

Reclassification of species of the spiral-shaped phototrophic purple non-sulfur bacteria of the α -Proteobacteria: description of the new genera *Phaeospirillum* gen. nov., *Rhodovibrio* gen. nov., *Rhodothalassium* gen. nov. and *Roseospira* gen. nov. as well as transfer of *Rhodospirillum fulvum* to *Phaeospirillum fulvum* comb. nov., of *Rhodospirillum molischianum* to *Phaeospirillum molischianum* comb. nov., of *Rhodospirillum salinarum* to *Rhodovibrio salinarum* comb. nov., of *Rhodospirillum sodomense* to *Rhodovibrio sodomensis* comb. nov., of *Rhodospirillum salexigens* to *Rhodothalassium salexigens* comb. nov. and of *Rhodospirillum mediosalinum* to *Roseospira mediosalina* comb. nov.

Johannes F. Imhoff, Ralf Petri and Jörg Süling

Author for correspondence: Johannes F. Imhoff. Tel: +49 431 597 3850. Fax: +49 431 565 876.
e-mail: jimhoff@ifm.uni-kiel.de

Institut für Meereskunde
Kiel, Düsternbrooker Weg
20, D-24105 Kiel, Germany

The 16S rDNA sequence of *Rhodospirillum mediosalinum* was determined and compared with corresponding sequences from other spiral-shaped purple non-sulfur bacteria classified as or related to the genus *Rhodospirillum* in the α subclass of the *Proteobacteria*. Sequence similarities separate the currently recognized *Rhodospirillum* species into five different groups with no more than 91% sequence similarity, clearly indicating the necessity to recognize these groups as different genera. Major diagnostic properties of these bacteria are compared and new genera *Phaeospirillum* gen. nov., *Roseospira* gen. nov., *Rhodothalassium* gen. nov. and *Rhodovibrio* gen. nov. are described with the species *Phaeospirillum fulvum* comb. nov., *Phaeospirillum molischianum* comb. nov., *Rhodovibrio salinarum* comb. nov., *Rhodovibrio sodomensis* comb. nov., *Rhodothalassium salexigens* comb. nov. and *Roseospira mediosalina* comb. nov. The genus *Rhodospirillum* is represented by *Rhodospirillum rubrum* and *Rhodospirillum photometricum* and an emended description of this genus is also given.

Keywords: *Rhodospirillum*, α -*Proteobacteria*, *Phaeospirillum* gen. nov., *Rhodovibrio* gen. nov., *Rhodothalassium* gen. nov., *Roseospira* gen. nov.

The EMBL accession numbers for the sequences reported in this paper are listed in the legend to Fig. 1.

INTRODUCTION

The genus *Rhodospirillum* at present consists of eight species, with *Rhodospirillum rubrum* as the type species. In addition, *Rhodocista centenaria* (Kawasaki *et al.*, 1992), which was originally described as *Rhodospirillum centenum* (Favinger *et al.*, 1989), has already been considered as a species of the new genus *Rhodocista* primarily on the basis of significant differences in rRNA gene sequences to recognized *Rhodospirillum* species (Kawasaki *et al.*, 1992). Another spiral-shaped species with a moderate relationship to *Rhodospirillum rubrum* is *Rhodospira trueperi*, which has been described as a new genus and species on the basis of phenotypic and genotypic properties (Pfennig *et al.*, 1997). On the basis of its spiral shape, *Rhodocyclus tenuis* had also originally been recognized as a species of the genus *Rhodospirillum* (*Rhodospirillum tenue*, Pfennig, 1969). However, this species belongs to the β subclass of the *Proteobacteria* and was the first species of spiral non-sulfur purple bacteria recognized not to be a *Rhodospirillum* species (Imhoff *et al.*, 1984). It became obvious from the work of Kawasaki *et al.* (1993) that, based on 16S rRNA gene sequences, the recognized species of the genus *Rhodospirillum* of the α subclass of the *Proteobacteria* are phylogenetically quite distantly related and do not warrant classification in the same genus. We report here on the 16S rDNA sequences of the type strains of *Rhodospirillum mediosalinum* and *Rhodospirillum photometricum*. On the basis of 16S rRNA gene sequences of all recognized species of the genus *Rhodospirillum* we propose a reclassification according to their phylogenetic relationship.

METHODS

DNA was extracted and purified from 2 ml of a culture using the Qiagen genomic DNA buffer set. Recombinant *Taq* polymerase was used for PCR (Mullis & Faloona, 1987) with the following primers: 5' GTTTGATCCTGGCTCAG 3' (position 11–27, *Escherichia coli* numbering) and 5' TACCTTGTTACGACTT 3' (1491–1506). Sequences were obtained by cycle sequencing with the SequiTherm sequencing kit (Biozym) and the chain-termination reaction (Sanger *et al.*, 1977) using an automated laser fluorescence sequencer (Pharmacia). Sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994). Distance matrices were calculated on the basis of the algorithm according to Jukes & Cantor (1969) with the DNADIST program within the PHYLIP program package (Felsenstein, 1989). The FITCH program in the PHYLIP package fitted a tree to the evolutionary distances.

RESULTS AND DISCUSSION

The phylogenetic relationship on the basis of 16S rRNA gene sequences of representative strains of species of the genus *Rhodospirillum* in comparison to *E. coli*, *Rhodospira trueperi* and *Rhodocista centenaria* is shown in the distance matrix (Table 1) and in the phylogenetic tree derived from this matrix (Fig. 1). The sequence of *Rhodospirillum mediosalinum* is not similar

to any of the considered sequences [similarity below 89%, except to *Rhodospira trueperi* (90.9%)], indicating that this species should be placed in a separate genus. Also, the type strain of *Rhodocista centenaria* and two additional strains identified as belonging to this species (Kawasaki *et al.*, 1992) are quite distant from all the other species considered, although they are very closely related to each other. A similarly large phylogenetic distance exists between *Rhodospira trueperi* and the *Rhodospirillum* species, supporting its recognition as a new genus (Pfennig *et al.*, 1997). According to the sequence dissimilarity between the other recognized *Rhodospirillum* species, separate genera are proposed for the *Rhodospirillum fulvum*/*Rhodospirillum molischianum* group, for *Rhodospirillum rubrum* and *Rhodospirillum photometricum*, for *Rhodospirillum mediosalinum*, for *Rhodospirillum salexigens* and for the *Rhodospirillum salinarum*/*Rhodospirillum sodomense* group. None of these groups share sequence similarities higher than 91% with each other.

This differentiation is supported by several phenotypic properties, such as major quinone components, fatty acid composition and salt requirement. These and other diagnostic features, including DNA base composition, structure of intracytoplasmic photosynthetic membranes and requirement of growth factors, are shown in Table 2. Fatty acid composition and major quinone components are well recognized as diagnostic properties for differentiation of species, genera and major bacterial groups. The presence of ubiquinones, menaquinones and rhodoquinones, as well as the isoprenoid chain length, have diagnostic value (Hiraishi *et al.*, 1984; Imhoff, 1984, 1988a, b; Imhoff & Bias-Imhoff, 1995). Fatty acids have been used to characterize species of the genus *Rhodobacter* (Imhoff, 1991), for example, and to differentiate species of *Ectothiorhodospira* and *Halorhodospira* (Imhoff & Süling, 1996; Thiemann & Imhoff, 1996). In general a good correlation is obtained between relationships on the basis of 16S rDNA sequence similarities and fatty acid composition as demonstrated with *Ectothiorhodospira* and *Halorhodospira* species (Imhoff & Süling, 1996; Thiemann & Imhoff, 1996).

On the basis of major quinone components and fatty acid composition, all proposed genera of the spiral-shaped purple non-sulfur bacteria can be distinguished (see Table 2). Furthermore, differentiation by these properties is in agreement with groups formed by 16S rDNA sequence similarities. Four of these genera are defined as salt-dependent and three as freshwater bacteria. The salt requirement is considered as a genus-specific property of these bacteria, which is in accordance with different phylogenetic lines forming freshwater and salt water representatives. The requirement for salt and the dependence on different salt concentrations for optimal growth have already been used as diagnostic tools to separate the marine species of *Rhodobacter* into the new genus *Rhodovulum*

Table 1. Levels of 16S rDNA sequence similarity and evolutionary distances of spiral-shaped phototrophic purple non-sulfur bacteria of the α -Proteobacteria with *E. coli* as reference species

The values on the upper right are the uncorrected percentages of sequence similarity and the values on the lower left are K_{nuc} values corrected for multiple base change by the method of Jukes & Cantor (1969).

Organism	Sequence similarity (%) and evolutionary distance (K_{nuc})											
	1	2	3	4	5	6	7	8	9	10	11	12
1 <i>Rhodocista centenaria</i> ATCC 43720 ^T		81.4	87.5	87.6	87.2	89.0	88.5	87.8	87.5	88.4	87.1	88.3
2 <i>Escherichia coli</i>	0.213		79.8	80.3	80.5	80.2	80.5	79.6	79.0	80.8	80.6	80.5
3 <i>Rhodospirillum salinarum</i> ATCC 35394 ^T	0.137	0.235		98.7	88.5	87.0	86.8	86.0	85.5	86.0	85.1	86.6
4 <i>Rhodospirillum sodomense</i> ATCC 51195 ^T	0.136	0.229	0.013		88.5	87.1	86.9	86.2	85.7	86.1	85.5	86.9
5 <i>Rhodospirillum salexigens</i> DSM 2132 ^T	0.140	0.226	0.125	0.125		85.5	85.3	86.0	85.5	86.8	86.8	87.6
6 <i>Rhodospirillum fulvum</i> DSM 113 ^T	0.118	0.230	0.143	0.142	0.161		99.1	88.8	88.3	89.1	88.0	88.2
7 <i>Rhodospirillum molischianum</i> ATCC 14031 ^T	0.125	0.226	0.146	0.144	0.163	0.009		88.6	88.1	88.8	88.0	88.1
8 <i>Rhodospirillum photometricum</i> E11	0.133	0.238	0.155	0.153	0.155	0.121	0.124		99.0	95.6	89.7	90.2
9 <i>Rhodospirillum photometricum</i> DSM 122 ^T	0.137	0.247	0.161	0.158	0.161	0.127	0.130	0.010		95.1	89.4	89.8
10 <i>Rhodospirillum rubrum</i> ATCC 11170 ^T	0.126	0.222	0.155	0.154	0.145	0.118	0.121	0.046	0.051		91.2	91.2
11 <i>Rhodospira trueperi</i> ATCC 700224 ^T	0.141	0.224	0.166	0.162	0.146	0.130	0.131	0.111	0.114	0.093		93.6
12 <i>Rhodospirillum mediosalinum</i> BN 280 ^T	0.127	0.225	0.148	0.144	0.135	0.129	0.129	0.105	0.109	0.094	0.067	

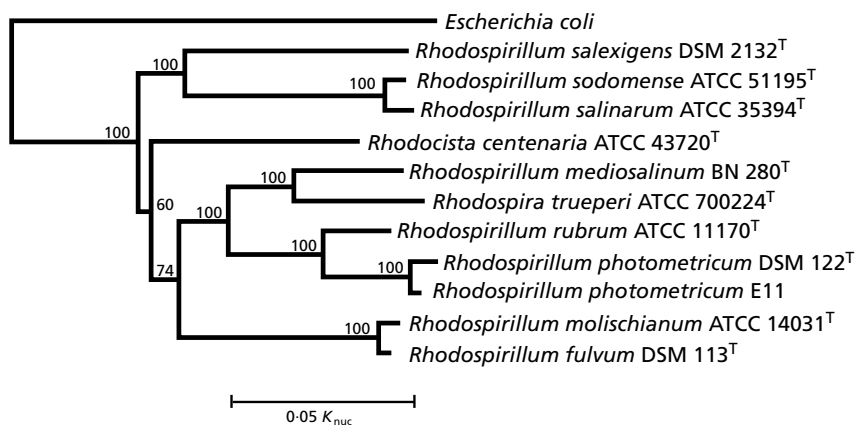


Fig. 1. Phylogenetic tree derived from the distance matrix (Table 1) and calculated as indicated in Methods. The following strains and sequences (accession numbers in brackets) have been included: *E. coli* (K02555), *Rhodospirillum mediosalinum* BN 280^T (AJ000989), *Rhodospira trueperi* ATCC 700224^T (X99671), *Rhodospirillum rubrum* ATCC 11170^T (D30778), *Rhodospirillum photometricum* DSM 122^T (AJ222622), *Rhodospirillum photometricum* E11 (D30777), *Rhodospirillum molischianum* ATCC 14031^T (M59067), *Rhodospirillum fulvum* DSM 113^T (D14433), *Rhodospirillum salexigens* DSM 2132^T (D14431), *Rhodospirillum sodomense* ATCC 51195^T (M59072), *Rhodospirillum salinarum* ATCC 35394^T (D14432) and *Rhodocista centenaria* ATCC 43720^T (D12701).

(Hiraishi & Ueda, 1994) and to classify *Ectothiorhodospira* and *Halorhodospira* species (Imhoff & Süling, 1996). Although the structure of the intracytoplasmic membrane system is not considered of primary importance to differentiate genera, according to their ultrastructure identical internal membrane systems are present in most of the proposed genera

(with the exception of *Rhodospirillum*). The DNA base ratio is fairly similar in all of the described genera, with values between 63 and 70 mol% G + C and is therefore not of diagnostic value for these bacteria. Similarly, the growth factor requirement varies significantly from species to species and cannot be regarded as a suitable tool to differentiate the genera.

Table 2. Diagnostic properties of spiral-shaped purple non-sulfur bacteria of the α -Proteobacteria

Data are from the species descriptions as cited in the text and from Kawasaki *et al.* (1992) and Trüper & Imhoff (1989); fatty acid composition is from Imhoff (1986) and Imhoff & Bias-Imhoff (1995). Abbreviations: ICM, intracellular membranes; lam, lamellae; sta, stacks; ves, vesicles; Q, ubiquinone; RQ, rhodoquinone; MK, menaquinone; ND, not determined.

Species	Salt required (%) [*]	ICM	Cell diam. (μ m)	G + C content (mol%) [†]	Growth factor	Major quinone	Major fatty acids				
							14:0	16:0	16:1	18:0	18:1
<i>Rhodospirillum rubrum</i>	None	ves	0.8–1.0	63.8–65.8	Biotin	Q-10, RQ-10	2.1	14.0	27.1	1.3	54.8
<i>Rhodospirillum photometricum</i>	None	sta	1.2–1.5	64.8–65.8	Niacin	Q-8, RQ-8	1.0	25.2	22.2	0.4	51.0
<i>Rhodospirillum fulvum</i>	None	sta	0.5–0.7	64.3–65.3	<i>p</i> -Aminobenzoic acid	Q-9, MK-9	0.6	15.1	25.8	1.2	54.5
<i>Rhodospirillum molischianum</i>	None	sta	0.7–1.0	60.5–64.8	Amino acids	Q-9, MK-9	0.7	18.1	36.5	0.7	43.5
<i>Rhodocista centenaria</i>	None	lam	1.0	69.9 nd	Biotin, B ₁₂	Q-9	ND	ND	ND	ND	ND
<i>Rhodospirillum salexigens</i>	7 (5–20)	lam	0.6–0.7	64.0	Glutamate	Q-10, MK-10	3.8	16.1	1.5	17.8	59.9
<i>Rhodospirillum salinarum</i>	4 (3–24)	ves	0.8–0.9	67.4	Yeast extract	Q-10, MK-10	1.0	7.4	0.3	23.0	35.2
<i>Rhodospirillum sodomense</i>	12 (6–20)	ves	0.6–0.7	66.2–66.6 nd	Complex nutrients	ND	ND	ND	ND	ND	ND
<i>Rhodospira trueperi</i>	2 (0.5–5)	ves	0.6–0.8	65.7	Biotin, thiamine, pantothenate	Q-7, MK-7	7.5	27.9	1.2	1.2	60.7
<i>Rhodospirillum mediosalinum</i>	4–7 (0.5–15)	ves	0.8–1.0	66.6 nd	Thiamine, <i>p</i> -aminobenzoic acid, niacin	ND	ND	ND	ND	ND	ND

* Optimum concentrations with growth ranges in parentheses.

† G + C content determined as buoyant density and by thermal denaturation (nd).

From the available data it is obvious that phylogenetic relationships based on 16S rDNA sequences of these bacteria are in good correlation with differences in major quinone and fatty acid composition and are in accordance with the requirement for NaCl or sea salt for growth. Therefore, these properties must be considered as being of primary importance in defining and differentiating the genera. Accordingly, the majority of these species must be placed into different genera and only *Rhodospirillum rubrum* and *Rhodospirillum photometricum* belong to the genus *Rhodospirillum*. Following Rule 39a of the International Code of Nomenclature of Bacteria (Lapage *et al.*, 1992), the genus name *Rhodospirillum* must be maintained with the type species of this genus, namely *Rhodospirillum rubrum*.

In contrast to the clear separation of the proposed genera, *Rhodospirillum fulvum* and *Rhodospirillum molischianum* are highly similar on the basis of their 16S rDNA sequences and hardly qualify as separate species. The same holds for *Rhodospirillum sodomense* and *Rhodospirillum salinarum*. On the basis of their phenotypic properties, however, they are well recognized species (Trüper & Imhoff, 1989; Nissen & Dundas, 1984; Mack *et al.*, 1993) and it is intended to maintain these. Therefore, we propose the following taxonomic changes.

1. Transfer of *Rhodospirillum fulvum* and *Rhodospirillum molischianum* to *Phaeospirillum* gen. nov. as *Phaeospirillum fulvum* comb. nov. and *Phaeospirillum molischianum* comb. nov.
2. Transfer of *Rhodospirillum salinarum* and *Rhodospirillum sodomense* to *Rhodovibrio* gen. nov. as *Rhodovibrio salinarum* comb. nov. and *Rhodovibrio sodomensis* comb. nov.
3. Transfer of *Rhodospirillum salexigens* to *Rhodothalassium* gen. nov. as *Rhodothalassium salexigens* comb. nov.

4. Transfer of *Rhodospirillum mediosalinum* to *Roseospira* gen. nov. as *Roseospira mediosalina* comb. nov.

Emended description of the genus *Rhodospirillum* (Molisch 1907)

Rhodospirillum (Rho.do.spi.ril'lum. Gr. n. *rhodon* the rose; M.L. neut. n. *Spirillum* a bacterial genus; M.L. neut. n. *Rhodospirillum* the rose *Spirillum*).

Cells are vibrioid- to spiral-shaped, 0.8–1.5 μ m wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. They belong to the α -Proteobacteria and have a G + C content between 63 and 66 mol%. Intracytoplasmic photosynthetic membranes are present as vesicles or lamellae, contain bacteriochlorophyll *a* and various carotenoids as photosynthetic pigments. Contain ubiquinones and rhodoquinones. Freshwater bacteria without a requirement for NaCl or sea salt for growth. Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but is also possible under micro-oxic to oxic conditions in the dark. Growth factors are required. Known species of the genus are *Rhodospirillum rubrum* (Molisch 1907) and *Rhodospirillum photometricum* (Molisch 1907). The type species is *Rhodospirillum rubrum*.

Description of *Phaeospirillum* gen. nov.

Phaeospirillum (Phae.o.spi.ril'lum. Gr. adj. *phaeos* brown; M.L. neut. n. *Spirillum* a bacterial genus; M.L. neut. n. *Phaeospirillum* brown *Spirillum*).

The name *Phaeospirillum* was proposed by Kluyver & van Niel (1936) for brown-coloured phototrophic spiral-shaped bacteria. It is not included in the *Approved Lists of Bacterial Names* and therefore has no standing in nomenclature. It is proposed to revive

this name for the brown-coloured spiral-shaped phototrophic purple non-sulfur bacteria of the α -*Proteobacteria* that do not belong to the genus *Rhodospirillum*. Cells are vibrioid- to spiral-shaped, 0.5–1.0 μm wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. They belong to the α -*Proteobacteria* and have a G+C content between 60 and 66 mol%. Intracytoplasmic photosynthetic membranes are present as lamellar stacks, contain bacteriochlorophyll *a* and various carotenoids as photosynthetic pigments. Contain ubiquinones and menaquinones Q-9 and MK-9 as major components. Freshwater bacteria without a requirement for NaCl or sea salt for growth. Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but is also possible in the dark at very low oxygen tensions. Growth factors are required. Known species of the genus are *Phaeospirillum fulvum* and *Phaeospirillum molischianum*. The type species is *Phaeospirillum fulvum*.

Description of *Phaeospirillum fulvum* comb. nov.

Phaeospirillum fulvum (*Rhodospirillum fulvum* van Niel 1944). The description is the same as that for *Rhodospirillum fulvum* (van Niel, 1944; Trüper & Imhoff, 1989).

Description of *Phaeospirillum molischianum* comb. nov.

Phaeospirillum molischianum (*Rhodospirillum molischianum* Giesberger 1947). The description is the same as that for *Rhodospirillum molischianum* (Giesberger, 1947; Trüper & Imhoff, 1989).

Description of *Rhodovibrio* gen. nov.

Rhodovibrio (Rho.do.vi'bri.o. Gr. n. *rhodon* the rose; M.L. masc. n. *Vibrio* a bacterial genus; M.L. masc. n. *Rhodovibrio* the rose *Vibrio*).

The genus name *Rhodovibrio* was proposed by Molisch (1907) for bacteria (*Rhodovibrio parvus*) later recognized as belonging to *Rhodopseudomonas palustris*. It is not included in the *Approved Lists of Bacterial Names* and therefore has no standing in nomenclature. It is proposed to revive this name for description of bacteria with the following properties. Cells are vibrioid- to spiral-shaped, 0.6–0.9 μm wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. They belong to the α -*Proteobacteria* and have a G+C content between 65 and 69 mol%. Intracytoplasmic photosynthetic membranes are present as vesicles, contain bacteriochlorophyll *a* and various carotenoids as photosynthetic pigments. Contain ubiquinones and menaquinones Q-10 and MK-10 as major components. Halophilic bacteria that require NaCl or sea salt for growth. Have salt optima above sea water salinity and tolerate up to 20% total salts. Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but is also possible under micro-

oxic to oxic conditions in the dark. Complex nutrients are required. Known species of the genus are *Rhodovibrio salinarum* and *Rhodovibrio sodomensis*. The type species is *Rhodovibrio salinarum*.

Description of *Rhodovibrio salinarum* comb. nov.

Rhodovibrio salinarum (*Rhodospirillum salinarum* Nissen and Dundas 1984). The description is the same as that for *Rhodospirillum salinarum* (Nissen & Dundas, 1984; Trüper & Imhoff, 1989).

Description of *Rhodovibrio sodomensis* comb. nov.

Rhodovibrio sodomensis (*Rhodospirillum sodomense* Mack *et al.* 1993). The description is the same as that for *Rhodospirillum sodomense* (Mack *et al.*, 1993).

Description of *Rhodothalassium* gen. nov.

Rhodothalassium (Rho.do.tha.las'si.um. Gr. n. *rhodon* the rose; Gr. adj. *thalassios* belonging to the sea; M.L. neut. n. *Rhodothalassium* the rose belonging to the sea).

Cells are vibrioid- to spiral-shaped, 0.5–1.0 μm wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. They belong to the α -*Proteobacteria* and have a G+C content between 63 and 66 mol%. Intracytoplasmic photosynthetic membranes are present as lamellar stacks. Contain bacteriochlorophyll *a* and various carotenoids as photosynthetic pigments. Contain ubiquinones and menaquinones Q-10 and MK-10 as major components. Halophilic bacteria that require NaCl or sea salt for growth. Have salt optima above sea water salinity and tolerate up to 20% or more total salts. Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but is also possible under micro-oxic to oxic conditions in the dark. Amino acids may be required as growth factors. The type species is *Rhodothalassium salexigens*.

Description of *Rhodothalassium salexigens* comb. nov.

Rhodothalassium salexigens (*Rhodospirillum salexigens* Drews 1981). The description is the same as that for *Rhodospirillum salexigens* (Drews, 1981; Trüper & Imhoff, 1989).

Description of *Roseospira* gen. nov.

Roseospira (Ro.se.o.spi'ra. L. adj. *roseus* rosy; Gr. n. *spira* the spiral; M.L. fem. n. *Roseospira* the rosy spiral).

Cells are vibrioid- to spiral-shaped, 0.6–1.0 μm wide, motile by means of polar flagella, multiply by binary fission and are Gram-negative. They belong to the α -*Proteobacteria* and have a G+C content between 65 and 68 mol%. Intracytoplasmic photosynthetic membranes are present as vesicles, contain bacterio-

chlorophyll *a* and various carotenoids as photosynthetic pigments. Halophilic bacteria that require NaCl or sea salt for growth. Have salt optima above sea water salinity and tolerate up to 15% total salts. Growth occurs preferably photo-organotrophically under anoxic conditions in the light, but is also possible under micro-oxic conditions in the dark. Growth factors are required. The type species is *Roseospira mediosalina*.

Description of *Roseospira mediosalina* comb. nov.

Roseospira mediosalina (*Rhodospirillum mediosalinum* Kompantseva and Gorlenko 1984). The description is the same as that for *Rhodospirillum mediosalinum* (Kompantseva & Gorlenko, 1984).

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