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Magnetospirillum sulfuroxidans sp. nov., capable of sulfur-dependent lithoautotrophy and a taxonomic reevaluation of the order *Rhodospirillales* [☆]

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ABSTRACT

A spiral-shaped, highly motile bacterium was isolated from freshwater sulfidic sediment. Strain J10^T is a facultative autotroph utilizing sulfide, thiosulfate, and sulfur as the electron donors in microoxic conditions. Despite high 16S rRNA gene sequence identity to *Magnetospirillum gryphiswaldense* MSR-1^T (99.6 %), digital DNA-DNA hybridisation homology and average nucleotide identity between the two strains was of the different species level (25 % and 83 %, respectively). Strain J10^T is not magnetotactic. The DNA G + C content of strain J10^T is 61.9 %. The predominant phospholipid ester-linked fatty acids are C18:1 ω 7, C16:1 ω 7, and C16:0. Strain J10^T (=DSM 23205^T = VKM B-3486^T) is the first strain of the genus *Magnetospirillum* showing lithoautotrophic growth and is proposed here as a novel species, *Magnetospirillum sulfuroxidans* sp. nov. In addition, we propose to establish a framework for distinguishing genera and families within the order *Rhodospirillales* based on phylogenomic analysis using the threshold values for average amino acid identity at ~72 % for genera and ~60 % for families. According to this, we propose to divide the existing genus *Magnetospirillum* into three genera: *Magnetospirillum*, *Paramagnetospirillum*, and *Phaeospirillum*, constituting a separate family *Magnetospirillaceae* fam. nov. in the order *Rhodospirillales*. Furthermore, phylogenomic data suggest that this order should accommodate six more new family level groups including *Magnetospiraceae* fam. nov., *Magnetovibrionaceae* fam. nov., *Dongiaceae* fam. nov., *Niveispirillaceae* fam. nov., *Fodinicurvataceae* fam. nov., and *Oceanibaculaceae* fam. nov.

Introduction

The order *Rhodospirillales* was proposed in 1971 (Pfennig and Truper, 1971), and currently consists of the families *Rhodospirillaceae*, *Acetobacteraceae* (Gillis and De Ley, 1986); *Geminicoccaceae* (Proença et al., 2018); *Kiloniellaceae* (Wiese et al., 2009); *Azospirillaceae*, *Reyranellaceae*, *Rhodovibrionaceae*, *Stellaceae*, *Terasakiellaceae*, *Thalassobaculaceae*, *Thalassospiraceae*, and *Zavarziniaceae* (Hördt et al., 2020). The family *Rhodospirillaceae* comprises 25 genera that include a broad spectrum of bacterial species with different metabolic properties, such as photoheterotrophy, photoautotrophy, and chemoheterotrophy.

The genus *Magnetospirillum* was proposed by Schleifer et al. (1991) and is currently classified in the family *Rhodospirillaceae*. Species of the genus *Magnetospirillum* are best known for their ability to produce intracellular magnetosomes - the membrane-enclosed highly crystalline magnetite mineral (Bazylinski and Frankel, 2004). Magnetosomes are arranged in chains that cause the bacterium to behave like a bar magnet. Magnetosome production is encoded by a so-called magnetosome genomic cluster (MGC) (Uebe and Schüler, 2016). Most previously described *Magnetospirillum* species were specifically enriched and isolated based on their magnetotactic property (Blakemore et al., 1979; Koziyeva et al., 2019; Schleifer et al., 1991) but the genus also includes several strains lacking magnetosomes

Abbreviations: AAI, average amino acid identity; ANI, average nucleotide identity; dDDH, digital DNA-DNA hybridization; OGRI, overall genomic relatedness index; POCP, percentage of conserved proteins.

[☆] The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA sequence and genome sequence of the strain J10^T are FJ860937, and JAGTUF000000000, respectively.

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(Gorlenko et al., 2011; Shinoda et al., 2005; Thrash et al., 2010). The magnetotactic strains are microaerophilic heterotrophs (Dziuba et al., 2016; Frankel et al., 1979; Koziaeva et al., 2019; Matsunaga et al., 1991; Schleifer et al., 1991), while some also use nitrate as the alternative electron acceptor (Bazylnski and Blakemore, 1983; Matsunaga et al., 1991; Shinoda et al., 2005). Recently, the genus *Magnetospirillum* was proposed to incorporate a related genus *Phaeospirillum* (Hördt et al., 2020) which includes anoxygenic purple nonsulfur bacteria lacking magnetosomes (Imhoff et al., 1998). At the same time, Monteil et al. suggested to divide the phenotypically similar *Magnetospirillum* species into two evolutionarily divergent lineages (Monteil et al., 2020).

This study describes properties of a previously isolated non-magnetotactic representative of the genus *Magnetospirillum*, strain J10^T, capable of lithoautotrophic growth with reduced sulfur compounds in microoxic conditions (Geelhoed et al., 2010; Geelhoed et al., 2009). We propose the species name *Magnetospirillum sulfuroxidans* sp. nov. for this strain. Furthermore, based on phylogenomic comparison and phenotypic differences, the genus *Magnetospirillum* is proposed to be reclassified into three genera, including *Magnetospirillum*, *Paramagnetospirillum* and *Phaeospirillum*, while the order *Rhodospirillales* is to be amended with several novel families.

Methods

Strain and culture conditions

Strain J10^T was isolated from sulfidic sediments of a freshwater ditch collected in Delft (the Netherlands) using sulfide-O₂ gradient cultivation technique as previously described (Geelhoed et al., 2009). Cell biomass of the strain J10^T for genomic DNA isolation and fatty acid analysis was obtained on the FSM Medium (Heyen and Schüler, 2003).

Phenotypic characterization

Utilization of alternative electron acceptors was tested batch cultures with acetate as the electron donor in the absence of reductants. Utilization of Fe(II) and sulfide was examined in agarose-stabilized gradient systems (Geelhoed et al., 2009), and other electron donors were tested in liquid batch incubations with 1–2 % O₂ in the headspace.

Chemotaxonomic characterization

Cells of strains J10^T, *M. gryphwaldense* MSR-1^T, and *M. kuznetsovii* BB-1^T for polar lipid fatty acid analysis were obtained at identical growth conditions and harvested at the mid-exponential growth phase. Fatty acid extraction and further identification using a Maestro gas chromatograph-mass spectrometer (Interlab, Russia) was performed as described previously (Koziaeva et al., 2019).

16S rRNA gene-based phylogeny

Genomic DNA was extracted using the UltraClean Soil DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA), and a 16S rRNA gene fragment (1225 bp) was amplified with primers 63F and 1387R. The GenBank/EMBL/DDBJ accession number of 16S rRNA gene sequence of strain J10^T is FJ860937. Phylogenetic analysis of 16S rRNA gene sequences was performed using the maximum-likelihood, neighbour-joining, and maximum parsimony algorithms. Alignment of 16S rRNA gene sequences was carried out using MUSCLE (Edgar, 2004) and the maximum-likelihood tree was constructed with the model recommended by ModelFinder (Kalyaanamoorthy et al., 2017) in IQ-Tree

(Nguyen et al., 2015). Maximum parsimony and neighbour-joining trees were reconstructed with MEGA-7 (Kumar et al., 2016).

Genome assembly

Genomic DNA for genome sequencing was purified with DNeasy UltraClean Microbial Kit (Qiagen). The whole nucleotide sequence of the genomic DNA of strain J10^T was determined using Illumina HiSeq platform with 150-bp pair-end reads. Raw sequence reads were trimmed using Trimmomatic version 0.36 (Bolger et al., 2014) with default settings. The quality-filtered reads were then assembled *de novo* using SPAdes version 3.15.0 with default settings (Bankevich et al., 2012). Protein-coding sequences were identified and annotated using the NCBI Prokaryotic Genome Annotation Pipeline (Tatusova et al., 2016). Whole-genome shotgun project was deposited in the DDBJ/ENA/GenBank under accession no. JAGTUF000000000.

Phylogenomic analysis

Phylogenomic analysis was performed using a concatenated alignment of 120 single-copy marker proteins obtained using GTDB-Tk software version 1.0.2 (Chaumeil et al., 2019) with a dataset of 197 genomes of *Rhodospirillales* strains (Supplementary Table 1) and the 151 genomes of type strains of the *Rhodobacterales*, *Caulobacterales*, *Hyphomonadales*, and *Hyphomicrobiales* as an outgroup. The maximum-likelihood tree was computed using IQ-Tree (Nguyen et al., 2015) with the model recommended by ModelFinder (Kalyaanamoorthy et al., 2017) and branch support was estimated using UFBoot2 (Hoang et al., 2017). Maximum parsimony and neighbour-joining trees were reconstructed using MPBoot (Hoang et al., 2018), and MEGA7 (Kumar et al., 2016), respectively.

Genome relatedness indices calculations

Average amino acid identity (AAI) values were calculated by CompareM 0.0.23 (<https://github.com/dparks1134/CompareM>) with default blastp parameters (i.e. e-value ≤ 0.001, percent identity ≥ 30 %, and alignment length ≥ 70 %). Pairwise percentages of conserved proteins (POCP) were calculated using the runPOCP.sh script (Grouzdev et al., 2018), based on a previously described approach (Qin et al., 2014). Average nucleotide identity (ANI) was calculated using the ANI calculator (<https://ani.jgi.doe.gov/html/calc.php?>) (Varghese et al., 2015). Digital DNA-DNA hybridization (dDDH) values were determined using the Genome-to-Genome Distance Calculator (GGDC) 2.1 online software (Meier-Kolthoff et al., 2013). ProK-lust ("Prokaryotic Clusters") service (Volpiano et al., 2021) was used to obtain AAI clusters.

Results and discussion

Phylogenetic analysis and taxonomy of strain J10^T

Phylogenetic analysis based on 16S rRNA sequence comparison showed (Fig. 1) that the closest relative of strain J10^T was *M. gryphwaldense* MSR-1^T (Schleifer et al., 1991) with the sequence identity of 99.6 % indicating that it might belong to the same species (Chun et al., 2018).

Complete genome sequencing and phylogenomic analysis was performed to clarify the taxonomic position of the strain J10^T. The final assembled genome size of 4,132,710 bp consisted of 60 scaffolds with an *N*₅₀ value of 150,424 bp and an average coverage of 373 ×. The G + C content of the genome is 61.9 %. The draft genome sequence of strain J10^T contained 3,930 genes, of which 3,846 were protein-coding sequences, 31 pseudogenes, and 53 coding RNAs. The 16S

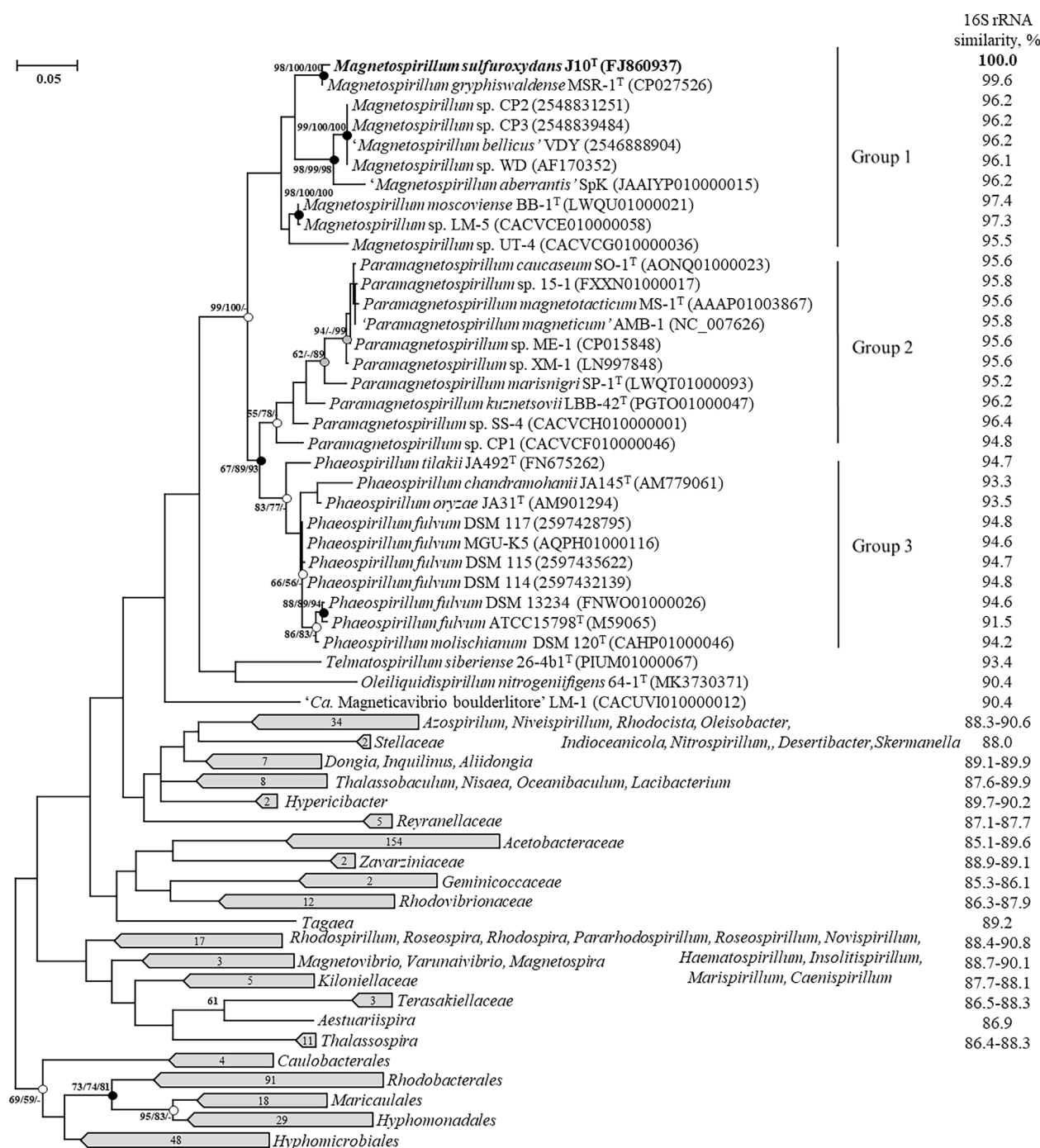


Fig. 1. Maximum-likelihood phylogenetic tree based on 16S rRNA gene sequences (1277 nucleotide sites) reconstructed with the GTR + F + R7 evolutionary model, showing the position of strain J10^T in relation to taxonomically characterized members of the order *Rhodospirillales*. White circles indicate that the corresponding nodes were recovered in the tree that was reconstructed based on the neighbour-joining algorithm; grey circles indicate that the corresponding nodes were recovered in the tree that was reconstructed based on the maximum parsimony algorithm; black circles indicate that the corresponding nodes were also recovered based on the neighbour-joining and maximum-parsimony algorithms. Bootstrap values (> 50 %) are listed as percentages at the branching points. Bar, 0.05 substitutions per nucleotide position. The right column shows 16S rRNA sequence similarity between strain J10^T and other species. The tree was rooted using type strains of the *Rhodobacterales*, *Caulobacterales*, *Hyphomonadales*, and *Hyphomicrobiales* orders as an outgroup. GenBank or IMG database accession numbers for 16S rRNA genes are indicated in brackets.

rRNA gene sequence detected in the genome was identical to that obtained by PCR and Sanger sequencing.

On the phylogenomic tree, strain J10^T formed a common branch with *M. gryphiswaldense* MSR-1^T (Fig. 2), similar to the 16S rRNA gene tree. However, the dDDH and ANI values between strains J10^T and MSR-1^T (25.0 and 83.0 %, respectively), were below the threshold for species delineation (Auch et al., 2010; Chun et al., 2018; Jain

et al., 2018) justifying classification of strain J10^T as a novel species in the genus *Magnetospirillum*.

Phenotypic properties of strain J10^T

A spiral-shaped motile bacterium was enriched in agarose-stabilized culture with opposing gradients of Fe(II) and sulfide

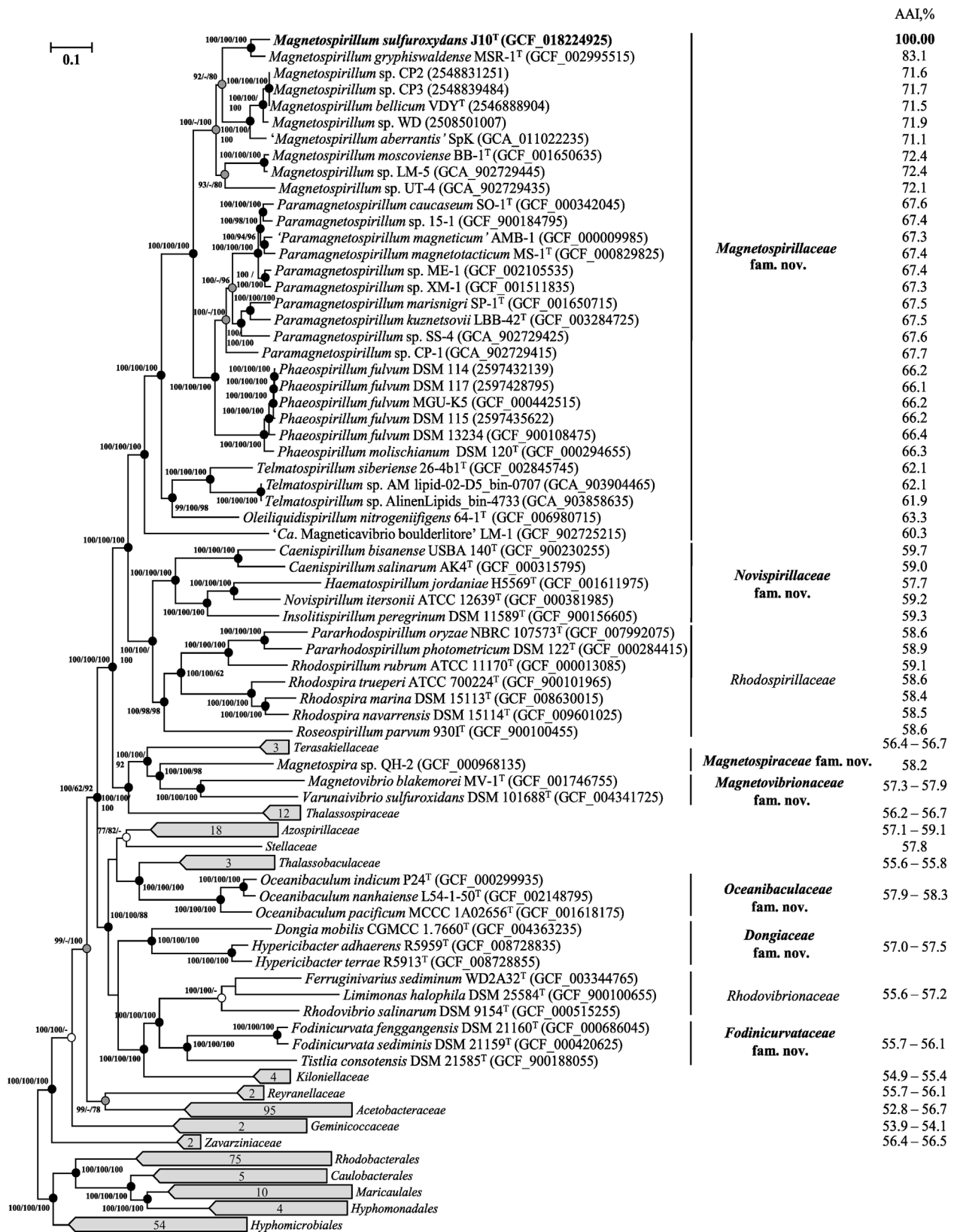


Fig. 2. Maximum-likelihood phylogenetic tree derived from concatenated 120 single copy marker proteins showing the position of strain J10^T in relation to members of the order Rhodospirillales. Phylogenetic analysis was performed using the LG + F + I + G4 model based on 34,747 amino acid positions. Bootstrap values (>50 %) are indicated as a branching point percentage. Bar, 0.1 amino acid substitutions per site. The tree was rooted using type strains of the orders Rhodobacterales, Caulobacterales, Hyphomonadales, and Hyphomicrobiales as an outgroup. Genome assembly numbers are given in brackets.

(derived from FeS) and oxygen. Cells developed as a visible band at the oxic-anoxic transition zone were serially diluted in gradient culture with sulfide as the electron donor and finally purified by serial dilutions in liquid culture with thiosulfate at microoxic conditions resulting in a pure culture strain J10^T (Geelhoed et al., 2009). The cells are motile by bipolar flagella. The cell length varied from 1 to 2 to 15 coils depending on substrate (acetate or thiosulfate, respectively). In terms of the key physiology, J10^T can be classified as sulfur-oxidizing facultative chemolithoautotroph (Geelhoed et al., 2009).

Cells of strain J10^T did not align in the magnetic field of a bar magnet like other *Magnetospirillum* strains 'M. aberrantis' SpK and *M. bellicum* VDY^T. Transmission electron micrographs did not show the inclusion of electron-dense magnetite crystals (Geelhoed et al., 2009) which is confirmed by the absence of genes encoding magnetosome formation. Strain J10^T is able to use N₂ as the N-source, a common property among the *Magnetospirillum* species (Bazylnski et al., 2000; Dziuba et al., 2016). Same is true for microaerophily. J10^T was only able to grow under air atmosphere in static heterotrophic conditions after a long lag phase. For chemolithoautotrophic growth of strain J10^T with sulfide or thiosulfate it was necessary to sequentially add O₂ to the head space at low amounts to avoid O₂ toxicity and limitation at the same time to achieve a complete oxidation to sulfate and prevent sulfur formation (Geelhoed et al., 2010).

The dominant fatty acids in the J10^T membrane phospholipids of were monounsaturated C18:1ω7 and C16:1ω7, and saturated C16:0, similar to *M. gryphiswaldense* MSR-1^T, *M. bellicum* YDY^T, and *M. moscowiense* BB-1^T (Table 1).

Another common properties of strain J10^T and the three species mentioned above is the full denitrification of nitrate (Dziuba et al., 2016; Thrash et al., 2010); while all three type strains tested negative for anaerobic growth with chelated Fe³⁺ (citrate and pyrophosphate), and sulfate as acceptors (Table 1). J10^T could not use chlorate as acceptor in contrast to strain YDY^T (Thrash et al., 2010). Strain J10^T grew heterotrophically with acetate, succinate, fumarate, pyruvate, lactate, butyrate, and propionate. Formate, H₂/CO₂, glucose and ribose did not support growth. This agrees with organic substrate utilization by *M. gryphiswaldense* MSR-1^T. None of the strains J10^T, MSR-1^T, and BB-1^T were able to oxidize Fe²⁺ in gel gradient cultures (Table 1).

An important characteristic of strain J10^T is its ability to grow lithoautotrophically with sulfide, thiosulfate, and elemental sulfur.

Autotrophic growth on thiosulfate was evidenced by incorporation of ¹³C labelled bicarbonate into biomass. In continuous culture studies, both strain J10^T and *M. gryphiswaldense* MSR-1^T utilized sulfide as energy source in mixotrophic growth conditions (acetate + sulfide) and were able to grow in autotrophic conditions with sulfide and thiosulfate (Geelhoed et al., 2010). Both strains have similar sulfur-oxidizing machinery including sulfide dehydrogenase FccAB, incomplete Sox cycle lacking thye SoxCD complex and rDSR system. In the original search for sulfite-oxidizing enzymes encoded in the genome of *M. gryphiswaldense* Geelhoed et al. (2010) did not find an appropriate candidates. A presence of rDSR system for sulfur oxidation to sulfite in sulfur-oxidizing bacteria (SOB) is normally accompanied by AprAB also working in reverse mode to oxidize sulfite to sulfate. But it was absent, as well as the aerobic cytochrome c-dependent sulfite dehydrogenase SorAB. The third type of such an enzyme - a quinone-dependent sulfite dehydrogenase SoeABC has not yet been discovered at that time. It is mainly found in the gammaproteobacterial SOB with the model protein fully functionally characterized in phototrophic purple bacterium *Allochrochromatium* (Dahl et al., 2013). It is this enzyme which is apparently encoded in both *M. gryphiswaldense* and J10^T. This combination is more common for the gammaproteobacterial than for alphaproteobacterial sulfur-oxidizers (Hallenbeck, 2017). *M. moscowiense* BB-1^T possesses the same set of genes for sulfur oxidation as MSR-1^T and J10^T but additionally has *aprAB*, *sat*, and *hdrA1A2CB* genes. No *sox* or *dsr* or *soe* genes were found in genome of *M. bellicum* YDY^T.

Taken together, the data indicate that strain J10^T represents a novel species. Strain J10^T is capable of autotrophic growth using energy derived from the oxidation of reduced inorganic sulfur compounds and therefore the name *Magnetospirillum sulfuroxidans* is proposed. The new species descriptions are given in Table 2.

Boundaries for genera and families deliniation in the order Rhodospirillales

To make a framework for genus and family demarcation within the order *Rhodospirillales*, we considered the genome-based phylogeny of core-genome proteins, 16S rRNA phylogeny and overall genome relatedness indices (OGRI) measurement. We considered AAI as a promising metric that is being used recently for delineation of genus and families (Koziaeva et al., 2019; Kuzmanović et al., 2022; Ma et al.,

Table 1

Traits characterising strain J10^T and *Magnetospirillum* type strains. 1. strain J10^T, 2. *M. gryphiswaldense* MSR-1^T, 3. *M. moscowiense* BB-1^T, 4. *M. bellicum* YDY^T. All strains have common characteristics: cell morphology – spirilla; utilization oxygen and nitrate as electron acceptors; electron acceptors sulfate, Fe(III), and fumarate could not be used; used electron donors – lactate, pyruvate, acetate, succinate, propionate, butyrate, and fumarate; not used – citrate, formate, glucose and benzoate. +, positive for utilisation; -, negative for utilization.

Characteristic	1	2	3	4 ^a
Cell size	2–12 × 0.2	1–20 × 0.7	2.0–4.0 × 0.3	3.0 × 0.5
T range (optimum), °C	4–35 (30)	– (28–34) ^b	18–38 (25–32)	10–42 (42)
pH range (optimum)	6–8.5 (6.8–7.2)	– (7–7.5)	5.5–8.5(6.5–6.8)	6.0–7.5(6.8)
DNA G + C content (%)	61.9	63.3	65.2	65.9
Magnetotaxis	–	+	+	–
Electron acceptors				
Perchlorate	–	–	–	+
Chlorate	–	–	–	+
Nitrite	–	–	–	+
Electron donors				
Sulfide	+	+	–	–
Thiosulfate	+	+	–	ND
H ₂ /CO ₂	–	–	–	+
Ethanol	–	–	–	+
FeCl ₂	–	–	–	+
Major fatty acids in the membrane phospho-lipids (% from total)	C _{16:0} – 13.3 C _{16:1ω7} – 30.6 C _{18:1ω7} – 45.2	C _{16:0} – 13.8 C _{16:1ω7} – 28.7 C _{18:1ω7} – 46.4	C _{16:0} – 16.5 C _{16:1ω7} – 31.2 C _{18:1ω7} – 45.6	C _{16:0} – 12.0 16:1ω7c/15iso 2OH – 10.5 18:1ω7c – 64.6

^a data were compiled from (Thrash et al., 2010)

^b data were compiled from (Schleifer et al., 1991)

Table 2*Magnetospirillum sulfuroxidans*: protologue.

Parameter	Species: <i>Magnetospirillum sulfuroxidans</i> sp. nov.
Author	Veronika V. Kozaieva
Species name	<i>sulfuroxidans</i>
Genus name	<i>Magnetospirillum</i>
Species status (SPST)	sp. nov.
Etymology	sul.fur.o.xi.dans. L. neut. n. <i>sulfur</i> ; sulfur; N.L. v. <i>oxido</i> , to oxidize; N.L. part. adj. <i>sulfuroxidans</i> , pertaining to the ability to oxidize sulfur
Description of the new taxon	Cells are helical spirilla, 2–12 × 0.2 µm, motile by bipolar flagella. Does not produce magnetosomes. Growth is observed at 4–35 °C (optimum 30°), 0–1 % NaCl (optimum 0–0.1 %) and pH range 6–8.5 (optimum 6.8–7.2). Grows microoxically with the following organic substrates: lactate, pyruvate, acetate, succinate, fumarate, propionate and butyrate. Glucose, ribose, benzoate, formate, H ₂ /CO ₂ and Fe(II) are not used. Can grow chemolithoautotrophically on sulfide, thiosulfate and elemental sulfur at microoxic conditions. Reduced inorganic sulfur compounds can also be used as auxiliary energy source for mixotrophic growth. Nitrate, but not chlorate, sulfate, Fe(III) and fumarate can serve as the electron acceptor for complete denitrification. Major membrane phospholipid fatty acids are C18:1ω7, C16:1ω7, and C16:0. The genomic DNA G + C content is 61.9 %. The type strain is J10 ^T (= DSM 23205 ^T = VKM B-3486 ^T) was isolated from freshwater sediment.
Authors	Veronika V. Kozaieva, Dmitry Y. Sorokin, Tatiana V. Kolganova, Denis S. Grouzdev
Title	<i>Magnetospirillum sulfuroxidans</i> sp. nov., capable of sulfur-dependent lithoautotrophy and a taxonomic reevaluation of the order <i>Rhodospirillales</i>
Journal	Systematic and Applied Microbiology
Corresponding author	Veronika V Kozaieva
of corresponding author	vkzaieva@mail.ru
Designation of the type strain	J10
Strain collection numbers	DSM 23205 ^T , VKM B-3486 ^T
16S rRNA gene accession numbers	FJ860937
Genome accession numbers	JAGTUF000000000 (type strain)
Genome status	Draft
G + C, %	61.9 (genome of type strain)
Country of origin	The Netherlands
Region of origin	Delft
Date of isolation	2009
Source of isolation	top layer of the sediment of a freshwater ditch in Delft
Number of strains in study	1
Growth medium, incubation conditions	FSM medium, pH 7; incubation – 30 °C; microaerobic
Conditions of preservation	Deep freezing in 15 % glycerol (v/v)
Gram stain	Negative
Cell shape	Spirillum
Cell size	2–12 × 0.2 µm
Motility (MOTY)	Motile, does not show magnetotactic behavior
Sporulation	None
Temperature range for growth	4–35
Lowest temperature for growth	4
Highest temperature for growth	35
Optimal temperature for growth	30
Lowest pH for growth	6.0
Highest pH for growth	8.5
Optimum pH for growth	6.8–7.2
pH category	neutrophilic
Relation to oxygen	microaerophilic
O ₂ conditions for strain testing	microaerobic
Carbon source used (class)	Carbonic acids or CO ₂
Terminal electron acceptor	O ₂ and NO ₃
Energy metabolism	Chemoorganotrophic and chemolithotrophic
Habitat (HABT)	Freshwater sulfidic sediments
Extraordinary features (EXTR)	Sulfur-based lithoautotrophy

2022). We reasoned that an AAI threshold for delimiting genera and families should correspond to values between intra- and inter-taxon pairwise comparisons; should be consistent with the branching of phylogenomic tree and should not cause major changes in the taxonomy of *Rhodospirillales*. We plotted histograms of all AAI pairwise comparisons to identify potential genera and family boundaries within *Rhodospirillales*. Initially, we used the ProKlust (“Prokaryotic Clusters”) service (Volpiano et al., 2021) to obtain clusters at different AAI values for determine possible framework. As a result, up to 59 % AAI majority of genera collapsed into a single family (Fig. Suppl1). Likewise, at 61 % AAI or higher, some families began to break up into multiple families (Fig. Suppl3). Thus, ~60 % AAI is a more suitable value for delineation of the families in the order *Rhodospirillales* (Fig. Suppl2). Fig. 3A, representing the current taxonomy, shows an overlap of

distributions at the 56–61 % AAI, whereas Fig. 3B and Fig. Suppl8 corresponding to family separation at 60 % AAI no overlap was observed.

The genus demarcation border was defined in the same way as for the families. According to ProKlust results, 70 % AAI is a weak boarder because many species formed single clusters, although they were on the different branches of the phylogenomic tree (Fig. Suppl4). At 72 % and further increase in AAI values, strains began to form an increasing number of clusters (Fig. Suppl6,7). Thus, ~71 % AAI proves to be a more suitable value for distinguishing genera in the order *Rhodospirillales* (Fig. Suppl5). When analyzed sequences according to current taxonomy (Fig. 4 A), in the graph showing the distribution of intra- and inter-genus AAI – POCF values there was an overlap at the 65–75 % AAI. At the same time, when species are divided at ~71 % AAI, no distribution overlap was found (Fig. 4 B).

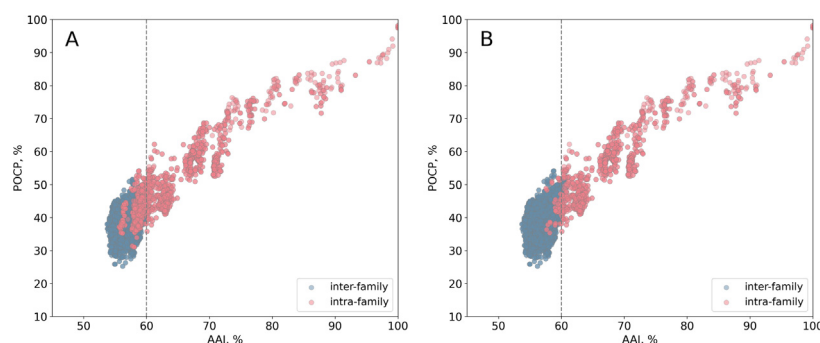


Fig. 3. Distribution of family comparisons according to AAI and POCP values. Pairwise comparisons within distinct families of the order *Rhodospirillales*. A) according to the current taxonomy. B) according to the proposed taxonomy are shown. Intra-family values are colored in pink, and inter-family values are colored in blue. Proposed AAI value distinguish families are shown with a vertical dotted line. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

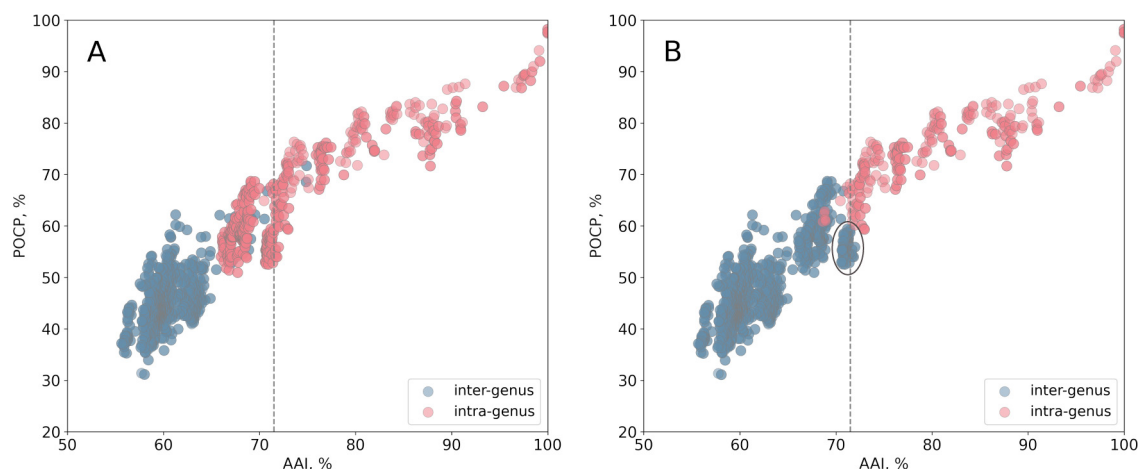


Fig. 4. Distribution of the comparisons of genera according to AAI and POCP values. Pairwise comparisons within distinct genera of the order *Rhodospirillales*. A) according to current taxonomy. B) according to proposed taxonomy are shown. Intra-genus values are colored in pink, and inter-genus values are colored in blue. Proposed AAI value for delimiting genera is shown as a vertical gray dashed line. Pairwise comparisons between *Phaeospirillum* and *Paramagnetospirillum* are marked with a black circle. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

An overall POCP threshold of 50 % was originally proposed as a boundary for delimitation of the genera (Qin et al., 2014). Within *Rhodospirillales* that threshold resulted in collapsing most genera of the family *Rhodospirillaceae* in a single genus. Therefore, we concluded that 50 % POCP is not a useful OGRI framework for the genera delimitation within the order *Rhodospirillales*. Similar observations were also made for some other taxa (Kuzmanović et al., 2022; Wirth and Whitman, 2018).

Using AAI boundary of ~60 % for the families and ~71 % for the genera together with the branching confidence of phylogenomic tree, allowed to largely preserve the current taxonomy of the order *Rhodospirillales* with a limited increase in the number of suggested novel families and genera as well as combining some species into single genera.

Taxonomy revision of the genus level

On the phylogenomic tree *Rhodospira trueperi* ATCC.700224^T (Pfennig et al., 1997) formed a clade with the species of the genus *Roseospira* (Imhoff et al., 1998). The AAI values between *Roseospira* and *Rhodospira* were ~75 %, while with all other species < 61 %. Therefore, we propose to transfer all five *Roseospira* species into the genus *Rhodospira* as new combinations (Table 3).

Within the family *Azospirillaceae* the species *Azospirillum halopraeferens* DSM 3675^T had AAI values ~68 % with other *Azospirillum* species. While within this genus, with exception of *A. halopraeferens*, values were < 73 %. Thus, *Azospirillum halopraeferens* DSM 3675^T should be reclassified as a separate new genus. However, more related isolates belonging to this prospective new genus need to be available for its formal description.

Taxonomy of the genus *Magnetospirillum*

After original characterization of strain J10^T by Geelhood et al. (2009; 2010), the genus *Magnetospirillum* was combined with *Phaeospirillum* based on the tree paraphylicity (Hördt et al., 2020). However, this study considered mainly mono- and paraphylicity of branches but not evolutionary distances and genomic indices. Accordingly, a follow up research suggested to divide this genus to two genera (Monteil et al., 2020), which is also in agreement with the GTDB classification: the current genus *Magnetospirillum* should be divided into two genera, *Magnetospirillum* (the MSR group on the tree) and *Phaeospirillum* with the inclusion in *Phaeospirillum* of some *Magnetospirillum* species from the AMB group (Fig. 1). To check all recent taxonomic changes and propositions related to the genus *Magnetospirillum*, the genome-based analyses were performed. On the 16S rRNA gene tree, species of the genus *Magnetospirillum* can be

Table 3Descriptions of the new combinations in the genus *Rhodospira*.

New name combination	Basonym	Description	Type strain and culture collection numbers
<i>Rhodospira marina</i>	<i>Roseospira marina</i> Guyoneaud et. al., 2003	The description of this taxon is as given by (Guyoneaud et al., 2002)	CE2105, ATCC BAA-447, DSM 15113
<i>Rhodospira goensis</i>	<i>Roseospira goensis</i> Kalyan Chakravarthy et al., 2007	The description of this taxon is as given by (Kalyan Chakravarthy et al., 2007)	JA135, ATCC BAA-1364, DSM 18985, JCM 14191
<i>Rhodospira mediosalina</i>	<i>Roseospira mediosalina</i> Imhoff et. al. 1998	The description of this taxon is as given by (Imhoff et al., 1998)	BN 280
<i>Rhodospira navarrensis</i>	<i>Roseospira navarrensis</i> Guyoneaud et. al., 2003	The description of this taxon is as given by (Guyoneaud et al., 2002)	SE 3104, ATCC BAA-448, DSM 15114
<i>Rhodospira visakhapatnamensis</i>	<i>Roseospira visakhapatnamensis</i> Kalyan Chakravarthy et al., 2007	The description of this taxon is as given by (Kalyan Chakravarthy et al., 2007)	JA131, ATCC BAA-1365, DSM 19947, JCM 14190

divided into three monophyletic groups, namely MSR, AMB, and Phaeo (Fig. 1). Group MSR comprised strains J10^T, magnetotactic strains *M. gryphiswaldense* MSR-1^T, *M. moscoviense* BB-1^T, and non-magnetotactic strains *M. bellicum* VDY^T and '*M. aberrantis*' SpK. Group AMB included '*M. magneticum*' AMB-1, *M. magnetotacticum* MS-1^T, *M. caucaseum* SO-1^T, *M. marisnigri* SP-1^T, and *M. kuznetsovii* LBB-42^T. Group Phaeo included only non-magnetotactic anoxygenic purple phototrophs of the genus *Phaeospirillum* before reclassification (Hördt et al., 2020). Interestingly, the magnetotactic AMB group has a later common ancestor with the non-magnetotactic group Phaeo, but not the MSR group. On the phylogenomic tree (Fig. 2) the subdivision of the genus *Magnetospirillum* into the same three groups was confirmed with the group AMB closest to the Phaeo group. Both the 16S rRNA-based and the core-genome trees clearly showed a division of *Magnetospirillum* into three clusters. The group MSR was separated with all AAI values outside the clade < 70 %, while the Phaeo and AMB groups showed borderline AAI values up to 72 %. Despite these significant differences in phenotypic properties provide strong evidence that the Phaeo and AMB groups belong to closely related, but still separate genus-level taxa. Organisms of these two groups show major difference in their lifestyle. While Phaeo group includes anoxygenic photoautotrophs, the AMB group mostly consists of chemoheterotrophs forming magnetosomes. Taking into account these reasoning, we argue that the proposal of Hördt et al. (2020) to unify the genera *Magnetospirillum* and *Phaeospirillum* are no longer supported. Instead, we

propose to split the genus into *Magnetospirillum sensu stricto*, represented by the MSR group; *Paramagnetospirillum* gen. nov., represented by the AMB group, as well as the original *Phaeospirillum* (Tables 4, 5).

Taxonomic revision of the Rhodospirillales families

Using the AAI threshold of 60 %, combined with the phylogenomic reconstructions (Fig. 2), formation of a new family *Magnetospirillaceae* is recommended. This is also supported by the analysis of RED distances according to the GTDB. On the 16S rRNA gene-based tree, the *Magnetospirillum* and *Rhodospirillum* branches are also well separated (Fig. 1). Thus, the family *Magnetospirillaceae* should include the following genera: *Magnetospirillum*, *Paramagnetospirillum*, *Phaeospirillum*, *Oleiliquidispirillum* (Li et al., 2020) and the genus *Telmatospirillum* (Sizova et al., 2007) currently classified in the family *Rhodospirillaceae*.

The clade consisting of the genera *Caenispirillum*, *Haematospirillum*, *Insolitispirillum* and *Novispirillum* has AAI values of 59–60 % with a nearest related group including the genera *Rhodospirillum*, *Pararhodospirillum*, *Rhodospira*, and *Roseospirillum*. These two groups have different lifestyle. Strains of the *Rhodospirillum* group are anoxygenic phototrophs, while the *Niveispirillum* group only includes chemoorganotroph. Thus, considering distinct physiology and phylogeny, these two clades can be regarded as two different families.

In addition, on the phylogenomic tree, the genera *Dongia* (Liu et al., 2010); *Hyperichibacter* (Noviana et al., 2020) and *Oceanibaculum*

Table 4Protologue of *Paramagnetospirillum* gen. nov., and emended description of the genus *Magnetospirillum* (Schleifer et al., 1991), emend. Hordt et al., 2020.

Genus name	<i>Paramagnetospirillum</i>	<i>Magnetospirillum</i>
Genus status	gen. nov.	emended
Genus etymology	Pa.ra. <i>mag.ne</i> .to.spi.ril.lum Gr. pref. [πάρα-] <i>para</i> -, beside, alongside of, near, like; M.L. neut. dim. n. <i>Magnetospirillum</i> , a genus name; M.L. neut. dim. n. <i>Paramagnetospirillum</i> , resembling the genus <i>Magnetospirillum</i>	<i>Ma.gne.to.spi.ril.lum</i> Gr. masc. n. <i>Magnês</i> (gen. <i>Magnêtos</i>), a magnet; N.L. pref. <i>magneto</i> -, pertaining to a magnet; Gr. fem. n. <i>spêtra</i> , a spiral; N.L. neut. dim. n. <i>spirillum</i> , a small spiral; N.L. neut. dim. n. <i>Magnetospirillum</i> , a small magnetic spiral
Type species of the genus	<i>Paramagnetospirillum magnetotacticum</i> comb. nov. (basonym: <i>Aquaspirillum magnetotacticum</i> (Maratea and Blakemore, 1981), <i>Magnetospirillum magnetotacticum</i> (Schleifer et al. 1992)	<i>Magnetospirillum gryphiswaldense</i> (Schleifer et al., 1992)
Description of the taxon and diagnostic traits	Helical (clockwise) spirilla, γ 1–6 × 0.2–0.6 μm. Gram-negative. Motile by means of a single flagellum at each pole. Magnetotactic cell contains enveloped magnetosomes which are arranged in a chain within the cytoplasm. Microaerophilic. Chemoorganotrophic. Catalase negative, oxidase can be present or absent. Growth occurs on various organic acids, carbohydrates are utilized only occasionally. The G + C content of DNA is 64 to 71 mol%. The type species is <i>Paramagnetospirillum magnetotacticum</i> .	The description is as given before (Schleifer et al., 1991) with additions. Some species does not form magnetosomes. Chemolithoautotrophic growth using reduced sulfur compounds as energy source. The G + C content is 61–66 %.
Genome size	4,406 to 4,967 kbp	4,132 to 4,506 kbp
GC content %	63.4 to 66.0 % retrieved from genomic data	61.9 to 65.9 % retrieved from genomic data

Table 5

Descriptions of the new combinations in genera *Paramagnetospirillum* gen. nov. and *Phaeospirillum*.

New name combination	Basonym	Description	Type strain and culture collection numbers
<i>Paramagnetospirillum magnetotacticum</i> (type species of the genus)	<i>Aquaspirillum magnetotacticum</i> (Maratea and Blakemore, 1981) <i>Magnetospirillum magnetotacticum</i> (Schleifer et al., 1991)	The description of this taxon is as given by (Maratea and Blakemore, 1981)	MS-1, ATCC 31632, DSM 3856, IFO 15272, JCM 21281, LMG10894, NBRC 15272
<i>Paramagnetospirillum marisnigri</i>	<i>Magnetospirillum marisnigri</i> (Dziuba et al., 2016)	The description of this taxon is as given by (Dziuba et al., 2016)	SP-1, DSM 29006, VKM B-2938
<i>Paramagnetospirillum caucaseum</i>	<i>Magnetospirillum caucaseum</i> (Dziuba et al., 2016)	The description of this taxon is as given by (Dziuba et al., 2016)	SO-1, DSM 28995, VKM B-2936
<i>Paramagnetospirillum kuznetsovii</i>	<i>Magnetospirillum kuznetsovii</i> Kozaeva et al., 2019	The description of this taxon is as given by (Kozaeva et al., 2019)	BB-1, KCTC 15749, VKM B-3270
' <i>Paramagnetospirillum magneticum</i> '	' <i>Magnetospirillum magneticum</i> ' Matsunaga et al., 1991	The description of this taxon is as given by (Kawaguchi et al., 1992; Matsunaga et al., 1991)	AMB-1, ATCC 700264
<i>Phaeospirillum molichianum</i>	<i>Rhodospirillum molischianum</i> Giesberger 1947, (Imhoff et al., 1998) <i>Magnetospirillum molischianum</i> (Hördt et al., 2020)	The description of this taxon is as given by (Hördt et al., 2020)	ATCC 14031; DSM 120; LMG 4354
<i>Phaeospirillum fulvum</i>	<i>Rhodospirillum fulvum</i> van Niel 1944, Imhoff et al. 1998 <i>Magnetospirillum fulvum</i> (Hördt et al., 2020)	The description of this taxon is as given by (Hördt et al., 2020)	ATCC 15798; ATCC 53113; DSM 113; Kleinkalden, 1360; SMG 113
<i>Phaeospirillum chandramohanii</i>	<i>Magnetospirillum chandramohanii</i> Anil Kumar et al. 2009, (Hördt et al., 2020)	The description of this taxon is as given by (Hördt et al., 2020)	JA145; JCM 14933; KCTC 5703; NBRC 104961
<i>Phaeospirillum oryzae</i>	<i>Magnetospirillum oryzae</i> Lakshmi et al. 2011, (Hördt et al., 2020)	The description of this taxon is as given by (Hördt et al., 2020)	JA317; KCTC 5704; NBRC 104938
<i>Phaeospirillum tilakii</i>	<i>Magnetospirillum tilakii</i> Raj et al. 2012, (Hördt et al., 2020)	The description of this taxon is as given by (Hördt et al., 2020)	JA492; KCTC 15012; NBRC 107650

Table 6

Protologue descriptions for the proposed new families.

Family name	Family status	Family etymology	Family diagnostic data
<i>Magnetospirillaceae</i>	fam. nov.	N.L. neut. dim. n. <i>Magnetospirillum</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Magnetospirillaceae</i> , the <i>Magnetospirillum</i> family	Gram-negative vibrio to spirilla. Nonmotile or motile by means of 1–2 polar or subpolar flagella. Some genera could form intracellular magnetosomes aligned in a single chain. Chemoorganotrophic, chemolithoautotrophic, or photoorganotrophic under anoxic conditions; some genera grow chemoheterotrophically under aerobic conditions in the dark. The family comprises of the genera <i>Magnetospirillum</i> , <i>Paramagnetospirillum</i> , <i>Phaeospirillum</i> , <i>Telmatospirillum</i> , and <i>Oleiliquidispirillum</i> . The type genus of the family is <i>Magnetospirillum</i>
<i>Magnetospiraceae</i>	fam. nov.	N.L. fem. n. <i>Magnetospira</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Magnetospiraceae</i> , the <i>Magnetospira</i> family.	The description of the family is identical to that given for the genus <i>Magnetospira</i> by Williams et al., 2012 (Williams et al., 2012). The type genus of the family is <i>Magnetospira</i> .
<i>Magnetovibrionaceae</i>	fam. nov.	N.L. masc. n. <i>Magnetovibrio</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Magnetovibrionaceae</i> , the <i>Magnetovibrio</i> family.	The description of the family is based on the shared phenotypic characteristics of its members, the type genus <i>Magnetovibrio</i> (Bazyliński et al., 2013 (Bazyliński et al., 2013) and genus <i>Varunaivibrio</i> (Patwardhan and Vetriani, 2016 (2016)). Currently, the family comprises of the genera <i>Magnetovibrio</i> and <i>Varunaivibrio</i> . The type genus of the family is <i>Magnetovibrio</i> .
<i>Oceanibaculaceae</i>	fam. nov.	N.L. neut. n. <i>Oceanibaculum</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Oceanibaculaceae</i> , the <i>Oceanibaculum</i> family	The description of the family is identical to that given for the genus <i>Oceanibaculum</i> by Lay et al., 2009 (Lai et al., 2009)The type genus of the family is <i>Oceanibaculum</i> .
<i>Dongiaceae</i>	fam. nov.	N.L. fem. n. <i>Dongia</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Dongiaceae</i> , the <i>Dongia</i> family.	The description of the family is based on the shared phenotypic characteristics of its members, the type genus <i>Dongia</i> (Liu et al., 2010 (Liu et al., 2010), and genera <i>Aliidongia</i> (Chen et al., 2017 (Chen et al., 2017) and <i>Hypericibacter</i> (Noviana et al., 2020, 2016 (Noviana et al., 2020). Currently, the family comprises the genera <i>Dongia</i> , <i>Aliidongia</i> , and <i>Hypericibacter</i> . The type genus of the family is <i>Dongia</i> .
<i>Novispirillaceae</i>	fam. nov.	N.L. fem. n. <i>Novispirillum</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Novispirillaceae</i> , the <i>Novispirillum</i> family.	The description of the family is based on the shared phenotypic characteristics of its members, the type genus <i>Novispirillum</i> (Yoon et al., 2007 (Yoon et al., 2007) and genera <i>Caenispirillum</i> (Yoon et al., 2007 (Yoon et al., 2007), <i>Haematospirillum</i> (Humrighouse et al., 2016 (Humrighouse et al., 2016), <i>Insolitispirillum</i> (Yoon et al., 2007 (Yoon et al., 2007), and <i>Marispirillum</i> (Lia et.

(continued on next page)

Table 6 (continued)

Family name	Family status	Family etymology	Family diagnostic data
Rhodospirillaceae	Emended Pfennig and Trueper 1971, Hordt et al., 2020	N.L. neut. dim. n. <i>Rhodospirillum</i> , type genus of the family; L. fem. pl. n. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Rhodospirillaceae</i> , the <i>Rhodospirillum</i> family	al., 2009 (Lia et al., 2009). The family includes the genera <i>Caenospirillum</i> , <i>Haematospirillum</i> , <i>Insolitispirillum</i> , <i>Marispirillum</i> , and <i>Novispirillum</i> (the type genus). The description is as given before (Pfennig and Trüper, 1971), with the following modification. This family houses <i>Pararhodospirillum</i> , <i>Phaeovibrio</i> , <i>Rhodospira</i> , <i>Rhodospirillum</i> (the type genus) and <i>Roseospirillum</i> .
Fodinicurvataceae	fam. nov.	N.L. fem. n. <i>Fodinicurvata</i> , type genus of the family; L. fem. pl. suff. -aceae, ending to denote a family; N.L. fem. pl. n. <i>Fodinicurvataceae</i> , the <i>Fodinicurvata</i> family	The description of the family is based on the shared phenotypic characteristics of its members, the type genus <i>Fodinicurvata</i> (Wang et al., 2009 (Wang et al., 2009)), <i>Pelagibius</i> (Choi et al., 2009 (Choi et al., 2009)), <i>Limibacillus</i> (Kim et al., 2015 (Kim et al., 2015) and genus <i>Tistlia</i> (Díaz-Cárdenas et al., 2010, 2016 (Díaz-Cárdenas et al., 2010)). The family consists of the type genus <i>Fodinicurvata</i> and the genus <i>Tistlia</i> .

Table 7

Proposed taxonomy of the order *Rhodospirillales*, with changes from the existing taxonomy. Hyphens (-) denote no change in classification.

Proposed family	Proposed genus	Proposed species	Current family	Current genus	Current species
<i>Magnetospirillaceae</i> fam. nov.	<i>Paramagnetospirillum</i> gen. nov.	<i>Paramagnetospirillum magnetotacticum</i> (T) comb. nov. <i>Paramagnetospirillum marisnigri</i> comb. nov. <i>Paramagnetospirillum caucaseum</i> comb. nov. <i>Paramagnetospirillum kuznetsovii</i> comb. nov. ' <i>Paramagnetospirillum magneticum</i> '	<i>Rhodospirillaceae</i>	<i>Magnetospirillum</i> (T)	<i>Magnetospirillum magnetotacticum</i> <i>Magnetospirillum marisnigri</i> <i>Magnetospirillum caucaseum</i> <i>Magnetospirillum kuznetsovii</i> ' <i>Magnetospirillum magneticum</i> '
	<i>Phaeospirillum</i>	<i>Phaeospirillum molichanum</i> <i>Phaeospirillum fulvum</i> <i>Phaeospirillum chandramohanii</i>			<i>Magnetospirillum molischianum</i> <i>Magnetospirillum fulvum</i> <i>Magnetospirillum chandramohanii</i>
	<i>Rhodospira</i> emend.	<i>Phaeospirillum oryzae</i> <i>Phaeospirillum tilakii</i> <i>Rhodospira marina</i> comb. nov. <i>Rhodospira goensis</i> comb. nov. <i>Rhodospira mediosalina</i> comb. nov. <i>Rhodospira navarrensis</i> comb. nov. <i>Rhodospira visakhapatnamensis</i> comb. nov.		<i>Roseospira</i>	<i>Magnetospirillum oryzae</i> <i>Magnetospirillum tilakii</i> <i>Roseospira marina</i> <i>Roseospira goensis</i> <i>Roseospira mediosalina</i> <i>Roseospira navarrensis</i> <i>Roseospira visakhapatnamensis</i>
<i>Novispirillaceae</i> fam. nov. <i>Dongiaceae</i> fam. nov. <i>Oceanibaculaceae</i> fam. nov. <i>Magnetospiraceae</i> fam. nov. <i>Magnetovibrionaceae</i> fam. nov. <i>Fodinicurvataceae</i> fam. nov.			<i>Thalassospiraceae</i> <i>Thalassospiraceae</i> <i>Rhodovibrionaceae</i>		

currently classified in the *Rhodospirillaceae*, are forming a separate clade paraphyletic to the other *Rhodospirillaceae* members. The AAI values between these three genera and the remaining *Rhodospirillaceae* were below 60 %. Therefore, *Dongia* and *Hyperichibacter* should be assigned to a new family *Dongiaceae*, while the genus *Oceanibaculum* is forming another new family *Oceanibaculaceae*.

By the same criteria, the family *Thalassospiraceae* should be splitted into three different families. The genus *Magnetospira* would form its own family *Magnetospiraceae*, while the genera *Magnetovibrio* and *Varunavibrio* are united in a new family *Magnetovibrionaceae*.

Inquilinus limosus DSM 16000^T had AAI values in the range of 57–60 % with the other *Azospirillaceae* genera and can be placed in a separate family. However, this needs to be postponed until more related genomes are available.

Within the family *Rhodovibrionaceae*, the genera *Tistlia* and *Fodinicurvata* had < 58 % AAI with the genera *Rhodovibrio*, *Limimonas*, and *Ferruginivarius*. Obviously, the former two need to be separated into a novel family *Fodinicurvataceae*.

Protologes of the new families and proposed changes in taxonomy of the order *Rhodospirillales* are provided below (Tables 6, 7).

Data availability

Data will be made available on request.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.syapm.2023.126406>.

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