Request for an Opinion

Proposal of *Spirillum winogradskyi* sp. nov., a novel microaerophilic species, an emended description of the genus *Spirillum* and Request for an Opinion regarding the status of the species *Spirillum volutans* Ehrenberg 1832

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A novel obligately organotrophic, facultatively microaerophilic spirillum, designated strain D-427^T, was isolated from sulfidic sludge of a municipal wastewater-treatment plant. Cells were Gram-negative, large and highly motile due to bipolar tufts of flagella covered with mucous sheaths. Coccoid cells were sometimes formed. Strain D-427^T grew optimally at pH 7.5–7.8 and 28 °C in the presence of 2 % O_2 in the gas phase. The organism showed oxidase and very low catalase activity. The isolate grew chemo-organotrophically with a limited number of organic acids as substrates. The DNA G+C content was 38.0 mol% (T_m). Phylogenetic analysis of the 16S rRNA gene sequence placed strain D-427^T in the genus *Spirillum* within the class *Betaproteobacteria*. The 16S rRNA gene sequence similarity between strain D-427^T and *Spirillum volutans* ATCC 19554^T, the type strain of the single species of the genus, was 98.6 %. The low level of DNA–DNA hybridization and different phenotypic properties indicate that strain D-427^T is clearly distinguishable from *Spirillum volutans*. No strain of *S. volutans* is available from any established culture collection or from the authors who described this species. Therefore, on the basis of phenotypic and genotypic data and the fact that the type and single species of the genus *Spirillum* cannot be included in any scientific study, since the type strain has been lost, we propose to assign strain D-427^T as a novel species of the genus *Spirillum*, *Spirillum winogradskyi* sp. nov. (type strain D-427^T =DSM 12756^T =VKM B-2518^T), and we request that the Judicial Commission place the name *Spirillum volutans* on the list of rejected names if a suitable type strain is not found or a neotype is not proposed within 2 years following the publication of this paper. An emended description of the genus *Spirillum* is also provided.

The genus *Spirillum* Ehrenberg 1832 is one of the oldest established bacterial genera. The formal description of the genus has been changed several times (Williams & Rittenberg, 1957). In 1973, the genus *Spirillum* was defined as encompassing large, obligately microaerophilic freshwater spirilla having a DNA base composition of 36–38 mol% G+C and included only a single species, the type species *Spirillum volutans* (Hylemon *et al.*, 1973a, b). Phylogenetic analysis based on 16S rRNA gene sequences showed that the genus *Spirillum* falls within the family *Spirillaceae* of the *Betaproteobacteria* (Krieg, 2005). At present, the description of the genus is based mainly on the

The GenBank/EMBL/DDBJ accession number for the 16S r RNA gene sequence of strain D-427^T is AY845251.

Phase-contrast and transmission electron micrographs of strain D-427^T and its detailed fatty acid profile are available as supplementary material with the online version of this paper.

phenotypic properties of a single species, which was represented by two strains (Rittenberg & Rittenberg, 1962; Wells & Krieg, 1965). In spite of the fact that, according to ecological observations, large spirilla are widespread in freshwater and anthropogenic water habitats representing niches with low levels of oxygen and sulfide (Dubinina *et al.*, 1993; Kuenen & Dubinina, 2005), other representatives of the genus *Spirillum* have not been obtained in pure culture.

We have isolated a heterotrophic, microaerophilic, spiral-shaped strain that was classified as a representative of the genus *Spirillum* (Podkopaeva *et al.*, 2006). Strain D-427^T was originally given the name '*Spirillum winogradskii*', but this name has not been validly published. In this paper, we present the results of a polyphasic taxonomic study of strain D-427^T and discuss the status of the type species of the genus *Spirillum*, *S. volutans*, the type strain (ATCC 19554^T) and reference strain ATCC 19553 both having been lost.

Samples from aeration tanks of a municipal wastewater-treatment plant containing sulfide at 1–2 mg l^{-1} and oxygen at 2.0–3.5 mg l^{-1} were used for inoculation of an enrichment using modified semi-liquid MPSS medium (Caraway & Krieg, 1974) with a freshly prepared FeS suspension (Kucera & Wolfe, 1957) of the following composition (l^{-1}): 1 g $(NH_4)_2SO_4$, 1 g $MgSO_4$, 0.03 g $CaCl_2 . 2H_2O$, 0.002 g $FeCl_3 . 6H_2O$, 0.002 g $MnSO_4$, 1 g sodium succinate, 1 g casein hydrolysate and 1 g agar (Difco). The medium was adjusted to pH 7.5. Vitamins and trace elements (Pfennig & Lippert, 1966) were added before inoculation. The incubation was carried out at 28 °C, corresponding to the temperature in the aeration tanks. After 3 days of incubation, thin white bands consisting of large motile spirilla with intracellular inclusions of elemental sulfur appeared in the medium about 0.5–1.0 cm below the surface. These cells were collected with a capillary tube and transferred to a test tube. Enrichment cultures were obtained by using serial 10-fold dilutions in test tubes. The last positive tube was used for isolation on the same medium solidified with 1.5 % agar (Difco) in Petri dishes. After 5 days of incubation at 28 °C, small, semi-transparent colonies of large spiral cells appeared only below the agar surface. Colonies were flat, of irregular shape with fringed edges, with a diameter ranging from 0.2 to 0.5 mm. A single colony was transferred into liquid MPSS medium without FeS. The purity of the culture was tested using phase-contrast microscopy (NU-2; Zeiss) and transfers into nutrient-rich media. Routine cultivation was carried out in liquid MPSS medium without FeS, casein hydrolysate being replaced by peptone at a concentration of 5 g l^{-1}. The purified organism was designated strain D-427^T.

The morphology of cells grown for 18 h was studied with a phase-contrast microscope and in a transmission electron microscope (JEM-100C; JEOL) as described elsewhere (Dubinina *et al.*, 1993). In order to study the effect of aeration conditions on the growth of spirilla, rubber-stoppered 50 ml vials with 10 ml MPSS medium were flushed with filter-sterilized argon and a calculated volume of sterile air was then injected to create final oxygen concentrations in the gas phase of 1, 2, 5, 10 and 20 %. Cultivation was performed in vials with agitation on a shaker at 28 °C. Physiological and biochemical properties were determined as described previously (Dubinina *et al.*, 1993). The ability to use different carbon and nitrogen sources was tested by removing peptone and succinate from the medium. Carbon and nitrogen sources were added to the medium at a concentration of 1 g l^{-1} or, in the case of amino acids, 0.25 g l^{-1}. All tests were performed in triplicate. Mucous polysaccharide capsules were revealed by staining with ruthenium red (Luft, 1971). The fatty acid content was determined from 4–6 mg lyophilized biomass after acid methanolysis. Fatty acid methyl esters were analysed on a specialized chromatograph from the Microbial Identification System (Sherlock; MIDI Inc.) (Stead *et al.*, 1992). DNA was isolated according to the method of Marmur (1961) from 5 l batch cultures grown aerobically on liquid MPSS medium in which casein hydrolysate was replaced by peptone at a concentration of 10 g l^{-1}. The DNA G+C content was determined by thermal denaturation as described previously (Owen & Lapage, 1976). DNA of *Escherichia coli* K-12 DSM 498 (51.7 mol%) was used as a reference. Levels of DNA–DNA binding were determined by measuring the renaturation rate of denatured DNA at the optimal renaturation temperature as recommended by De Ley *et al.* (1970). For the DNA–DNA hybridization analysis, lyophilized DNA from *S. volutans* ATCC 19553 was used.

The almost-complete 16S rRNA gene sequence of strain D-427^T was amplified by PCR with the universal eubacterial primers 27f and 1492r (Medlin *et al.*, 1988; Lane, 1991) and aligned with related sequences using CLUSTAL_X software (Thompson *et al.*, 1997). An evolutionary-distance matrix was calculated using the algorithm of Jukes & Cantor (1969). The phylogenetic tree was constructed using the neighbour-joining (Saitou & Nei, 1987) and maximum-parsimony (Fitch, 1971) methods. Bootstrap analyses were based on 1000 resamplings. The PAUP 4.0b10 (Swofford, 1998) and TREECON (Van de Peer & De Wachter, 1994) software packages were used for the analysis.

Cells of strain D-427^T were spiral-shaped and actively motile, with polar tufts of flagella that were visible as a single flagellum under a phase-contrast microscope. Thin-section electron micrographic preparation revealed a Gram-negative cell-wall structure and that the flagella formed several tufts covered with sheaths. Cell morphology of the isolate is shown in Supplementary Fig. S1 (available in IJSEM Online). Spores were never observed. Coccoid bodies, 7–10 μm in diameter, appeared in cultures during the stationary growth phase. Intracellular polyhydroxyalkanoates and volutin were formed. In the presence of sulfide and polysulfide in the medium, elemental sulfur accumulated in the cells. Strain D-427^T oxidized thiosulfate

quantitatively to tetrathionate, which accumulated in the medium (Podkopaeva *et al.*, 2005).

The isolate was facultatively microaerophilic. Although growth occurred under an air atmosphere (20 % O_2), the optimum O_2 concentration for growth, as determined by the maximum increase in cell biomass, was 2 %. When inoculated into liquid or semi-liquid MPSS medium under an air atmosphere, growth of strain D-427^T occurred only in a zone located 0.5–1.0 cm below the surface, and massive cell lysis was observed microscopically during the exponential growth phase. Electron micrographs revealed that the cells were surrounded by polysaccharide capsules. The capsule size varied according to the oxygen regime used for incubation, and it was minimal at low oxygen partial pressure.

Strain D-427^T grew within a temperature range of 10–35 °C, with optimal growth at 28 °C. The pH range for growth was pH 6.5–8.5, with an optimum at pH 7.5–7.8. No growth was observed above 0.5 % NaCl in the medium. The cells showed oxidase activity and very low catalase activity. A comparative characterization of all tested physiological properties of strain D-427^T, as well as some other characteristics, is given in the species description and also in Table 1. The strain utilized a limited number of organic acids, mainly intermediates of the tricarboxylic acid cycle. The isolate did not grow with nitrate, nitrite or individual amino acids (glutamate, aspartate, serine, methionine or cysteine) as nitrogen sources. It had a strictly respiratory metabolism, using only oxygen as electron acceptor.

The G+C content of the genomic DNA was 38 mol%. Major cellular fatty acids were 16 : 0, 16 : 1 and 18 : 1. The detailed fatty acid profile of strain D-427^T is shown in Supplementary Table S1.

Comparative analysis of 16S rRNA gene sequences revealed that strain D-427^T belonged to the class *Betaproteobacteria* and was most closely related to the type strain of *S. volutans*, ATCC 19554^T (98.6 % similarity) (Fig. 1). The level of DNA–DNA hybridization between strain D-427^T and *S. volutans* ATCC 19553 was 12 %. The low value of DNA–DNA binding between these two strains together with some phenotypic properties clearly indicated that strain D-427^T represents a novel species within the genus *Spirillum*, according to the criteria for differentiation of bacterial species (Wayne *et al.*, 1987), although most physiological characteristics of strain D-427^T were essentially the same as those displayed by *S. volutans* ATCC 19553. The isolate differed from the type species by several phenotypic characteristics, such as its non-obligate microaerophily, the formation of coccoid bodies, growth at 0.5 % NaCl and the ability to assimilate acetate and the inability to utilize lactate as a carbon source (Table 1). The new isolate also differed from *S. volutans* by its ability to grow in liquid medium under an atmosphere of air without addition of catalase or superoxide dismutase to MPSS medium. The strain seems to achieve a suitable environment for microaerophilic growth through the production of extracellular polysaccharides during aerobic growth.

Table 1. Differential phenotypic characteristics of strain D-427^T and *S. volutans* ATCC 19553

+, Positive; w, weakly positive; −, negative; ND, no data available. Both strains were able to utilize fumarate, malate, oxaloacetate, pyruvate and succinate; the latter was used especially well by both strains. Neither was able to utilize some alcohols, sugars or amino acids. Strain D-427^T was unable to utilize benzoate, oxalate, formate, salicylate, glyoxylate, some alcohols (mannitol, glycerol, ethanol and butanol), sugars (glucose, galactose, sorbose, maltose, arabinose, sucrose, fructose and rhamnose) or amino acids (serine, lysine, tryptophan, threonine, histidine, phenylalanine, methionine, tyrosine, proline, hydroxyproline, ornithine, glutamine, glutamate, aspartate, asparagine, leucine, cysteine, cystine, alanine, arginine and valine). Data for *S. volutans* ATCC 19553 were taken from Caraway & Krieg (1974), Cole & Rittenberg (1971) and Krieg (2005).

Characteristic	Strain D-427^T	*S. volutans* ATCC 19553
Cell size (μm)		
Cell width	1.7–2.1	1.4–1.7
Cell length	23.0–49.0	14.0–60.0
Wavelength	15.0–23.0	16.0–28.0
Helix diameter	6.1–10.3	5.0–8.0
Presence of sheathed flagella	+	−
Formation of coccoid bodies in old cultures	+	−
Formation of sulfur globules from sulfide	+	ND
Resistance to NaCl (%)	0.5	0.02
O_2 concentration in the gas phase (%)		
Range	1–20	1–12*
Optimum	2.0	6.0
Vitamin requirement	+	−
Temperature optimum (°C)	28	30
Assimilation of:		
Acetate	+	−
Aconitate	w†	w
Citrate	w†	w
Isocitrate	w†	w
Lactate	−	+
2-Oxoglutarate	+	w
Source of isolation	Wastewater	Freshwater

*Growth occurs under an air atmosphere following addition of catalase or superoxide dismutase to the medium.
†Utilized only in the present of yeast extract (100 mg l^{-1}).

Unfortunately, both strains of *S. volutans* which conform to the original description of the species have been lost and do not exist in any established culture collection. A more detailed comparison of their physiological as well as chemotaxonomic features therefore cannot be achieved. Therefore, on the basis of the data presented, we propose strain D-427^T as the type strain of a novel species of the

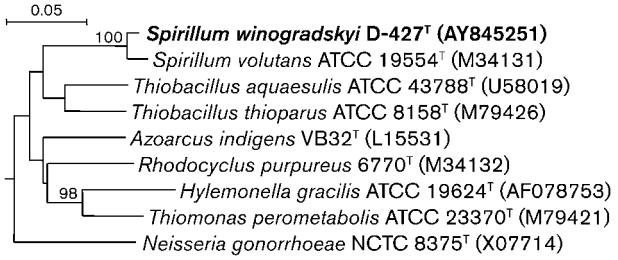

Fig. 1. Phylogenetic tree showing the evolutionary position of strain D-427^T based on 16S rRNA gene sequence analysis. The tree was constructed by the neighbour-joining method. Bar, 5 nucleotide substitutions per 100 bases. Numbers on branches represent bootstraps percentages from 1000 replicates in a full heuristic search; only values above 70 % are shown.

genus *Spirillum* with the name *Spirillum winogradskyi* sp. nov. On the basis of the results obtained in this study, an emended description of the genus *Spirillum* is also presented.

Request for an Opinion regarding the status of the species *Spirillum volutans* Ehrenberg 1832

Since the type species of the genus *Spirillum* is not represented by a type strain which conforms to the description of the taxon, we suggest that the Judicial Commission consider the following points.

(i) Based on our findings that the type strain (ATCC 19554^T) and the known reference strain (ATCC 19553) of *S. volutans* are not currently available, we propose that a search should be made for a suitable replacement strain, or a neotype should be designated according to Rule 18c of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992).

(ii) If a suitable replacement type strain or a neotype of *S. volutans* cannot be found or proposed, respectively, within 2 years of the publication of this paper, we suggest that the Judicial Commission should place the name *Spirillum volutans* Ehrenberg 1832 on the list of rejected names and designate *Spirillum winogradskyi* as a new type species and use the description of *Spirillum winogradskyi* as a description for the genus *Spirillum*.

Emended description of the genus *Spirillum* Ehrenberg 1832, 38^{AL}

The description is as given by Krieg (2005) with the following additional features. Cells form coccoid bodies. Motile by bipolar tufts of sheathed flagella; each tuft is covered with an individual sheath and is easily visible as an apparently single thick polar flagellum by phase-contrast microscopy. During growth on sulfide or polysulfide, cells accumulate globules of elemental sulfur. Catalase-negative or exhibit very low catalase activity. Cells are obligately or facultatively microaerophilic. Growth is observed only at low NaCl concentrations (below 0.5 %). Peptone and casein hydrolysate are used as nitrogen sources. Growth factors and vitamins are required. Major fatty acids are 16 : 0, 16 : 1 and 18 : 1. Representatives of the genus are widespread in freshwater and anthropogenic aquatic habitats containing sulfide.

Description of *Spirillum winogradskyi* sp. nov.

Spirillum winogradskyi (wi.no.grad′sky.i. N.L. masc. gen. n. *winogradskyi* of Winogradsky, named after Sergey N. Winogradsky, a Russian microbiologist who made a great contribution to the study of chemolithoautotrophic microorganisms).

Spiral cells, 1.7–2.1 μm in diameter, with one to three helices; helix diameter 6.1–10.3 μm. Cells are motile by means of bipolar tufts of flagella. Each tuft is covered with an individual sheath and looks like a thick polar flagellum under the phase-contrast microscope. Accumulates hydroxyalkanoates and volutin and forms globules of elemental sulfur in the presence of sulfide or polysulfide. Cells are facultatively microaerophilic, with optimal growth at an O_2 concentration of 2 %. Activity of oxidase and very low activity of catalase are present. Growth occurs within a pH range of 6.5–8.5, with an optimum at pH 7.5–7.8. The optimum growth temperature is at 28 °C. Chemo-organotrophic. Utilizes the following organic acids as carbon and energy sources: 2-oxoglutarate, succinate, fumarate, malate, oxaloacetate, pyruvate and acetate. Citrate, aconitate and isocitrate are utilized in the presence of yeast extract. Does not utilize amino acids, sugars or alcohols. Vitamins are required. Uses ammonium salts, casein hydrolysate, yeast extract and peptone as nitrogen sources. Does not hydrolyse casein or starch. Does not use nitrate, fumarate, sulfate, thiosulfate or elemental sulfur as terminal electron acceptors. Forms sulfide from cysteine. Predominant cellular fatty acids are 16 : 0, 16 : 1 and 18 : 1. The DNA G+C content of the type strain is 38.0 mol% (T_m).

The type strain, D-427^T (=DSM 12756^T =VKM B-2518^T), was isolated in Russia from sediments of an aeration tank for the treatment of municipal wastewater containing sulfide.

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REFERENCES

Caraway, B. H. & Krieg, N. R. (1974). Aerotaxis in *Spirillum volutans*. *Can J Microbiol* **20**, 1367–1377.

Cole, J. A. & Rittenberg, S. C. (1971). A comparison of respiratory processes in *Spirillum volutans*, *Spirillum itersonii* and *Spirillum serpens*. *J Gen Microbiol* **69**, 375–383.

De Ley, J., Cattoir, H. & Reynaerts, A. (1970). The quantitative measurement of DNA hybridization from renaturation rates. *Eur J Biochem* **12**, 133–142.

Dubinina, G. A., Grabovich, M. Y., Churikova, V. V., Lysenko, A. M. & Chernych, N. A. (1993). Reevaluation of the taxonomic status of the colorless sulphur spirilla belonging to the genus *Thiospira* and description of new species *Aquaspirillum bipunctata* comb. nov. *Microbiology* (English translation of *Mikrobiologiia*) **62**, 1101–1112.

Ehrenberg, C. G. (1832). Beiträge zur Kenntnis der Organization der Infusorien und ihrer geographischen Verbreitung besonders in Sibirien. *Abh K Akad Wiss Berlin* **1830**, 1–88 (in German).

Fitch, W. M. (1971). Toward defining the course of evolution: minimum change for a specific tree topology. *Syst Zool* **20**, 406–416.

Hylemon, P. B., Wells, J. S., Jr, Bowdre, J. H., MacAdoo, T. O. & Krieg, N. R. (1973a). Designation of *Spirillum volutans* Ehrenberg 1832 as type species of the genus *Spirillum* Ehrenberg 1832 and designation of the neotype strain of *S. volutans*. Request for an Opinion. *Int J Syst Bacteriol* **23**, 20–27.

Hylemon, P. B., Wells, J. S., Jr, Krieg, N. R. & Jannasch, H. W. (1973b). The genus *Spirillum*: a taxonomic study. *Int J Syst Bacteriol* **23**, 340–380.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Krieg, N. R. (2005). Genus I. *Spirillum* Ehrenberg 1832, 38^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 870–880. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Kucera, S. & Wolfe, R. S. (1957). A selective enrichment method for *Gallionella ferrugineae*. *J Bacteriol* **74**, 344–349.

Kuenen, J. G. & Dubinina, G. A. (2005). Genus VIII. *Thiospira* Visloukh 1914, 48^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 178–179. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Lane, D. J. (1991). 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*, pp. 115–175. Edited by E. Stackebrandt & M. Goodfellow. Chichester: Wiley.

Lapage, S. P., Sneath, P. H. A., Lessel, F. F., Skerman, V. B. D., Seeliger, H. P. R. & Clark, W. A. (editors) (1992). *International Code of Nomenclature of Bacteria (1990 Revision)*. Bacteriological Code. Washington, DC: American Society for Microbiology.

Luft, J. U. (1971). Ruthenium red and violet. II. Fine structure localization in animal tissues. *Anat Res* **171**, 369–415.

Marmur, J. A. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Medlin, L., Elwood, H. J., Stickel, S. & Sogin, M. L. (1988). The characterization of enzymatically amplified eukaryotic 16S-like rRNA-coding regions. *Gene* **71**, 491–499.

Owen, R. J. & Lapage, S. P. (1976). The thermal denaturation of partly purified bacterial deoxyribonucleic acid and its taxonomic applications. *J Appl Bacteriol* **41**, 335–340.

Pfennig, N. D. & Lippert, K. D. (1966). Über das Vitamin B_{12}-Bedürfnis phototropher Schwefelbakterien. *Arch Mikrobiol* **55**, 245–256 (in German).

Podkopaeva, D. A., Grabovich, M. Yu. & Dubinina, G. A. (2005). The functional role of reduced inorganic sulfur compounds in the metabolism of the microaerophilic bacterium *Spirillum winogradskii*. *Microbiology* (English translation of *Mikrobiologiia*) **74**, 12–19.

Podkopaeva, D. A., Grabovich, M. Yu., Dubinina, G. A., Lysenko, A. M., Tourova, T. P. & Kolganova, T. V. (2006). Two new species of microaerophilic sulfur spirilla, *Spirillum winogradskii* sp. nov. and *Spirillum kriegii* sp. nov. *Microbiology* (English translation of *Mikrobiologiia*) **75**, 172–179.

Rittenberg, B. T. & Rittenberg, S. C. (1962). The growth of *Spirillum volutans* in mixed and pure cultures. *Arch Mikrobiol* **42**, 138–153.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Stead, D. E., Sellwood, J. E., Wilson, J. & Viney, I. (1992). Evaluation of a commercial microbial identification system based on fatty acid profiles for rapid, accurate identification of plant pathogenic bacteria. *J Appl Bacteriol* **72**, 315–321.

Swofford, D. L. (1998). PAUP*. Phylogenetic analysis using parsimony (* and other methods), version 4. Sunderland, MA: Sinauer Associates.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Van de Peer, Y. & De Wachter, R. (1994). TREECON for Windows: a software package for the construction and drawing of evolutionary trees for the Microsoft Windows environment. *Comput Appl Biosci* **10**, 569–570.

Wayne, L. G., Brenner, D. J., Colwell, R. R., Grimont, P. A. D., Kandler, O., Krichevsky, M. I., Moore, L. H., Moore, W. E. C., Murray, R. G. E. & other authors (1987). International Committee on Systematic Bacteriology. Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* **37**, 463–464.

Wells, J. S., Jr & Krieg, N. R. (1965). Cultivation of *Spirillum volutans* in a bacteria-free environment. *J Bacteriol* **90**, 817–818.

Williams, M. A. & Rittenberg, S. C. (1957). A taxonomic study of the genus *Spirillum* Ehrenberg. *Int Bull Bacteriol Nomencl Taxon* **7**, 49–110.