions.

All *Spirillum* strains were negative for indole production, sulfatase activity, and hydrolysis of hippurate, casein, and starch.

Colonies ranged from 0.5 to 1.5 mm in diameter, except for strains 2 to 4 (*S. gracile*), 9 (*S. polymorphum*), 16 (*S. anulus*), and 35 (strain 101), which formed pinpoint colonies. All colonies were white, circular, and convex with smooth edges, except for strains 2 to 4, which formed white colonies with fimbriated

edges. The colonies of strains 2 to 4 were also formed partially below the agar surface. When originally isolated (7), these strains formed subsurface, spreading, semitransparent colonies, the spreading occurring within the agar. After prolonged subculturing, some of the spirilla in each of these three strains were reported to have lost their ability to diffuse through agar and formed instead small, nonspreading colonies similar to those occurring in our study.

All of the marine strains failed to grow in PSS broth in the absence of seawater. None of the freshwater strains, in contrast, were able to grow in the presence of seawater. The growth of all freshwater strains was prevented by the addition of 3% NaCl to PSS broth. Tolerance to lower concentrations was determined for several strains in defined or semidefined media (Table 5). The results confirm the sensitivity to Na⁺ reported by Skerman (33).

Giesberger (12) reported that Ca²⁺ improved the growth responses of spirilla. In contrast, all of the strains we have tested in this regard (Table 6) grew in defined or semidefined media to higher turbidities in the absence of 0.05% added CaCl₂. Moreover, no change in the kinds of sole carbon sources used by each of these strains occurred by addition of the Ca²⁺.

Coccol bodies were predominant in old cultures of all of the marine strains studied except strain 38 (*S. japonicum*). Among the freshwater spirilla, coccol bodies occurred only with strains 9 (*S. polymorphum*), 10 (*S. peregrinum*), and 11 to 13 (*S. iersonii*).

The mol % G + C (T_m) for the DNA of each strain is indicated in Table 7. The values ranged from 42 to 65%. Cole (10) determined the DNA base compositions of strains 13 (*S. iersonii*) and 29 (*S. seipens*) by T_m and buoyant density methods; these values were 59.3% (T_m) and 61.2% (buoyant density) for strain 13, and 47.4% (T_m) and 48.8% (buoyant density) for strain 29. Our determinations for these strains have yielded slightly higher values.

A summary of the morphology, physiological characteristics, and DNA base compositions of

TABLE 4. *Spirillum* strains which form water-soluble pigments on PSS agar containing 0.2 % aromatic amino acids

Strain no.	Designation	Tyrosine	Phenylalanine	Tryptophan
10	<i>S. peregrinum</i>	Brown	Yellow	Brown
11, 12, 13	<i>S. iersonii</i>	Brown		Brown
32	<i>S. linum</i>	Brown		Brown
37	206	Brown		Brown
33	<i>S. atlanticum</i>	Brown		Brown
34	<i>S. bejerinckii</i>	Brown		Brown
39	<i>S. halophilum</i>	Brown		

TABLE 5. Tolerance of freshwater strains of spirilla to low concentrations of NaCl^a

Strain no.	NaCl (%)	Maximal growth (%)	Strain no.	NaCl (%)	Maximal growth (%)
1	2.0 ^b	25	18	0.5	29
6	2.0 ^b	11		0.7	0
7	1.0	42	19	1.5	15
	1.5	0		2.0	0
10	2.0 ^b	35	24	1.5	22
12	1.5	25		2.0	0
	2.0	0	25	1.5	20
13	1.5	15		2.0	0
	2.0	0	26	1.5	16
15	0.3	13		2.0	0
	0.5	0	27	1.5	19
16	0.1	61		2.0	0
	0.3	0	28	1.5	21
17	0.7	66		2.0	0
	1.0	0			

^a Strains 15 to 18 were tested in DBM + 0.1% succinate + 0.1% Casamino Acids (Difco). All other strains were tested in DBM + succinate + pyruvate + L-glutamate (0.1% each).

^b 2.0% was the highest concentration tested under the above conditions; however, no strain grew in PSS broth containing 3.0% added NaCl.

TABLE 6. Effect of added CaCl₂ on growth of spirilla^a

Strain no.	Carbon source ^b				
	A	B	C	D	E
1	0 0	15 43	18 40	10 30	10 29
7	0 0	8 16	0 21	0 0	10 20
10	0 8	0 10	0 15	0 10	10 21
16	0 0	0 0	0 0	0 0	10 21
12	25 35	20 27	25 21	0 0	18 21
19	0 0	0 24	0 25	10 20	10 28
25	0 0	0 0	0 0	0 0	24 45
26	0 0	0 0	0 0	0 0	10 20

^a Test medium was DBM + carbon source, with and without CaCl₂. Numerators indicate turbidity (Klett units) at 420 nm with 16-mm cuvettes, from cultures having 0.05% added CaCl₂. Denominators indicate corresponding turbidities from parallel cultures having no added CaCl₂.

^b A, D-fructose; B, succinate; C, L-lactate; D, acetate E, L-glutamate.

the *Spirillum* strains is presented in Tables 8 and 9.

The growth responses of *Spirillum* strains for various sole carbon sources, with (NH₄)₂SO₄ supplied as a nitrogen source, are indicated in Table 10. The following compounds failed to serve as sole carbon sources for any strain: D-glucose, D-glucconate, D-mannitol, D-galactose, D-mannose, D-ribose, D-xylose, maltose, lactose, sucrose, 2-ketogluconate, sedoheptulose, D-erythrose, D-tartrate, L-tartrate, anthranilate, *p*-hydroxybenzoate, phenol, *t*-butanol, spermine, spermidine, and L-tryptophan. Strains 2 to 4 (*S. gracile*), 20 and 21 (*S. serpens*), and 33 (*S. atlanticum*) failed to yield a positive growth response (as defined in Materials and Methods) with any sole carbon source tested. Strain 33 did grow in a defined medium when succinate plus malate were supplied as carbon sources and L-methionine as the nitrogen source (see Table 11), but strains 2 to 4, 20, and 21 did not grow under these conditions.

The growth responses of *Spirillum* strains for various sole nitrogen sources, with succinate plus malate supplied as carbon sources, are indicated in Table 11. Strains 2 to 4 (*S. gracile*), 15 (*S. giesbergii*), 18 (*S. sinuosum*), 20 and 21 (*S. serpens*), and 34 (*S. beijerinckii*) failed to grow with any of the sole nitrogen sources tested. However, growth of strains 15, 18, and 34 did occur in defined media with (NH₄)₂SO₄ as the nitrogen source when certain sole carbon sources were provided (see Table 10). Strains 2 to 4, 20, and 21 did not exhibit a positive growth response with any sole carbon or

TABLE 7. *DN_A base composition of Spirillum strains*

Strain no.	Designation	T _m	G + C (mol %)
2	<i>S. gracile</i>	96.2	65
1	104 <i>S. gracile</i>	96.1	65
3	<i>S. gracile</i>	96.1	65
4	<i>S. gracile</i>	96.1	65
20	<i>S. serpens</i>	95.8	65
5	<i>S. delicatum</i>	95.5	63
6	103 <i>S. metanorphum</i>	95.4	63
7	<i>S. metanorphum</i>	95.4	63
9	<i>S. polymorphum</i>	94.9	62
11	<i>S. tiersoni</i>	94.8	62
12	<i>S. tiersoni</i>	94.7	62
10	<i>S. peregrinum</i>	94.6	62
13	<i>S. tiersoni</i>	94.6	62
43	<i>S. anulus</i>	93.3	59
15	<i>S. giesbergeri</i>	93.2	58
16	<i>S. anulus</i>	93.1	58
21	<i>S. serpens</i>	93.0	58
17	<i>S. graniferum</i>	92.8	57
18	<i>S. sinuosum</i>	92.8	57
19	<i>S. putridiconchylum</i>	90.6	52
22	<i>S. serpens</i>	90.3	51
23	<i>S. serpens</i>	90.1	51
24	<i>S. serpens</i>	90.0	50
25	<i>S. serpens</i>	90.0	50
26	<i>S. serpens</i>	90.0	50
27	<i>S. serpens</i>	89.9	50
28	<i>S. serpens</i>	89.9	50
29	<i>S. serpens</i>	89.9	50
30	<i>S. serpens</i>	89.9	50
31	<i>S. serpens</i>	89.7	50
32	<i>S. linum</i>	89.0	48
33	<i>S. atlanticum</i>	88.9	48
34	<i>S. bejerinckii</i>	88.6	47
35	101 <i>S. japonicum</i>	88.0	46
36	102 <i>S. japonicum</i>	87.9	45
38	206 <i>S. halophilum</i>	87.9	45
37	<i>S. halophilum</i>	87.8	45
39	<i>S. minutulum</i>	86.7	42
40	<i>S. minutulum</i>	86.6	42

nitrogen source under the testing conditions, and it is likely that these strains have complex nutritional requirements.

DISCUSSION

DNA base composition. Because of the wide spectrum of values for the DNA base compositions of *Spirillum* strains, including strains of *S. volutans* (16), it would appear that these strains do not have a relatively recent common ancestry. From compiled data (34), the average span of % G + C values for bacterial genera is approximately 7%, with very few genera having spans of more than 15%, and none having spans of more than 21%. Even for genera containing a

large number of species, relatively narrow spans occur (e.g., *Pseudomonas*, 12%). The genus *Vibrio* possessed a span of 35% prior to 1963 (15), but it was later divided into more than one genus on the basis of DNA base composition and other factors (31). Accordingly, the range of % G + C values for the genus *Spirillum* of 38% (for *S. volutans*) to 65% (for *S. gracile*), representing a span of 27%, suggested to us that this genus also might be divided, and a consideration of other characteristics of spirilla supports this view.

Analysis of phenotypic characteristics. Stanier et al. (37), in an extensive characterization and classification of aerobic pseudomonads, preferred not to employ the techniques of numerical taxonomy to make phenotypic comparisons among their strains. This viewpoint was based on the fact that many characteristics to which equal weight would be assigned may not in fact be equivalent when examined in terms of their underlying enzyme mechanisms and numbers of genes involved, and in many cases the basic mechanisms are not known. We have preferred not to employ numerical taxonomy for similar reasons. These reasons seem especially applicable to the characteristics discussed below.

Obligate microaerophilism versus aerobiosis. The fundamental nature of the obligate microaerophilism of *S. volutans* is not yet known. One possibility is that an inability to synthesize sufficient levels of succinic dehydrogenase may impair the rapid utilization of oxygen via the terminal respiratory chain, resulting in toxic levels of intracellular oxygen (11). A second possibility concerns the low level of catalase activity found for *S. volutans* (16), which might permit toxic levels of peroxide to accumulate. This is supported by our findings that increased growth of *S. volutans* occurs when exogenous catalase is provided, but aerobic growth still fails to occur. Moreover, other spirilla (strains 33, 34, and 38) exhibit low levels of catalase activity and yet are not obligately microaerophilic. McCord et al. (23) found that aerobic and aerotolerant bacteria possessed superoxide dismutase activity, in contrast to the prime anaerobes. They proposed that the prime physiological function of this enzyme is protection of oxygen-utilizing organisms against the potentially detrimental effects of the superoxide free-radical. Our preliminary studies of this enzyme in *S. volutans* indicate that an activity higher than that found in *Escherichia coli* is present. Another possible explanation of obligate microaerophilism is that hypernormal ratios of adenosine 5'-triphosphate to adenosine diphosphate plus inorganic phosphate may

TABLE 8. Summary of the morphology, physiological characteristics, and DNA base composition of freshwater *Spirillum* strains^a

Test	Strain no. and designation															
	22–31 (<i>S. serpens</i>)															
	7 (<i>S. metamorphum</i>)															
	19 (<i>S. putridiconchylum</i>)															
	15, 17 (<i>S. giesbergeri</i> , <i>S. graniferum</i>)															
	16, 43 (<i>S. anulus</i>)															
	18 (<i>S. sinuosum</i>)															
	2–4 (<i>S. gracile</i>)															
	11–13 (<i>S. itersonii</i>)															
	10 (<i>S. peregrinum</i>)															
	5 (<i>S. delicatum</i>)															
	9 (<i>S. polymorphum</i>)															
	1, 6 (104, 103)															
	20 (<i>S. serpens</i>)															
	21 (<i>S. serpens</i>)															
Mol % G + C of DNA	50–51	63	52	57–58	58–59	57	65	62	62	63	62	63–65	65	58		
Cell diameter ^b	L	L	L	L	L	L	S	M	M	S	S	M	M	L		
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Phosphatase	+	+	W	+	+	+	+	+	+	+	+	+	+	+		
Sulfide from cysteine	+	+	+	d	+	+	+	+	+	+	+	+	+	+		
Indole	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
Casein, starch, hippurate	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
Sulfatase	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
Urease	d	–	–	+	–	+	+	–	+	–	–	–	–	–		
Esculin	–	–	–	–	–	–	–	+	+	–	–	–	–	–		
Gelatin	d	+	–	–	–	–	–	–	–	–	–	–	–	–		
Nitrate to nitrite	d	–	–	–	–	–	–	–	–	–	–	–	–	–		
Nitrate reduced beyond nitrite stage	–	–	–	–	–	–	+	–	–	+	–	+	–	–		
Anaerobic growth with nitrate	–	–	–	–	–	–	–	+	–	–	–	–	–	–		
Selenite reduction	–	–	–	–	–	–	–	d	+	–	+	–	+	–		
DNase	d	–	–	–	–	–	+	+	+	–	–	–	–	–		
RNase	d	+	+	+	d	–	+	+	–	+	–	–	+	–		
Acid reaction from sugars	–	–	–	–	–	–	+	+	+	+	+	+	+	+		
1% bile (growth)	+	+	+	+	+	+	–	+	+	–	+	+	+	+		
1% glycine (growth)	+	+	+	+	–	+	–	–	–	–	–	–	–	–		
TSL agar (growth)	d	+	+	–	–	–	–	–	+	–	–	+	+	+		
EMB agar (growth)	+	+	+	–	–	–	–	+	–	–	–	+	+	–		
MacConkey agar (growth)	d	–	–	–	–	–	–	+	–	–	–	+	+	–		
MRVP broth (growth)	d	–	–	+	–	–	–	+	–	+	–	+	+	–		
Pigment from aromatic amino acids	–	–	–	–	–	–	–	+	–	–	–	–	–	–		
Coccioid bodies formed	–	–	–	–	–	–	–	+	–	–	–	–	–	–		
Seller agar (growth)	d	+	–	–	–	–	–	+	+	–	–	+	+	–		
Fluorescent pigment	d	+	–	–	–	–	d	+	+	–	–	+	+	+		
Litmus milk alkaline	–	–	–	–	–	–	–	–	+	–	–	–	–	–		
3% NaCl (growth)	–	–	–	–	–	–	–	–	–	–	–	–	–	–		

^a Symbols: +, all strains positive; –, all strains negative; W, weak reaction; d, differing reactions. Where the latter symbol appears, the following strains were positive: sulfide from cysteine, 15; urease, 28; gelatin, 23 to 28, 30, 31; nitrate to nitrite, 22; selenite reduction, 12; DNase, 26, 29, 31; RNase, 16, 26, 29, 31; TSL, 22 to 25, 27, 28, 30, 31; MacConkey agar, 23 to 25, 27, 28, 30, 31; MRVP broth, 23 to 25, 27, 28, 30; Seller agar, 23, 24, 30; fluorescent pigment, 4, 23 to 31.

^b L, Large; M, medium; S, small. (See Table 2 for diameter in μm .)

occur in the presence of 21% oxygen atmospheres. Such ratios could affect the intracellular regulation of respiration and other metabolic processes. The inhibitory effect of low-phosphate concentrations on the growth of *S. volutans* (16) may have significance in this regard. Although it is difficult to assess the number of genes associated with obligate microaerophilism at present, it is noteworthy that *S. volutans*, in addition to being the only

TABLE 9. *Summary of the morphology, physiological characteristics, and DNA base composition of marine strains of Sphillum*^a

Test	Strain no. and designation				
	39, 40 (<i>S. halophilum</i> , <i>S. minutum</i>)	32, 33 (<i>S. linum</i> , <i>S. atlanticum</i>)	38 (<i>S. japonicum</i>)	34 (<i>S. bel-jerickii</i>)	35-37 (101, 102, 103)
Mol % G + C of DNA	42	48	45	47	45-46
Cell diameter ^b	S	M	L	L	L
Catalase	+	+ or W	W	W	+
Phosphatase	-	+	+	+	-
Sulfide from cysteine	-	d	-	+	+
Indole and sulfatase	-	-	-	-	-
Casein, starch, hippurate hydrolysis	-	-	-	-	-
Urease	-	-	-	-	-
Esculin	-	-	-	-	-
Gelatin liquefaction	-	-	-	-	-
Nitrate to nitrite	+	-	-	-	-
Nitrate reduced beyond nitrite stage	-	-	-	-	-
Anaerobic growth with nitrate	-	-	-	-	-
Selenite reduction	+	+	+	-	-
DNase	-	-	-	+	-
RNase	-	d	-	+	-
Acid reaction from sugars	-	-	-	-	-
1% bile (growth)	+	+	+	+	+
1% glycine (growth)	+	+	-	-	+
TSI agar (growth)	+	+	-	-	+
EMB agar (growth)	-	-	-	-	d
MacConkey agar (growth)	-	-	-	-	-
Seller agar (growth)	-	-	-	-	-
MRVP broth (growth)	-	+	-	-	+
Pigment from aromatic amino acids	d	+	-	+	d
Coccol bodies predominant in older cultures	+	+	-	+	+
Fluorescent pigment	-	+	-	-	d
9.75% total NaCl (growth)	+	+	+	+	+
12.75% total NaCl (growth)	d	-	-	-	-

^a Symbols: +, all strains positive; -, all strains negative; W, weak reaction; d, differing reactions. Where the latter symbol appears, the following strains are positive: sulfide from cysteine, 32; RNase, 33; EMB agar, 37; pigment from aromatic amino acids, see Table 4; fluorescent pigment, 37; 12.75% total NaCl, 39.

^b L, Large; M, medium; S, small. (See Table 2 for diameter in μ m).

obligately microaerophilic *Sphillum* species, possesses the lowest G + C content (38%) of any *Sphillum* (in fact, 12% lower than for any other freshwater *Sphillum*).

Cell diameter. The span of values for cell diameter within a bacterial genus usually does not exceed 1.0 μ m (4). This is in contrast to the span of 1.5 μ m (0.2 to 1.7 μ m) found for organisms in the genus *Sphillum*. Strains of *S. volutans* possess the greatest diameter of any *Sphillum* strains (16), and this can be corre-

lated with their low G + C value and their obligate microaerophilism.

Freshwater versus marine habitat. The fundamental difference between a marine bacterium versus a freshwater bacterium is not yet known. According to Stanier (36), the central problem of marine microbiology is the question of the existence of specific marine bacteria. Macleod (21) summarized a review of the question by noting that marine bacteria had special requirements for inorganic ions, partly to supply the

9		x x				x x x	x		
1	x x x x x x x x x x x x x x x					x x x	x	x x	
6	x x x x x x x x x x x x x x x					x	x	x x	
20									
21									
39		x x x x x x	x			x	x	x	
40	x x x x x x x	x				x		x	
32			x						
33									
38		x x x x				x x	x		
34		x	x						
35			x			x			
36		x x				x			
37			x			x		x	

^a ×, positive growth response = 10 Klett units or more.

TABLE 11. *Sole nitrogen sources for Spirillum strains with succinate plus malate as carbon sources^a*

Strain no.	L-Alanine	L-Glutamate	L-Aspartate	L-Glutamine	L-Asparagine	L-Proline	L-Hydroxyproline	L-Histidine	L-Tyrosine	L-Tryptophan	L-Phenylalanine	L-Ornithine	L-Citrulline	L-Lysine	L-Arginine	L-Methionine	L-Serine	L-Cysteine	Glycine	L-Leucine	L-Isoleucine	L-Valine	L-Threonine	Urea	Ammonium sulfate	Potassium nitrate
22																										
23	x	x	x	x	x	x	x					x	x	x												
24	x	x	x	x	x	x	x	x				x	x	x												
25	x	x	x	x	x	x	x					x	x	x												
26	x	x	x	x	x	x	x					x	x	x												
27	x	x	x	x	x	x	x					x	x	x												
28	x	x	x	x	x	x	x	x				x	x	x												
29	x	x	x	x	x	x	x					x	x	x												
30	x	x	x	x	x	x	x					x	x	x												
31	x	x	x	x	x	x	x	x				x	x	x												
7									x																	
19	x	x	x	x	x	x	x																			
15																										
17			x	x	x	x																				
16																										
43	x	x																								
18																										
2																										
3																										
4																										
11	x	x	x	x	x	x	x	x				x	x	x												
12	x	x	x	x	x	x	x	x				x	x	x												
13	x	x	x	x	x	x	x	x				x	x	x												
10	x	x	x	x	x	x	x	x				x	x	x												
5			x																							
9	x	x	x	x	x	x																				
1	x	x	x	x	x	x																				
6	x	x	x	x	x	x																				
20																										
21																										
39	x	x																								
40	x	x	x	x	x	x																				
32																										
33																										
38	x	x																								
34																										
35																										
36	x	x	x	x	x	x																				
37	x	x	x	x	x	x																				

^a x, positive growth response = 10 Klett units or more.

needs of the organism for growth and metabolism and partly to maintain the structural integrity of the cell. The possession of a requirement for Na^+ distinguishes marine bacteria from most nonmarine species, and the need of marine bacteria for a combination of Mg^{2+} and Ca^{2+} exceeds that of most terrestrial species. MacLeod stated that: "Although the marine bacteria examined have a number of characteristics in common, the only one which clearly distinguishes them from bacteria in other habitats is a capacity to survive and grow in the sea. In this respect, then, marine bacteria are unique." This view was further developed by Baumann et al. (2), who recognized two ecologically distinct groups in the genus *Vibrio*: straight and curved rods of marine origin, and *Vibrio cholerae* and related strains, which are inhabitants of freshwater and of the human intestine. They suggested that the ecological distinctions may reflect differences in the cell-wall and permease systems and may therefore reflect profound biochemical dissimilarity between the two groups. This view, supported by phenotypic differences and lack of significant DNA hybridization, led to a transfer of *Vibrio parahaemolyticus* to the genus *Be-necke*a, a genus containing only marine bacteria. Such considerations are applicable to the question of the distinction of freshwater spirilla from marine spirilla.

Strains of *S. volutans* are freshwater strains but are distinguishable from all other freshwater strains of *Spirillum* by their large size, obligate microaerophilism, and low G + C value. The remaining freshwater strains collectively have a cell-diameter range of 0.2 to 1.5 μm and a range of G + C values from 50 to 65%. The marine strains have a cell-diameter range of from 0.3 to 1.2 μm and a range of G + C values from 42 to 48%.

Salt tolerance. All of the marine spirilla could grow in the presence of 9.75% total NaCl, whereas none of the freshwater strains could tolerate a concentration of 3% NaCl. The tolerance of the marine strains to salt must not be assumed to be a consequence of the fact that these spirilla live in the sea (21), just as the sensitivity of the freshwater spirilla cannot be attributed merely to the fact that these strains are not marine.

In summary, on the basis of ecological differences, salt tolerance, and % G + C, we believe that the marine spirilla (strains 32 to 40) represent a group distinct from both the obligately microaerophilic freshwater spirilla and the aerobic freshwater spirilla.

Evaluation of nutritional spectra. In addition

to a lack of appropriate enzymes, inability of a strain to use a given sole carbon source or sole nitrogen source might be attributable to impermeability or to toxic effects of the compounds at the concentrations used (37). For example, Phibbs and Hylemon (Abstr. Annu. Meet. Amer. Soc. Microbiol., 1973, p. 187) demonstrated that enzymes for catabolism of glucose and fructose occur in strain 13 (*S. itersonii*), but only fructose could serve as a sole source of energy and carbon because the cells were impermeable to glucose. The mode of regulation of a metabolic pathway might also be such as to allow utilization of a compound for anabolism or catabolism without allowing it to serve as a sole carbon source or sole nitrogen source for growth (26). Some insight into the latter possibility could be obtained by testing sole carbon or nitrogen sources with a medium containing enough peptone or yeast extract to permit a low basal level of growth. Stimulation of growth may reflect an ability to utilize a carbon source, even though the compound might not be able to support growth in the absence of the supplements. Supplements providing a low basal level of growth were not employed in our study, and therefore the nutrition of spirilla cannot yet be said to be thoroughly understood. Our nutritional data should be applied with caution as a measure of enzymatic complements, and we believe the data to be useful at present only at the lower levels of classification unless they correlate well with a variety of other characterization tests. Nevertheless, the fact that most *Spirillum* strains were able to grow with at least some sole carbon or sole nitrogen sources indicates that *Spirillum* nutrition is usually simple, with only a few strains standing out as having more complex requirements.

Division of the genus *Spirillum*. From the above considerations, three major groups of spirilla appear to exist: (i) the very large, obligately microaerophilic freshwater spirilla having a % G + C of 38; (ii) the aerobic freshwater spirilla having a % G + C of 50 to 65; and (iii) the marine spirilla which possess a % G + C of 42 to 48. From the previous discussion, these groups appear to be distinguishable at the generic level rather than at the specific level, and consequently we propose a division of the genus *Spirillum* into three genera, with retention of the name *Spirillum* for that genus containing the type species of the original genus.

Genus I. *Spirillum* Ehrenberg 1832.

Description. Large, rigid, helical cells, 1.4 to 1.7 μm in diameter. Obligately microaerophilic.

FIG. 31 to 48. Electron micrographs of various strains of *Aquaspirillum* and *Oceanospirillum* showing types of flagellation. Preparations were shadowed with tungsten oxide and were all photographed at the same magnification, with the exception of Fig. 47, which was a negatively stained preparation photographed at a higher magnification.

FIG. 31. Strain 29 (ATCC 12638), *A. serpens*.

FIG. 32. Strain 39 (ATCC 19192), *O. minutulum* (previously *S. halophilum*).

FIG. 33. Strain 20 (ATCC 11330), *A. aquaticum*.
FIG. 34. Strain 13 (ATCC 12639), *A. itersonii*.

FIG. 35. Strain 35 (ATCC 27509), *O. maris*.
FIG. 36. Strain 33 (ATCC 12753), *O. immun* (previously *S. atlanticum*).

FIG. 37. Strain 7 (ATCC 15280), *A. metamorphum*.

FIG. 38. Strain 32 (ATCC 11336), *O. tinum*.

Motile by means of bipolar fascicles of flagella. Gram negative. Chemo-organotrophic, having a strictly respiratory metabolism with oxygen as the terminal electron acceptor. Oxidase and catalase positive (the latter may be very weak). Carbohydrates are neither oxidized nor fermented. Found in freshwater. The G + C content of the DNA is 36 to 38 mol %.

Species of the genus *Spirillum*. At present we include only the type species, *S. volutans* (16, 38), in this genus. This species and its neotype strain (ATCC 19554) have been described recently in detail (16) (see also Fig. 21).

Genus II. *Aquaspirillum* gen. nov. (Aq. ua. spi. ril' lum. L. noun *aqua* water; Gr. noun *spira* a spiral; M. L. dim. neut. noun *spirillum* a small

spiral; M. L. neut. noun *Aquaspirillum* a small water spiral.)

Description. Rigid, helical or helically curved cells, 0.2 to 1.5 μ m in diameter. Motile by means of fascicles of flagella at one or both poles. In one species, although multiflagellate cells occur, cells with bipolar single flagella predominate. Gram negative. Chemo-organotrophic, having a strictly respiratory metabolism with oxygen as the terminal electron acceptor. Some species can grow anaerobically with nitrate as the electron acceptor. Oxidase positive. Catalase positive, but may be weak. Indole and sulfatase negative. Casein, starch, or hippurate not hydrolyzed. No growth in the presence of 3% NaCl. Carbohydrates not

FIG. 39. Strain 21 (ATCC 12289), unclassified.

FIG. 40. Strain 19 (ATCC 15279), *A. putridiconchylium*.

fermented; a few species can oxidize a limited variety of carbohydrates. Intracellular PHB present in all but one species. Found in freshwater. The G + C content of the DNA ranges from 49 to 65 mol %. Type species: *Aquaspirillum serpens*.

Species of the genus *Aquaspirillum*. We have included strains 1 to 7, 9 to 13, 15 to 31, and 43 in this genus at present. On the basis of DNA base composition, morphology, physiology and nutrition, and serology (24), we have grouped these strains into the several species

FIG. 41. Strain 3 (ATCC 19624), *A. gracile*.
FIG. 42. Strain 1 (ATCC 27510), *A. dispar*.

indicated below.

Strains 22 to 31. The cell diameters of these strains all were in the range 0.6 to 1.1 μm . Furthermore, the G + C values of these strains ranged from 49 to 51 mol% (although our T_m

determinations indicated a minimal value of 50%, Cole [10] found strain 31 to have a value of 49% by buoyant density methods). These values were 6% lower than for any other *Aquasprillum* strains except strain 19. The

FIG. 43 and 44. *Strain 38 (ATCC 19191), O. japonicum*.
FIG. 45. *Strain 40 (ATCC 19193), O. mitsuokii*.

latter strain differed from strains 22 to 31 mainly in being able to use a wide variety of tricarboxylic acid cycle intermediates and related compounds as sole carbon sources (Table 10) and also in giving only a very weak catalase reaction. Strains 22 and 31 resembled each other in that they all utilized L-glutamate and

L-aspartate as sole carbon sources, and most utilized L-alanine; very few other carbon sources were utilized. Other characteristics reinforce the above similarities. Eight of the ten strains liquefied gelatin in 4 days, and this was in contrast to all other spirilla studied except strain 7 (which had a much greater % G + C

FIG. 46. Strain 9 (NCIB 9072), *A. polymorphum*.
FIG. 47 and 48. Strain 5 (ATCC 14667), *A. delicatum*.

value). All of the strains grew on EMB agar and in the presence of 1% bile, were phosphatase positive, and produced sulfide from cysteine. Eight of ten strains grew on TSI slants, and seven of ten grew on MacConkey agar. None of

the strains reduced selenite, grew with 1% glycine, or grew anaerobically with nitrate. Only one strain, 22, reduced nitrate to nitrite, and the reaction was weak. Other reactions listed in Table 8 are less consistent; moreover,

McElroy and Krieg (24) showed that, although strains 22 and 25 were serologically related, strains 26 and 29 were not related to them or to each other. A further consideration is that strains 23, 24, 27, 28, and 30 are said to be derivatives of strain 25 (see Table 1), and consequently it is not surprising that these strains should be very similar (but not identical) to each other. Even with this consideration, however, and despite some differences among strains 22 to 31, it is our opinion that the many similarities among the strains justify placing them together in a single species, *Aquaspirillum serpens*.

Aquaspirillum serpens (Müller) comb. nov. (Basionym: *Vibrio serpens* Müller 1786.)

Description of species. Cell diameter, 0.6 to 1.1 μm . Possess a fascicle of flagella at each pole. No growth anaerobically with nitrate. Acid reactions not produced from carbohydrates. Utilize few or no tricarboxylic acid cycle intermediates or related compounds as sole carbon sources when ammonium ions are supplied as the nitrogen source. Nitrate, urea, tyrosine, glycine, and hydroxyproline are not utilized as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Growth occurs in the presence of 1% bile and on EMB agar. No growth occurs in the presence of 1% glycine. Phosphatase activity present. Selenite not reduced. Intracellular PHB present. Sulfide is produced from cysteine. Esculin is not hydrolyzed. Pigments are not formed from aromatic amino acids. Coccoid bodies are not predominant in older cultures. Gelatin is liquefied by most strains in 4 days. The G + C values of the DNA range from 49 to 51 mol %.

Neotype strain. Müller (25), who provided the original description of the species *V. serpens*, did not designate a type strain. His original strains are not extant. Although Williams and Rittenberg (49) designated ATCC 11330 as the neotype strain, Williams later retracted this designation in a personal communication to the American Type Culture Collection (1, 32), saying that she intended to suggest ATCC strain 12638 as the neotype. We concur in this suggestion, and propose here that this strain be designated as the neotype strain since its characteristics agree with Müller's description of the species (25). It should be noted that Müller's description is not very definitive (e.g., no dimensions are provided), but as Williams has pointed out (46): "Although it would be most difficult at present to identify an isolate of the spiral organisms from the descriptions and drawings given by Müller,

the drawings do show that his organisms were morphologically similar to those observed today."

Description of the neotype strain. Cell diameter, 0.8 to 1.1 μm . Wavelength, 3.5 to 10.0 μm . Helix diameter, 2.1 to 2.5 μm . Length, 3.5 to 16.0 μm (see also Fig. 13 and 31.) Other characteristics are as given for the genus and species, with the following additional information. Negative reactions: urease production, gelatin liquefaction, nitrate reduction, growth on TSI slants, growth on MacConkey agar, growth in MRVP broth, growth on Sella agar. Positive reactions: production of deoxyribonuclease (DNase), ribonuclease (RNase), and a water-soluble, yellow-green, fluorescent pigment. The compounds serving as sole carbon sources when ammonium ions are supplied as a nitrogen source are listed for the strain in Table 10. Compounds serving as sole nitrogen sources when succinate plus malate are supplied as carbon sources are listed for the strain in Table 11. The G + C of the DNA is 50 mol % (T_m).

Strains 7, 15 to 17, 19, and 43. These strains all possessed large cell diameters (0.8 to 1.5 μm). Strain 19 differed from strains 15 to 17 and 43 in having a lower G + C (52% compared to 57 to 59%), and it also had a lower % G + C than strain 7 (52% compared to 63%); strain 19 also was only weakly catalase positive. This strain resembled the strains of *A. serpens* except for the weak catalase test and for its ability to utilize a wide variety of compounds as sole carbon sources. Strain 7 differed from strains 15 to 17 and 43 not only in its higher % G + C but also in its greater nutritional versatility—growing with most tricarboxylic acid cycle intermediates and several amino acids as sole carbon sources. Furthermore, strain 7 differed from all other spirilla studied here, except strains of *A. serpens*, in being able to liquefy gelatin rapidly. It differed from strains 15 to 17 and 43 in its production of fluorescent pigment and in its ability to grow on Sella agar. Strain 19 differed from strains 7, 15 to 17, and 43 in its ability to use a wide variety of amino acids as sole carbon sources. There appears to be no serological relationship between strain 7 and strain 19 or between these strains and strains 15 to 17 and 43. In our opinion, the above considerations appear to justify placing strains 7 and 19 in separate species (*A. metamorphum* and *A. puridiconchylum*, respectively) and excluding strains 15 to 17 and 43 from these species.

Aquaspirillum metamorphum (Terasaki) comb. nov. (Basionym: *Spirillum metamorphum* Terasaki 1961.)

Description of species. Cell diameter, 1.1 to 1.3 μm . Cells generally have less than one complete turn. Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no anaerobic growth with nitrates. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. Gelatin liquefied within 4 days. A wide variety of tricarboxylic acid cycle intermediates and related compounds and amino acids can serve as sole carbon sources when ammonium ions are supplied as the nitrogen source. Only a few amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources; nitrate and urea cannot be used. Growth occurs in the presence of 1% bile but not 1% glycine. Growth occurs on Sella slants but not in MRVP broth. Urease negative. Intracellular PHB is present. Coccioid bodies not predominant in older cultures. Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 63 mol %.

Type strain. ATCC 15280 was designated as the type strain of this species by Terasaki in a personal communication to the ATCC (1), and we formally designate it so here. It is the only strain presently included in the species. The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 15, 16, and 37.

Aquaspirillum putridiconchyllum (Terasaki) comb. nov. (Basionym: *Spirillum putridiconchyllum* Terasaki 1961).

Description of species. Cell diameter, 0.9 to 1.1 μm . Number of turns: from less than 1 to a maximum of 4, usually 3. Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no growth anaerobically with nitrate. Catalase weakly positive. Phosphatase positive. No acid reactions from sugars. Gelatin not liquefied in 4 days. A wide variety of tricarboxylic acid cycle intermediates and related compounds can serve as sole carbon sources when ammonium ions are supplied as the nitrogen source. A wide variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources; nitrate and urea are not utilized. Growth occurs in the presence of 1% bile but not 1% glycine. Growth does not occur on Sella slants or in MRVP broth. Urease negative. Intracellular PHB is present. Coccioid bodies are not predominant in older cultures. Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 52%.

Type strain. ATCC strain 15279 was designated as the type strain by Terasaki in a personal communication to the ATCC (1), and we formally designate it so here. It is the only strain presently included in the species. The

characteristics of this strain are indicated in Tables 2, 8, 10, and 11, and Fig. 14 and 40.

Strains 15 to 18 and 43. All five strains had a similar G + C content (57 to 59 mol %) and gave negative results for a number of tests: gelatin liquefaction in 4 days, nitrate and selenite reduction, acid reactions from carbohydrates, anaerobic growth with nitrate, fluorescent pigment production, growth on EMB, MacConkey, TSI, and Sella media and with 1% glycine, and formation of coccioid bodies. Moreover, McElroy and Krieg (24) showed strains 15, 17, and 18 to belong to the same serological group; they also reported a reciprocal cross-agglutination between strains 16 and 18, but the heterologous titers were so low as to preclude grouping the strains together. Despite all the above similarities, some differences did occur. Strain 18 had a smaller cell diameter than did the others (see Table 2). Strains 16 and 43 were urease negative. Strains 15 and 17 grew with 1% bile, strain 17 failed to form H_2S from cysteine, strain 43 failed to exhibit DNase activity, and strain 18 did not exhibit RNase activity (see Table 8). The five strains were similar in their failure to utilize a wide range of sole carbon sources, and the few sources utilized differed among the strains (see Table 10).

Strains 15 and 17 appeared nearly identical, except for H_2S production from cysteine and utilization of fumarate as a sole carbon source, and in our opinion they should be included in a single species. The names *S. giesbergi* and *S. graniferum* (applied to strains 15 and 17, respectively) were proposed by Williams and Rittenberg in the same paper (49), the former on page 88 and the latter on page 92. The name *S. giesbergi*, therefore, has priority over *S. graniferum*. Consequently, we apply the name *Aquaspirillum giesbergi* to the species resulting from the combination of the type strains 15 and 17.

Although strain 16 differed from strain 43 by its possession of RNase activity, its utilization of pyruvate as a sole carbon source, and its failure to utilize alanine and asparagine as sole nitrogen sources, a common ability to use glutamate as a sole carbon source or as a sole nitrogen source as well as a great number of other similarities evident from a consideration of Table 8 appear to justify inclusion of both strains within a single species, *Aquaspirillum anulus*.

Because of its smaller diameter, strain 18 differs from strains 15, 16, 17, and 43, and we assign it to a separate species, *Aquaspirillum sinuosum*.

On the basis of their numerous similarities, it is tempting to combine strains 15 to 18 and 43 into a single species, but this would be premature in view of the smaller cell diameter of strain 18 and the fact that many of the similarities are based on a common failure to yield positive tests. DNA hybridization studies would be most helpful in deciding the extent of relationship among the five strains.

Aquaspirillum giesbergeri (Williams and Rittenberg) comb. nov. (Basionym: *Spirillum giesbergeri* Williams and Rittenberg 1957.)

Description of species. Cell diameter, 1.0 to 1.4 μm . Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. Gelatin not rapidly liquefied. A very limited number of tricarboxylic acid cycle intermediates and related compounds and no amino acids can serve as sole carbon sources when ammonium ions are supplied as the nitrogen source; pyruvate can be used. Only a few amino acids can serve as sole nitrogen sources when succinate plus malate are supplied as carbon sources; nitrate cannot be used. Growth occurs in the presence of 1% bile but not 1% glycine. Growth occurs in MRVP broth but not on Seller slants. Urease positive. Intracellular PHB is present. Coccoid bodies are not predominant in older cultures. Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 57 to 58 mol %.

Type strain. ATCC strain 11334 (our strain 15) was designated as the type strain of this organism by Williams and Rittenberg (49). The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 18 and 19. It differs from the only other strain presently included in the species (strain 17) in that it produces sulfide from cysteine, cannot use fumarate as a sole carbon source, and cannot use any amino acid or urea as a sole nitrogen source.

Aquaspirillum anulus (Williams and Rittenberg) comb. nov. (Basionym: *Spirillum anulus* Williams and Rittenberg 1957.)

Description of species. Cell diameter, 1.1 to 1.5 μm . Cells are half-moon or S-shaped. Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. Gelatin not rapidly liquefied. Extremely limited in ability to use tricarboxylic acid cycle intermediates or related compounds or amino acids as sole carbon sources when ammonium ions are supplied as the nitrogen source; glutamate can be used. Only a limited variety of

amino acids but not nitrate or urea can serve as sole nitrogen sources when succinate plus malate are supplied as carbon sources; glutamate can be used. Growth does not occur in the presence of 1% bile or 1% glycine. Growth does not occur in MRVP broth or on Seller slants. Urease negative. Intracellular PHB is present. Coccoid bodies not predominant in older cultures. Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 57 to 58 mol %.

Type strain. ATCC strain 11879 (equivalent to NCIB 9012) was designated as the type strain by Williams and Rittenberg (49). The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 20. It differs from the only other strain we presently include in the species (strain 16) in that it is RNase negative, cannot use pyruvate as a sole carbon source, and cannot use alanine or aspartate as a sole nitrogen source. Its average cell diameter is also slightly smaller.

Aquaspirillum sinuosum (Williams and Rittenberg) comb. nov. (Basionym: *Spirillum sinuosum* Williams and Rittenberg 1957.)

Description of species. Cell diameter, 0.6 to 0.9 μm . Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. Gelatin is not liquefied in 4 days. Only a limited variety of tricarboxylic acid cycle intermediates and related compounds and no amino acids can serve as sole carbon sources when ammonium ions are supplied as a nitrogen source. Amino acids, nitrate, or urea cannot serve as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Growth occurs in the presence of 1% bile but not 1% glycine. Growth does not occur in MRVP broth or on Seller slants. Urease positive. Intracellular PHB is present. Coccoid bodies are not predominant in older cultures. Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 57 mol %.

Type strain. ATCC strain 9786 was designated by Williams and Rittenberg (49) as the type strain of this organism. The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 9. It is presently the only strain included in the species.

Strain 21. This strain appears to be related to strains 15 to 18 and 43. It was sent to us by the American Type Culture Collection as an unlisted strain of *S. serpens*, but it is clearly not closely related to strains 22 to 31, which we have placed in the species *A. serpens*. The latter

strains possessed a G + C value of 50 to 51%, compared to 58% for strain 21; such a difference would seem to preclude any extensive degree of DNA homology. The cell diameter and DNA base composition of strain 21 suggested a relationship to strains 15 to 18 and 43. However, it differed from these strains in that it could use none of the sole carbon or sole nitrogen sources tested, it produced a fluorescent pigment, and it was one of the few freshwater strains to grow in the presence of 1% glycine. It did resemble strains 15 to 18 and 43 in its generally negative responses to characterization tests (see Table 8). Although the overall characteristics of strain 21 indicate some relationship to strains 15 to 18 and 43, the differences listed above make us hesitate to place strain 21 in any of the species which include these strains. DNA hybridization studies would be most helpful in deciding the degree of relationship.

Strains 2, 3, and 4. Cells of these three strains possessed the smallest diameter of any spirilla (0.2 to 0.3 μm). They possessed a G + C value of 65% and were identical by all tests except fluorescent pigment production. Of all the spirilla studied here, only strains 2, 3, and 4 attacked pentoses (Table 3). McElroy and Krieg (24) showed that these strains were serologically related to each other but not to any other *Spillum* strains. Of all freshwater spirilla studied, only strain 3 failed to exhibit intracellular PHB (strains 2 and 4 were not tested). In our opinion, the ability of strains 2, 3, and 4 to attack carbohydrates appears to set them apart from other strains having a small cell diameter (strains 5 and 9), and they should be grouped together in a single species, *Aquaspirillum gracile*.

Aquaspirillum gracile (Canale-Parola et al.) comb. nov. (Basionym: *Spillum gracile* Canale-Parola et al. 1966.)

Description of species. Cell diameter, 0.2 to 0.3 μm . Motile by means of bipolar fascicles of flagella. Nitrate reduced to nitrite. No growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions produced from D-glucose, D-galactose, and L-arabinose, but from no other carbohydrates. No growth with any of a wide spectrum of sole carbon sources when ammonium ions are supplied as the nitrogen source. No growth with any of a wide spectrum of sole nitrogen sources when succinate plus malate are supplied as carbon sources. Growth occurs in the presence of 1% bile but not 1% glycine. Urease positive. Intracellular PHB appears to be absent. Coccoid bodies are not predominant in older cultures.

Pigments are not formed from aromatic amino acids. The G + C content of the DNA is 65%.

Type strain. Canale-Parola et al. (7) did not designate a type strain for this species; however, strains 2, 3, and 4 of our study were used as a basis for the original description by Canale-Parola et al. and were designated as strains D5, D4, and D6, respectively, by these authors. We therefore designate strain 3 (strain D4 of Canale-Parola et al.), ATCC 19624, as the type strain of the species.

Description of the type strain. Cell diameter, 0.2 to 0.3 μm . Wavelength, 2.8 to 3.5 μm . Helix diameter, 0.5 to 2.1 μm . Length, 3.5 to 14.0 μm (see also Fig. 1 and 41). Other characteristics are as for the genus and species, with this additional information: sulfide produced from cysteine, esculin not hydrolyzed; gelatin not liquefied in 4 days; selenite not reduced; DNase and RNase produced; growth occurs on EMB agar but not on TSI, MacConkey, or Sella agars or in MRVP broth; fluorescent pigment not produced.

Strains 11, 12, and 13. These three strains were similar in cell diameter (0.4 to 0.6 μm), DNA base composition (62 mol % G + C), reduction of nitrate beyond the nitrite stage, production of acid reactions from glycerol and D-fructose (but not from other carbohydrates), ability to grow anaerobically with nitrate, failure to grow in the presence of 1% glycine, ability to form a water-soluble fluorescent pigment, RNase and DNase activity, an ability to grow on EMB, MacConkey, TSI and Sella agars and in MRVP broth, and predominance of coccoid bodies in older cultures. The three strains produced brown, water-soluble pigments with tyrosine and tryptophan. The variety of sole carbon sources and sole nitrogen sources used by the three strains was similar (see Tables 10 and 11), but a few differences occurred. McElroy and Krieg (24) found the three strains to belong to the same serological group, and they were not antigenically related to other spirilla.

The ability of these strains to oxidize carbohydrates was shared only by strains 2, 3, 4, and 10. The former were of much smaller cell diameter, attacked a different spectrum of carbohydrates, and did not grow anaerobically with nitrate or reduce nitrate beyond the nitrite stage. Strain 10 differed from strains 11, 12, and 13 in its failure to attack glycerol, to reduce nitrate, to grow on MacConkey agar or in MRVP broth, to exhibit DNase or RNase activity, and to use the following compounds as sole carbon sources: ethanol, propanol, butanol, and a number of amino acids (see Table

10). Strain 10, unlike strains 11, 12 and 13, was urease positive. Although strain 10 resembled strains 11 to 13 in cell diameter, formation of coccoid bodies, and ability to attack carbohydrates, it nevertheless appeared to be distinguishable from these strains at the species level because of the many differences cited above. Consequently, in our opinion, strains 11 to 13 should be grouped together within a single species, *Aquaspirillum itersonii*.

Aquaspirillum itersonii (Giesberger) comb. nov. (Basionym: *Spirillum itersonii* Giesberger 1936.)

Description of species. Cell diameter, 0.4 to 0.6 μm . Motile by means of bipolar fascicles of flagella. Nitrate reduced beyond the nitrite stage; grows anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions produced from glycerol and D-fructose, but from no other carbohydrates. A wide variety of sole carbon sources can be used when ammonium ions are supplied as the nitrogen source. A wide variety of sole nitrogen sources can be used when succinate plus malate are supplied as carbon sources. Grows in the presence of 1% bile but not 1% glycine. Grows on EMB, TSI, MacConkey, and Sella agar and in MRVP broth. Brown, water-soluble pigments are produced from tyrosine and tryptophan. Urease negative. Coccoid bodies predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 6.2%.

Neotype strain. The strains on which Giesberger (Ph.D. thesis, Delft Univ., Netherlands, 1936) based his original description of *S. itersonii* are no longer available, and a neotype strain must be designated. Williams and Rittenberg (49) distinguished two subspecies: *S. itersonii* subsp. *vulgatum*, which could use nitrates as sole nitrogen sources, and *S. itersonii* subsp. *itersonii*, which could not use nitrates. They designated ATCC 11331 (our strain 11) as the type strain for the first subspecies; although they did not designate a neotype strain for *S. itersonii* subsp. *itersonii*, M. A. Williams deposited a representative strain with the American Type Culture Collection (1), ATCC 12639 (our strain 13). We have found this strain to be capable of using nitrate as a sole nitrogen source, and therefore we have been unable to confirm the existence of two subspecies within the species.

In his original description of the species, Giesberger (Ph.D. thesis, Delft Univ., Netherlands, 1936) referred to a cell diameter of 0.5 μm , a wavelength of 3.0 to 3.5 μm , bipolar tufts of flagella, intracellular granules, gram-negative reaction, positive catalase test, white

colonies, rich growth on peptone agar, inability to liquefy gelatin, an ability to use a wide variety of tricarboxylic acid cycle intermediates and related compounds as sole carbon sources, an ability to use fructose as a sole carbon source, an ability to use a wide variety of complex nitrogen compounds as sole nitrogen sources, and ability to grow anaerobically with nitrate in complex media. In all these respects, strain 13 appears to correspond exactly. Although Giesberger found glucose to be used as a sole carbon source, strain 13 (as well as strains 11 and 12) failed to use this compound. With this exception, strain 13 appears to fit closely Giesberger's description of this species; therefore, we formally designate this strain (ATCC 12639) as the neotype strain for *A. itersonii*.

Description of the neotype strain. Cell diameter, 0.5 to 0.6 μm . Wavelength, 3.0 to 3.5 μm . Helix diameter, 1.2 to 1.6 μm . Length, 2.0 to 5.6 μm (see also Fig. 4 and 34). Other characteristics are as given for the genus and species, with the following additional information. Negative reactions: gelatin liquefaction (4 days), selenite reduction, alkaline reaction in litmus milk. Positive reactions: sulfide production from cysteine, esculin hydrolysis, DNase and RNase activity, water-soluble yellowish-green fluorescent pigment production. Compounds serving as sole carbon sources when ammonium ions are supplied as the nitrogen source are listed in Table 10. Compounds serving as sole nitrogen sources are listed for this strain in Table 11. The G + C of the DNA is 6.2 mol % (T_m).

Strain 10. This strain resembled the strains of *A. itersonii* and *A. gracile* in its ability to attack carbohydrates. Its cell diameter, however, was greater than that of *A. gracile*, and the many ways in which it differs from *A. itersonii* have already been discussed; moreover, McElroy and Krieg (24) found strain 10 to be serologically unrelated to other spirilla. Because of the extensive differences, we believe that strain 10 should remain separated from the strains of *A. itersonii* in a distinct species, *Aquaspirillum peregrinum*.

Aquaspirillum peregrinum (Pretorius) comb. nov. (Basionym: *Spirillum peregrinum* Pretorius 1963).

Description of species. Cell diameter, 0.5 to 0.7 μm . Motile by means of bipolar fascicles of flagella. Nitrate not reduced; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reaction produced from D-fructose but from no other carbohydrate. Ethanol, propanol, butanol, ornithine, citrulline, arginine, lysine, methionine, serine, cysteine, gly-

cine, leucine, isoleucine, and valine do not serve as sole carbon sources when ammonium ions are supplied as a nitrogen source; a number of tricarboxylic acid cycle intermediates and related compounds, as well as fructose, can be used. A wide variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources; urea can be used. Grows in the presence of 1% bile but not 1% glycine. Can grow on EMB, TSI, and Sella agar, but not on MacConkey agar or in MRVP broth. A yellow, water-soluble pigment is formed from phenylalanine; no pigments are formed from tryptophan or tyrosine. Urease positive. Coccoid bodies predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 62 mol % (T_m).

Type strain. ATCC 15387 was designated as the type strain of *Spirillum peregrinum* by Pretorius (27). The characteristics of this strain are given in Tables 2, 8, 10, and 11, and also in Fig. 8. It is presently the only strain included in the species.

Strains 5 and 9. Both of these strains have a very small cell diameter (0.3 to 0.5 μ m) and a similar DNA base composition (62 to 63 mol % G + C). Strain 9, however, was the only freshwater strain of *Spirillum* to lack phosphatase activity, and strain 5 was the only strain lacking bipolar flagellation. Strain 5 differed from strain 9 in its ability to hydrolyze esculin and to reduce selenite, and also in its positive RNase reaction, its ability to grow on TSI agar and in MRVP broth, its inability to grow on EMB or MacConkey agars, and its lack of predominance of coccoid bodies in older cultures. Strain 5 also was much more versatile in its ability to use tricarboxylic acid cycle intermediates and related compounds as sole carbon sources (Table 10). McElroy and Krieg (24) found these strains to be serologically unrelated to each other or to other spirilla. We believe that, in view of the many differences between strains 5 and 9, these strains should be retained in separate species (*Aquaspirillum delicatum* and *Aquaspirillum polymorphum*, respectively).

Aquaspirillum delicatum (Leifson) comb. nov. (Basionym: *Spirillum delicatum* Leifson 1962).

Description of species. Cell diameter, 0.3 to 0.5 μ m. Motile, possessing from 1 to 6 flagella at one pole, usually 1 to 2 flagella. Nitrate reduced to nitrite; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. A wide variety of tricarboxylic acid cycle intermediates and related compounds, but

no amino acids, can serve as sole carbon sources when ammonium ions are supplied as the nitrogen source. A wide variety of amino acids and also nitrate can serve as sole nitrogen sources when succinate plus malate are supplied as carbon sources. No growth in the presence of 1% bile or 1% glycine. Grows on TSI agar and in MRVP broth, but not on EMB, MacConkey or Sella agars. Pigments not formed from aromatic amino acids. Urease negative. Coccoid bodies do not predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 63 mol % (T_m).

Type strain. ATCC 14667 was designated as the type strain of *Spirillum delicatum* by Leifson (19). The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 2, 47, and 48. It is presently the only strain included in the species.

Aquaspirillum polymorphum (Williams and Rittenberg) comb. nov. (Basionym: *Spirillum polymorphum* Williams and Rittenberg 1957).

Description of species. Cell diameter, 0.3 to 0.5 μ m. Motile, possessing predominantly bipolar single flagella. Nitrates reduced to nitrite, but cannot grow anaerobically with nitrate. Catalase positive. Phosphatase negative. Acid reactions not produced from carbohydrates. Only malonate, acetate, and a limited variety of amino acids are used as sole carbon sources when ammonium ions are supplied as the nitrogen source. A wide variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Can grow in the presence of 1% bile but not 1% glycine. Can grow on EMB and MacConkey agar, but not on TSI or Sella agar or in MRVP broth. Pigments not formed from aromatic amino acids. Urease negative. Coccoid bodies predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 62 mol % (T_m).

Type strain. ATCC 11332 (identical to NCIB 9072) was designated as the type strain of *Spirillum polymorphum* by Williams and Rittenberg (49). The characteristics of this strain are given in Tables 2, 8, 10, and 11, and Fig. 3 and 46. It is presently the only strain included in this species.

Strains 1 and 6. These two strains were nearly identical in their characteristics (Tables 2 and 8). Only a few differences occurred in regard to the kinds of sole carbon or sole nitrogen sources used (Tables 10 and 11). A cell diameter of 0.5 to 0.7 μ m and a DNA base composition of 63 to 65 mol % G + C suggest a possible relationship to *A. tiersonii* or *A. peregrinum*; however, acid reactions were not

produced from carbohydrates, coccoid bodies were not predominant in older cultures, and growth occurred in the presence of 1% glycine. Unlike *A. peregrinum*, but like *A. itersonii*, nitrate was reduced beyond the nitrite stage; however, unlike *A. itersonii*, growth did not occur anaerobically with nitrate. In contrast to *S. peregrinum*, strains 1 and 6 were urease negative and did not hydrolyze esculin. In contrast to *A. itersonii*, pigments were not produced from aromatic amino acids. The ability to grow in the presence of 1% glycine suggests a relationship to strain 21, but their % G + C was 5% lower than that of strain 21, and a number of other differences were evident: ability to reduce nitrate, ability to use a wide variety of sole carbon and sole nitrogen sources, etc. Strains 1 and 6 appear to represent a unique group of spirilla, and in our opinion they should be placed together in a new species, *Aquaspirillum dispar*.

Aquaspirillum dispar sp. nov. (dis³ par. L. adj. *dispar* unlike.)

Description of species. Cell diameter, 0.5 to 0.7 μm . Motile, possessing bipolar fascicles of flagella. Nitrate reduced beyond the nitrite stage, but no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. A wide variety of tricarboxylic acid cycle intermediates and related compounds and a few amino acids can be used as sole carbon sources when ammonium ions are supplied as a nitrogen source. A wide variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources; nitrate cannot be used. Grows in the presence of 1% bile and 1% glycine. Grows on EMB, TSI, MacConkey and Sella agars and in MRVP broth. Pigments not formed from aromatic amino acids. Urease negative. Coccoid bodies do not predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 63 to 65 mol % (T_m).

Type strain. We designate strain 1 as the type strain. This has been deposited with the American Type Culture Collection under the number ATCC 27510.

Description of the type strain. Cell diameter, 0.5 to 0.7 μm . Wavelength, 2.0 to 2.5 μm . Helix diameter, 1.0 to 1.4 μm . Length, 3.0 to 3.8 μm (see also Fig. 7 and 42). Other characteristics are as for the genus and species, with the following additional information. Negative reactions: esculin hydrolysis, gelatin liquefaction, DNase and RNase activity, alkaline reaction in litmus milk. Positive reactions: selenite reduc-

tion, production of a yellowish-green, water-soluble, fluorescent pigment. Compounds serving as sole carbon sources are listed in Table 11. The G + C of the DNA is 56 mol % (T_m).

Strain 20. This strain was indicated by Williams and Rittenberg (49) to be the type strain of *Spirillum serpens*, but this was later retracted (see footnote, Table 1). It is clearly not closely related to strains 22 to 31, which we have placed in *A. serpens*, from a consideration of its smaller cell diameter and higher % G + C. These considerations suggest a possible relationship to strains 1, 6, and 10 to 13. A further resemblance to strains 1 and 6 (*A. dispar*) was an ability to grow with 1% glycine. Unlike strains 1, 6, and 10 to 13, however, all of which grew with a wide variety of sole carbon or nitrogen sources (Tables 10 and 11), strain 20 failed to grow with any sole carbon or sole nitrogen source provided. Unlike strains 1 and 6, strain 20 failed to reduce nitrate beyond the nitrite stage; it also reduced selenite and exhibited DNase and RNase activity, and it formed a fluorescent, water-soluble pigment. Strain 20 differed from strains 10 to 13 by its failure to attack carbohydrates and failure to exhibit a predominance of coccoid bodies in older cultures. It differed from strains 11 to 13 by its inability to grow anaerobically with nitrate and by its failure to hydrolyze esculin. McElroy and Krieg (24) found strain 20 to differ serologically from all other spirilla they tested. From all these considerations, we believe that strain 20 should be assigned to a new species, for which we propose the name *Aquaspirillum aquaticum*.

Aquaspirillum aquaticum sp. nov. (aqua ti.cum. L. adj. *aquaticus* living in water).

Description of species. Cell diameter, 0.5 to 0.6 μm . Motile, possessing bipolar fascicles of flagella. Nitrate reduced to nitrite, but not beyond the nitrite stage; no growth anaerobically with nitrate. Catalase and phosphatase positive. Acid reactions not produced from carbohydrates. None of a wide variety of sole carbon sources can be used when ammonium ions are supplied as a nitrogen source. None of a wide variety of sole nitrogen sources can be used when succinate plus malate are supplied as carbon sources. Grows in the presence of 1% bile or 1% glycine. Grows on EMB, TSI, MacConkey and Sella agars and in MRVP broth. Pigments are not formed from aromatic amino acids. Urease negative. Coccoid bodies do not predominate in older cultures. Intracellular PHB is present. The G + C content of the DNA is 65 mol % (T_m).

Type strain. We designate strain 20 (ATCC 11330) as the type strain of the species. It is presently the only strain included in this species.

Description of the type strain. Cell diameter, 0.5 to 0.6 μm . Wavelength, 2.0 to 5.0 μm . Helix diameter, 0.8 to 1.0 μm . Length, 2.5 to 13.5 μm (see also Fig. 5 and 33). Other characteristics are as for the genus and species, with the following additional information. Negative reactions: esculin hydrolysis, gelatin liquefaction. Positive reactions: sulfide production from cysteine; selenite reduction, DNase and RNase activity; water-soluble, yellowish-green, fluorescent pigment production; alkaline reaction in litmus milk. The G + C of the DNA is 65 mol % (T_m).

Genus III. *Oceanospirillum* gen. nov. (Oceano, *o.spirillum*. M. L. noun *oceanus* ocean; Gr. noun *spira* a spiral; M. L. neut. noun *spirillum* a small spiral; M. L. neut. noun *Oceanospirillum* a small ocean spiral.)

Description. Rigid, helical cells, 0.3 to 1.2 μm in diameter. Able to grow aerobically. Motile by means of bipolar fascicles of flagella. Gram negative. Chemo-organotrophic, having a strictly respiratory metabolism with oxygen as the terminal electron acceptor. No growth anaerobically with nitrate. Intracellular PHB is present. Oxidase positive. Catalase positive, but may be very weak. Indole and sulfatase negative. Casein, starch, or hippurate not hydrolyzed. Seawater required for growth. Growth can occur with 9.75% total NaCl. Carbohydrates neither oxidized nor fermented. Found in marine environments. The G + C content of the DNA ranges from 42 to 48 mol % (T_m). Type species: *Oceanospirillum linum*.

Species of the genus *Oceanospirillum*. We have included strains 32 to 40 in this genus and have grouped them into several species on the basis of DNA base composition, morphology, physiology, and nutrition.

Strains 39 and 40. These two strains had the smallest cell diameter of any marine spirilla and also the lowest % G + C. They were nearly identical in their characteristics; however, strain 39 differed from strain 40 in its production of a brown, water-soluble pigment from tryptophan and its ability to grow with 12.75% total NaCl. The two strains were able to use a similar variety of sole carbon or sole nitrogen sources (see Tables 10 and 11), but a few differences occurred. The ability of strains 39 and 40 to reduce nitrate was unique among the marine spirilla. McElroy and Krieg (24) found the two strains to be serologically indistinguishable

from each other, yet distinct from all other spirilla studied. In view of the above considerations, we propose that strains 39 and 40 be placed together in a distinct species.

Strain 39 was isolated by Watanabe and deposited in the American Type Culture Collection under the number 19192 as a representative strain of *Spirillum halophilum* Watanabe 1959. In his original description of this species, however, Watanabe (44) referred to a cell diameter of 0.5 to 0.6 μm , somewhat larger than the diameter of strain 39 (0.3 to 0.6 μm , but usually 0.3 to 0.4 μm). Moreover, Watanabe referred to the inability of the species to exhibit catalase activity and reduce nitrates; he also noted that the species could produce slight acid from glucose. Strain 39 does not correspond to these characteristics. Because of the several points of difference and because strain 39 so closely resembles strain 40 (a strain deposited in the American Type Culture Collection as a representative strain of the species *Spirillum minutum*), we consider strain 39 not to be a strain of *S. halophilum* but rather a strain of *S. minutum*.

In his description of *Spirillum minutum*, Watanabe (44) referred to the lack of catalase activity in this species; however, strain 40 (ATCC 19193) is catalase positive. Nevertheless, in all other respects, strain 40 does correspond to the original description of the species. Furthermore, because it was deposited with the American Type Culture Collection by Watanabe as a strain of *S. minutum*, we believe that strain 40 does represent this species. Strain 39 and 40 are nearly identical, and therefore we propose that these two strains be placed together in a single species, *Oceanospirillum minutum*.

Oceanospirillum minutum (Watanabe) comb. nov. (Basionym: *Spirillum minutum* Watanabe 1959.)

Description of species. Cell diameter, 0.3 to 0.4 μm . Other characteristics are as for the genus, with the following additional information. Negative reactions: phosphatase, sulfide production from cysteine, urease production, esculin hydrolysis, gelatin liquefaction, DNase and RNase production, growth on EMB, MacConkey, and Sella agars, growth in MRVP broth, fluorescent pigment production. Positive reactions: nitrate reduction to nitrite (but not beyond the nitrite stage), catalase (strongly positive), selenite reduction, growth in the presence of 1% bile and 1% glycine, growth on TSI agar, coccoid bodies predominant in older cultures. A wide variety of tricarboxylic acid

cycle intermediates and related compounds and a limited variety of amino acids can be used as sole carbon sources when ammonium ions are supplied as a nitrogen source. A moderately large variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Intracellular PHB is present. The G + C content of the DNA is 42 mol % (T_m).

Neotype strain. Because a type strain was not previously designated for this species, and because we have been unable to determine if strain 40 was used as a basis for the original species description, we therefore designate strain 40 (ATCC 19193) as the neotype strain of *Oceanospirillum minimum*.

Description of the neotype strain. Cell diameter, 0.3 to 0.4 μm . Wavelength, 2.0 to 3.0 μm . Helix diameter, 0.6 to 0.8 μm . Length, 3.0 to 8.0 μm (see also Fig. 22 and 45). Other characteristics are as for the genus and species, with the following additional information. No pigments are produced from aromatic amino acids. Growth does not occur in the presence of 12.75% total NaCl. Compounds serving as sole carbon sources when ammonium ions are supplied as a nitrogen source are listed for the strain in Table 10. Compounds serving as sole nitrogen sources when succinate plus malate are provided as carbon sources are listed in Table 11. The G + C content of the DNA is 42 mol % (T_m).

Strains 32 and 33. These two strains were of moderate cell diameter, whereas all other marine spirilla possessed larger or smaller diameters. They differed from all other marine strains in the genus in that they utilized only L-methionine as a sole nitrogen source when succinate plus malate were supplied as carbon sources (Table 11). However, a slight growth response by strain 32 with acetate serving as the sole carbon source (Table 10) indicated that ammonium ions could also serve as a sole nitrogen source for this strain. Strain 32 differed in only a few respects from strain 33: it formed a brown, water-soluble pigment from tryptophan (although both strains formed a brown pigment from tyrosine), was strongly catalase positive (strain 33 gave a weak reaction), produced sulfide from cysteine, and failed to exhibit RNase activity. Except for these differences, strains 32 and 33 closely resemble each other; furthermore, McElroy and Krieg (24) found that they were serologically indistinguishable from each other, yet were distinct from all other spirilla studied. Their DNA base composition of 48 mol % G + C was 6% greater than that for strains 39 and 40 (but

only 1 to 3% greater than that for other marine spirilla). Strains 32 and 33 differed from strains 34 to 36 and 38 to 40 by producing a fluorescent pigment. Unlike strains 39 to 40, they failed to reduce nitrate, and unlike strains 34 and 38, they grew in the presence of 1% glycine and on TSI agar. They differed from strains 34 to 37 by reducing selenite. In contrast to strains 34 and 38 to 40, they grew in MRVP broth, and unlike strains 37 to 40, they produced a brown pigment from tyrosine. The overall pattern of characteristics for strains 32 and 33 is unique and appears to justify their placement together in a separate species.

The names *Spirillum linum* and *Spirillum atlanticum* (the type strains of which are strains 32 and 33, respectively) were proposed by Williams and Rittenberg in the same paper (49), the former on page 82 and the latter on page 91. The name *S. linum*, therefore, has priority over *S. atlanticum*; consequently, we apply the name *Oceanospirillum linum* to the species resulting from the combination of the type strains 32 and 33.

Oceanospirillum linum (Williams and Rittenberg) comb. nov. (Basionym: *Spirillum linum* Williams and Rittenberg 1957.)

Description of species. Cell diameter, 0.4 to 0.6 μm . Negative reactions: urase production, esculin hydrolysis, gelatin liquefaction, nitrate reduction, DNase production, growth on EMB, MacConkey, and Sella agar, growth in the presence of 12.75% NaCl. Positive reactions: catalase (may be weak) and phosphatase production, selenite reduction, growth in the presence of 1% bile and 1% glycine, growth on TSI agar and in MRVP broth, production of a brown, water-soluble pigment from tyrosine, fluorescent pigment production, and predominance of coccoid bodies in older cultures. Very few, if any, tricarboxylic acid cycle intermediates or related compounds and no amino acids are used as sole carbon sources when ammonium ions are supplied as a nitrogen source. Only L-methionine can be used as a sole nitrogen source when succinate plus malate are supplied as carbon sources. Intracellular PHB is present. The G + C content of the DNA is 48 mol % (T_m).

Type strain. ATCC 11336 (our strain 32) was designated as the type strain of *Spirillum linum* by Williams and Rittenberg (49). The characteristics of this strain are indicated in Tables 2, 9, 10, and 11, and Fig. 24 and 38, with the following additional information. Catalase reaction: strongly positive. Sulfide is produced from cysteine. RNase negative. Brown, water-soluble pigment produced from tryptophan.

Strain 38. This strain had the largest cell diameter of any marine spirillum (1.0 to 1.2 μm). Its DNA base composition of 45 mol % G + C did not sharply distinguish it from other *Oceanospirillum* strains, however. Unlike other marine spirilla, strain 38 did not exhibit a predominance of coccoid bodies in older cultures. Unlike strains 35 to 37 and 39 to 40, it was phosphatase positive, and it differed from strains 32 to 37 in being able to use a wider variety of tricarboxylic acid cycle intermediates as sole carbon sources (Table 10), although the variety was still rather limited. Unlike strains 39 and 40, it did not reduce nitrate, and in contrast to strains 32, 33, 35 to 37, 39 and 40, it failed to grow in the presence of 1% glycine. It differed from strains 32, 33, and 37 by its failure to form a fluorescent pigment, and it differed from strains 32 to 34, 37 and 39 in its failure to form pigments from any aromatic amino acid. Unlike strains 35 to 37, it reduced selenite. Further comparisons substantiate a unique pattern of characteristics for this strain; moreover, McElroy and Krieg (24) found this strain to be serologically unrelated to any other spirilla studied. In view of these considerations, we believe it should be placed in a separate species, *Oceanospirillum japonicum*.

Oceanospirillum japonicum (Watanabe) comb. nov. (Basionym: *Spirillum japonicum* Watanabe 1959.)

Description of species. Cell diameter, 1.0 to 1.2 μm . Negative reactions: sulfide from cysteine, urease production, gelatin liquefaction, esculin hydrolysis, nitrate reduction, DNase and RNase production, growth in the presence of 1% glycine, growth on TSI, EMB, MacConkey, and Sella agar, and in MRVP broth, pigment production from aromatic amino acids, fluorescent pigment production, growth in the presence of 12.75% total NaCl, and predominance of coccoid bodies in older cultures. Positive reactions: catalase (very weak) and phosphatase production; selenite reduction, growth in the presence of 1% bile. A limited variety of tricarboxylic acid cycle intermediates and related compounds and a few amino acids can be used as sole carbon sources when ammonium ions are supplied as a nitrogen source. A wide variety of amino acids can be used as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Intracellular PHB is present. The G + C content of the DNA is 45 mol % (T_m).

Neotype strain. A type strain has not previously been designated for the species, although Watanabe deposited a representative

strain with the American Type Culture Collection under the number 19191 (our strain 38). We have been unable to determine if this strain was used as a basis for the original species description (44). In this description, Watanabe referred to a negative catalase reaction; however, strain 38 is catalase positive. Because it is only very weakly catalase positive (with an accumulation of bubbles appearing only after 15 to 30 min) and because strain 38 appears to fit closely the original species description with respect to other characteristics, we designate strain 38 (ATCC 19191) as the neotype strain of the species.

Description of the neotype strain. Cell diameter, 1.0 to 1.2 μm . Wavelength, 8.0 to 20.0 μm . Helix diameter, 2.0 to 3.0 μm . Length, 11.0 to 75.0 μm . (See also Fig. 30, 43, and 44). Other characteristics are as for the genus and species. Compounds serving as sole carbon sources are listed in Table 10. Compounds serving as sole nitrogen sources are listed in Table 11. The G + C content of the DNA is 45% (T_m). This strain is presently the only one included in this species.

Strain 34. This strain possessed a cell diameter similar to that for strains 35 to 37, but larger than that for strains 39 and 40 and smaller than that for strain 38. Its DNA base composition was 5% greater in G + C than that for strains 39 and 40, but was not sharply distinguishable from that for other marine spirilla. Strain 34 was the only *Oceanospirillum* strain to exhibit DNase activity, and it was also the only strain to fail to grow with any sole nitrogen source provided (Table 11) when a combination of succinate plus malate was supplied as carbon sources. A slight growth response, however, with malate or pyruvate as a carbon source, did occur with ammonium ions (Table 10). Unlike strains 39 and 40, strain 34 did not reduce nitrate. In contrast to strains 32, 33, and 38 to 40, it did not reduce selenite, and it differed from strains 35, 36, and 38 to 40 in that it produced a brown pigment from tyrosine. Unlike strains 32, 33, and 37 it failed to form a fluorescent pigment, and it differed from strains 32, 33, 35 to 37, and 39 to 40 by its failure to grow in the presence of 1% glycine or on TSI agar. It produced sulfide from cysteine, thus differing from strains 33 and 38 to 40. McElroy and Krieg (24) found strain 34 to be serologically unrelated to any other spirilla studied, and its unique pattern of characteristics appears to justify its placement in a separate species, *Oceanospirillum bejerinckii*.

Oceanospirillum bejerinckii (Williams and

Rittenberg) comb. nov. (Basionym: *Spirillum beierneckii* Williams and Rittenberg 1957.)

Description of species. Cell diameter, 0.7 to 1.0 μm . Negative reactions: urease production, esculin hydrolysis, gelatin liquefaction, nitrate reduction, selenite reduction, growth in the presence of 1% glycine, growth on TSI, EMB, MacConkey, or Sella agar or in MRVP broth, fluorescent pigment production, growth in the presence of 12.75% NaCl. Positive reactions: catalase (weak) and phosphatase production, sulfide production from cysteine, DNase and RNase production, growth in the presence of 1% bile, production of a water-soluble, brown pigment from tyrosine, predominance of coccoid bodies in older cultures. Very few tri-carboxylic acid cycle intermediates and no amino acids are utilized as sole carbon sources when ammonium ions are supplied as a nitrogen source. No amino acids are used as sole nitrogen sources when succinate plus malate are provided as carbon sources. Intracellular PHB is present. The G + C content of the DNA is 47 mol % (T_m).

Type strain. ATCC strain 12754 (our strain 34) was designated by Williams and Rittenberg (49) as the type strain of the species. The characteristics of this strain are indicated in Tables 2, 9, 10, and 11, and Fig. 26. It is presently the only strain included in this species.

Strains 35 to 37. These new isolates were similar to each other in DNA base composition (45 to 46 mol % G + C), cell diameter (0.7 to 1.0 μm), and in most physiological and nutritional tests, although strain 37 was somewhat more versatile than the others by its ability to grow on EMB agar, its formation of brown, water-soluble pigments from tyrosine and tryptophan, and formation of a fluorescent pigment. Strain 37 was also more versatile in its utilization of sole nitrogen sources (Table 11), but it resembled strains 35 and 36 in its limited spectrum of sole carbon sources (Table 10). The three strains were all negative for phosphatase, thereby differing from all other *Oceanospirillum* strains except strains 39 and 40; their G + C contents and cell diameters were greater than those of strains 39 and 40. Strains 35 to 37 differed from strains 39 and 40 also by their failure to reduce nitrate and by their limited variety of sole carbon sources. Unlike strains 32, 33, and 38 to 40, they failed to reduce selenite, and unlike strains 34 and 38, they grew in the presence of 1% glycine and on TSI agar. They produced sulfide from cysteine in contrast to strains 33 and 38 to 40. Strains 35 to 37 also used a much wider variety of sole

nitrogen sources than did strains 32 to 34. From their overall pattern of characteristics, we believe that strains 35 to 37 should be placed together in a new species, *Oceanospirillum maris*.

Oceanospirillum maris sp. nov. (maris. L. noun *mare* the sea; L. gen. noun *maris* of the sea.)

Description of species. Cell diameter, 0.7 to 1.0 μm . Negative reactions: phosphatase and urease production, esculin hydrolysis, gelatin liquefaction, nitrate reduction, selenite reduction, sulfide production from cysteine, growth on TSI agar and in MRVP broth, predominance of coccoid bodies in older cultures. Only a very limited variety of tri-carboxylic acid cycle intermediates and amino acids can serve as sole carbon sources when ammonium ions are supplied as a nitrogen source. A moderately large variety of amino acids can serve as sole nitrogen sources when succinate plus malate are supplied as carbon sources. Intracellular PHB is present. The G + C content of the DNA is 45 to 46 mol % (T_m).

Type strain. Strain 35 is designated as the type strain of *O. maris*. This strain has been deposited with the American Type Culture Collection under the number 27509. The description of this strain is as for the genus and species, with the following additional information. Cell diameter, 0.8 to 0.9 μm . Wavelength, 3.5 to 5.0 μm . Helix diameter, 1.4 to 2.0 μm . Length, 7.0 to 15.0 μm (see also Fig. 28 and 35). No growth on EMB agar. No pigment production from aromatic amino acids. No fluorescent pigment produced. Compounds serving as sole carbon sources are listed in Table 10. Compounds serving as sole nitrogen sources are listed in Table 11. The G + C content of the DNA is 46 mol % (T_m).

A summary of the differential characteristics of the genera *Spirillum*, *Aquaspirillum*, and *Oceanospirillum* is as follows. Those characteristics which these genera have in common include: rigid helical or helically curved bacteria. Motile by means of polar flagella. Gram negative. Chemo-organotrophic, having a strictly respiratory metabolism. Oxidase positive. Catalase positive (may be weak). Indole and sulfatase negative. Cannot hydrolyze casein, starch or hippurate.

The genera *Spirillum* and *Aquaspirillum* differ from the genus *Oceanospirillum* in that they cannot grow in media containing 3% NaCl

TABLE 12. *Differentiation among the species of the genera Spirillum and Aquaspirillum*^a

Species	Cell diameter, μ m	% G + C of DNA	Obligately microaerophilic	Acid from carbohydrates	Phosphatase	Anaerobic growth with nitrate	Gelatin liquefaction, 4 days	Coccoid bodies	Nitrate reduced to nitrite	Nitrate reduced beyond nitrite stage	Esculin hydrolysis	Urease	1% bile—growth	1% glycine—growth	Pigments from aromatic amino acids	Versatile with respect to tricarboxylic acid cycle intermediates and related compounds as sole C sources	Versatile with respect to sole N sources	Bipolar flagella	TSI agar, growth	EMB agar, growth	MacConkey agar, growth	Seller agar, growth	MRVP broth, growth	Fluorescent pigment	Intracellular PHB	Litmus milk, alkaline	Catalase	DNase	RNase
<i>A. gracile</i>	0.2–0.3	65	–	–	+	+	–	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–	d	–	–	+	–	+
<i>A. delicatum</i>	0.3–0.4	63	–	–	–	+	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	+
<i>A. polymorphum</i>	0.3–0.5	62	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. itersonii</i>	0.4–0.6	62	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. peregrinum</i>	0.5–0.7	62	–	–	+	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. dispar</i>	0.5–0.7	63–65	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. aquaticum</i>	0.5–0.6	65	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. sinuosum</i>	0.6–0.9	57	–	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. serpens</i>	0.6–1.1	50–51	–	–	–	–	–	–	d	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. putridiconchylum</i>	0.9–1.1	52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	d	–	–	–	–	–	–	–	–	–
<i>A. metamorphum</i>	1.1–1.3	63	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. giesbergeri</i>	1.0–1.4	57–58	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>A. anulus</i>	1.1–1.5	58–59	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>S. volutans</i>	1.4–1.7	38	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

^a +, all strains positive; –, all strains negative; d, difference among strains; W, weak reaction; i, intermediate.

or seawater and are found in freshwater sources, whereas *Oceanospirillum* requires seawater, can grow in media containing 9.75% total NaCl, and is found in marine sources.

Characteristics attributed to *Spirillum* include the following, as well. Obligately microaerophilic. Cell diameter greater than 1.5 µm. Motile by means of bipolar fascicles of flagella. Cannot grow anaerobically with nitrate. Cannot oxidize carbohydrates. Intracellular PHB is present. G + C of the DNA is 36 to 38 mol %.

Additional characteristics of *Aquaspirillum* are as follows. Aerobic. Cell diameter from 0.2 to 1.5 µm. Motile by means of polar fascicles of flagella at one or both poles. In one species, although fascicles of flagella occur, bipolar, single flagella predominate. One species can grow anaerobically with nitrate. One species lacks intracellular PHB. Some species can oxidize a limited variety of carbohydrates. G + C of the DNA is 49 to 65 mol %.

Oceanospirillum has, in addition to the above mentioned, the following characteristics. Aerobic. Cell diameter from 0.3 to 1.2 µm. Motile by means of bipolar fascicles of flagella. Cannot grow anaerobically with nitrate. Cannot oxidize carbohydrates. Intracellular PHB present. G + C of the DNA is 42 to 48 mol %.

Within *Spirillum* and *Aquaspirillum*, differentiation of the species is indicated in Table 12. The differentiation of the species within the genus *Oceanospirillum* is given in Table 13.

Limits of variation and future changes in descriptions. Because the descriptions presented here are in many cases based only upon a single strain, the degrees of variation within the species and genera remain to be defined. As new strains are isolated and compared to those

already described, and as data from DNA hybridization studies become available, modification of species and genus descriptions will undoubtedly follow—just as our study has modified previous descriptions of spirilla. Moreover, although a consistent methodology should be used for characterizing spirilla in order to provide a suitable basis for comparison, new methods or improvements of existing methods may reveal unsuspected relationships. For example, although we used the highest commercially available grade of compounds for determination of nutritional requirements, some scant growth responses may possibly be found to be attributable to low levels of impurities in these preparations.

Several species of spirilla have not been treated within the framework of our study. In many cases, no type strain or reference strain for a species appeared to be available; for example, the type strain of *Spirillum man-cuniense* Cayton and Preston 1955 (9) has been lost. In the case of *Spirillum virginianum* Dmitroff 1926 (13), no type strain was thought to exist, but in 1960 M. A. Williams deposited a strain said to be a lineal descendant of Dmitroff's culture with the National Collection of Industrial Bacteria; we have only recently obtained this strain (NCIB 9075) and have not yet characterized it.

In the case of *Spirillum lunatum* Williams and Rittenberg 1957 (49), Terasaki (43) has noted several characteristics of the type strain (ATCC 11337) which are not consistent with the original description of this marine species. These characteristics include an ability to grow in either the presence or absence of seawater, a small cell diameter, and unipolar single or

TABLE 13. *Differentiation among the species of the genus Oceanospirillum*^a

Species	Cell diameter, µm	% G + C of DNA	Phosphatase	Catalase	Sulfide from cysteine	Nitrate reduced to nitrite	Selenite reduction	DNase	RNase	1% glycine—growth	TSI agar—growth	EMB agar—growth	MRVP broth—growth	Pigments from aromatic amino acids	Coccoid bodies	Fluorescent pigment	Growth with 12.75% NaCl	Versatile with respect to tricarboxylic acid cycle intermediates and related compounds as sole C sources	Versatile with respect to sole N sources
<i>O. minutulum</i>	0.3-0.4	42	-	+	-	+	-	-	+	+	-	-	d	+	+	-	d	+	+
<i>O. lunum</i>	0.4-0.6	48	+	+ / W	d	+	+	d	+	+	-	+	+	+	+	+	-	-	-
<i>O. bejerinckii</i>	0.7-1.0	47	+	W	+	-	+	+	+	-	-	-	-	+	+	+	-	-	-
<i>O. maris</i>	0.7-1.0	45-46	-	+	+	-	-	-	-	+	+	d	+	d	+	d	-	-	+
<i>O. japonicum</i>	1.0-1.2	45	+	W	-	-	+	-	-	-	-	-	-	-	-	-	-	i	+

^a +, all strains positive; -, all strains negative; d, difference among strains; W, weak reaction; i, intermediate.

bipolar single flagella (see reference 47). We have confirmed these results in a preliminary study of the species and have also determined the % G + C of the DNA of strain 11337 to be 63 mol % (T_m), far higher than for any other marine spirillum we have examined. Terasaki (43) also noted the ability of ATCC 11337 to attack carboxylates; again, this is not characteristic of other marine spirilla studied. *S. lunatum* strain 54 of the National Collection of Marine Bacteria was deposited by M. A. Williams in 1958, and a comparison of this strain with ATCC 11337 may be useful in attempting to resolve the problem.

With regard to *Spirillum minor* Carter 1888 (8), the causative agent of one form of rat-bite fever in man, this organism has never been cultivated on artificial media. Morphologically, it appears to be a spirillum (29). It is said to be a natural parasite of rats, which act as healthy carriers. Although this organism would seem not to fit into the genera discussed in our study because of its parasitic nature, this cannot yet be certain. Moreover, because of its habitat and widespread distribution, it has been described under many names, and some of these may indicate separate species. Further information is required before its taxonomic status can be clarified.

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