Roseospira visakhapatnamensis sp. nov. and *Roseospira goensis* sp. nov.

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Two Gram-negative, vibrioid, phototrophic, purple non-sulfur strains, JA131^T and JA135^T, were isolated from marine habitats. Strain JA131^T is non-motile but strain JA135^T is motile by means of a pair of monopolar flagella. Both strains have an obligate requirement for NaCl for growth. The intracellular photosynthetic membranes of the two novel strains are of the vesicular type. Bacteriochlorophyll *a* and probably rhodovibrine are present as photosynthetic pigments. Niacin, thiamine and *p*-aminobenzoic acid are required as growth factors for both novel strains. Based on 16S rRNA gene sequence analysis, morphological and physiological characteristics, strains JA131^T and JA135^T are significantly different from each other and from other species of the genus *Roseospira* and thus represent two novel species for which the names *Roseospira visakhapatnamensis* sp. nov. and *Roseospira goensis* sp. nov. are proposed, respectively. The type strain of *Roseospira visakhapatnamensis* sp. nov. is JA131^T (=ATCC BAA-1365^T=JCM 14190^T) and the type strain of *Roseospira goensis* sp. nov. is JA135^T (=ATCC BAA-1364^T=JCM 14191^T).

The genus *Roseospira* comprises three species with validly published names: *Roseospira mediosalina* (Imhoff *et al.*, 1998a) (originally described as '*Rhodospirillum mediosalinum*'; Kompantseva & Gorlenko, 1984), *Roseospira marina* and *Roseospira navarrensis*. Another species of the genus *Roseospira*, '*Roseospira thiosulfatophila*', has been proposed (Guyoneaud *et al.*, 2002) but, to date, the name has not been validly published. In this communication, we propose two novel species of the genus *Roseospira*.

Strain JA131^T was isolated from a water sample [pH ~6.8, 30 °C, 2–3 % (w/v) salinity] which was collected on 25 March 2004 from the fishing harbour at Visakhapatnam, India (17° 41' N 83° 18' E). Strain JA135^T was isolated from a sediment sample [pH ~6.8; 30 °C, 6–7 % (w/v) salinity] collected on 12 February 2005 from Kurka saltern, Goa, India (15° 29' N 73° 49' E). Original enrichments of both strains were from photolithoheterotrophic media [anaerobic, 1 mM Na₂S·9H₂O+0.3 % (w/v) pyruvate/malate]. Strain JA131^T was isolated from an enrichment

culture containing 2 % NaCl and strain JA135^T was isolated from an enrichment containing 8 % NaCl. Subsequent culturing, purification and characterization were performed in Biebl & Pfennig (1981) medium with the following modifications (l⁻¹); 1 g MgSO₄·7H₂O, 0.15 g CaCl₂·2H₂O and 20 g NaCl supplemented with Na₂S₂O₃·5H₂O (2 mM). Strain purification and a taxonomic study based on a polyphasic approach were carried out as described earlier (Srinivas *et al.*, 2007).

Cell material for 16S rRNA gene sequencing was taken from 1–2 ml well-grown liquid culture. DNA was extracted and purified by using the Qiagen genomic DNA extraction kit. PCR amplification and 16S rRNA gene sequencing were performed as described previously (Imhoff *et al.*, 1998b). Recombinant *Taq* polymerase was used for PCR. The PCR was started with the primers 5'-GTTTGA-TCTGCTCAG-3' and 5'-TACCTTGTTACGACTTCA-3' (positions 11–27 and 1489–1506, respectively, according to the *Escherichia coli* 16S rRNA numbering system of the International Union of Biochemistry). 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_X program (Thompson *et al.*, 1997) and the alignment was corrected manually. The CLUSTAL_X alignment file was used as the input file to the

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JA131^T and JA135^T are AM282560 and AM283537, respectively.

Phase contrast images and transmission electron micrographs of cells of strains JA131^T and JA135^T and plots of the absorption spectra of extracted pigments from the two strains are available with the online version of this paper.

SEQBOOT program in the PHYLIP package (Felsenstein, 1989) and the output file from SEQBOOT was used as the input file for maximum-likelihood analysis with 100 datasets and five times jumbling. One single tree was produced using 100 trees generated during maximum-likelihood analysis using the CONSENSE program. A final dendrogram with evolutionary distances was constructed by taking the alignment.phy file as the infile and the consensus tree as the intree in the maximum-likelihood program of the PHYLIP package

While cells of strain JA131^T are vibrioid, 0.5–0.9 µm wide and 2–6 µm long (see Supplementary Fig. S1a in IJSEM Online), cells of strain JA135^T are vibrioid to crescent-shaped (short to very long spirals of 12–30 µm occur rarely), 0.8–1.0 µm wide and 3–8 µm long (see Supplementary Fig. S1b in IJSEM Online). Both strains multiply by binary fission. Strain JA131^T is non-motile. Strain JA135^T is motile by means of a pair of monopolar flagella (see Supplementary Fig. S2 available with the online version of this paper). Transmission electron micrographs of ultrathin sections of both novel strains revealed a vesicular type of internal membrane structure (see Supplementary Fig. S3a, b in IJSEM Online). Both strains were able to grow photoorganoheterotrophically [anaerobic, light (2400 lx), with pyruvic acid (0.3 % w/v)]. Chemoorganoheterotrophic [aerobic, dark and pyruvate (0.3 % w/v)] growth was observed in both strains, however, the biomass yields were poor. Photolithoautotrophy [anaerobic, light (2400 lx), Na₂S·9H₂O, Na₂S₂O₃·5H₂O (0.5 mM) and NaHCO₃ (0.1 % w/v)], chemolithoautotrophy [aerobic, dark, Na₂S₂O₃·5H₂O (0.5 mM) and NaHCO₃ (0.1 % w/v)] and fermentative growth (anaerobic, dark, with pyruvate [0.3 % w/v]) could not be demonstrated. Organic substrates utilized/not utilized by the novel strains are given in Table 1. Both novel strains could utilize ammonium chloride, glutamate and glutamine as nitrogen sources, while urea, nitrate and nitrite did not support growth. Diazotrophic growth and acetylene reduction could not be demonstrated. Niacin, thiamine and *p*-aminobenzoic acid are required as growth factors. Sulfate assimilation is absent and sulfide, thiosulphate and thioglycolate are used as sulfur sources by both novel strains; in addition, strain JA135^T could utilize elemental sulfur. Salt (NaCl) is essential for the growth of both novel strains; the salinity range was from 1–5 % (w/v) and the optimum level was 2 % (w/v) for strain JA131^T and 1–3 % (w/v) for strain JA135^T. The pH range for growth of strain JA131^T is 6.5–8.0, with an optimum at pH 7.0. For strain JA135^T, the pH range for growth is 7.0–8.0, with an optimum at pH 7.5. The temperature range for growth for both strains is 25–35 °C and the optimum is 30 °C.

Cell suspensions of both novel strains were red–brown when grown photosynthetically. The whole cell absorption spectrum of strain JA131^T showed absorption maxima at 371, 473, 503, 540, 590, 803, 847 and 900 nm and strain JA135^T showed maxima at 377, 473, 503, 540, 590, 803 and 857 nm, confirming the presence of bacteriochlorophyll *a*

and most probably carotenoids of the spirilloxanthin series (rhodovibrine) (see Supplementary Fig. S4a, b in IJSEM Online). An unusual shoulder peak at ~909 nm was observed with strain JA131^T as previously observed with '*Roseospira thiosulfatophila*' (Guyoneaud *et al.*, 2002).

The DNA G+C contents of strains JA131^T and JA135^T were 67 and 71 mol% (by HPLC), respectively. The phylogenetic relationships of strains JA131^T and JA135^T to other purple nonsulfur bacteria were examined using 16S rRNA gene sequences. The data obtained revealed that the novel isolates clustered with the type strains of species of the genus *Roseospira* but were distinct from other genera of purple nonsulfur bacteria. Strains JA131^T and JA135^T showed the highest gene sequence similarities to the type strains of *Roseospira navarrensis* (95.9 %), *Roseospira marina* (95.5 %), *Roseospira mediosalina* (94.2 %) and '*Roseospira thiosulfatophila*' (96.1 %). The sequence similarity between strains JA131^T and JA135^T is 96.6 % (Fig. 1). Apart from differences in 16S rRNA gene sequences, strains JA131^T and JA135^T also showed clear phenotypic differences to other species of the genus *Roseospira* (Table 1) that justify their description as representatives of two separate novel species, *Roseospira visakhapatnamensis* sp. nov. and *Roseospira goensis* sp. nov., respectively.

Description of *Roseospira visakhapatnamensis* sp. nov.

Roseospira visakhapatnamensis (vi.sa'kha.pat.nam.en.sis. N.L. fem. adj. *visakhapatnamensis* pertaining to Visakhapatnam, a port city in Andhra Pradesh, India, from where the type strain was isolated).

Cells are vibrioid, 0.5–0.9 µm wide and 2–6 µm long. Cells are non-motile and divide by binary fission. Growth occurs under anaerobic conditions in the light (photoorganoheterotrophy) or under aerobic conditions in the dark (chemoorganoheterotrophy). Internal photosynthetic membranes are of the vesicular type. Phototrophic cultures are red–brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 371, 473, 503, 540, 590, 803, 847 and 900 nm, confirming the presence of bacteriochlorophyll *a*, with an unusual absorption shoulder at 900 nm. The type strain is mesophilic (30 °C), with a pH optimum of 7.0 and requires 2 % NaCl (w/v) for optimal growth. The preferred mode of growth is photoorganoheterotrophy with a few organic compounds. Pyruvate, aspartate and peptone are good carbon sources. Growth also occurs on mannitol and cysteine. Photoautotrophic and chemoautotrophic growth are not possible in the presence of sulfide/thiosulfate/hydrogen as electron donors and NaHCO₃ as the carbon source. Fermentative growth is not possible in the presence of pyruvate as a fermentable carbon source. Sulphate assimilation is absent. Sulfide, thiosulfate and thioglycolate are used as sulfur sources. Niacin, *p*-aminobenzoic acid and thiamine are required as growth factors. The DNA G+C base composition is 67 mol% (by HPLC). Natural habitat is marine waters.

Table 1. Differentiating characteristics of species of the genus *Roseospira*

Taxa: 1, *Roseospira marina*; 2, *Roseospira navarrensis*; 3, '*Roseospira thiosulfatophila*'; 4, *Roseospira mediosalina* (Kompantseva and Gorlenko, 1984); 5, strain JA131^T; 6, strain JA135^T. Data for taxa 1–3 are from Guyoneaud *et al.* (2002). +, Substrate utilized or present; –, substrate not utilized or absent; (+), weak growth; μ , growth under micro-oxic conditions; ND, not determined. Pyruvate was utilized by all the strains. Caproate, caprylate, glycolate, tartrate, methionine, ethanol and propanol were not utilized by any of the strains.

Characteristic	1	2	3	4	5*	6*
Cell size (μm)	0.4–0.8 $\times$ 1.5–6.0	0.6–0.9 $\times$ 3.5–6.5	0.5–0.8 $\times$ 2.5–6.5	0.8–1.0 $\times$ 2.2–6.0	0.5–0.9 $\times$ 2–6	0.8–1.0 $\times$ 3.0–8.0
Motility	+	+	+	+	–	+
Colour of cell suspensions	Red	Brown–red	Red	Brown–red	Red–brown	Red–brown
Main carotenoids	Rhodovibrine, Rhodopine	Rhodopine, lycopene	Rhodovibrine, spirilloxanthin	Rhodopine, lycopene	Rhodovibrine	Rhodovibrine
Shoulder peak at ~909 nm	–	–	+	–	+	–
DNA G + C content (mol%)	68.8–69.4	66.8	71.9–72.3	66.6	67	71
Growth factors	Niacin, thiamine, <i>p</i> -aminobenzoic acid	Yeast extract	Yeast extract	Niacin, thiamine, <i>p</i> -aminobenzoic acid	Niacin, thiamine, <i>p</i> -aminobenzoic acid	Niacin, thiamine, <i>p</i> -aminobenzoic acid
Optimal NaCl concentration (% w/v)	2–4	3–4	0.5	4–7	2	1–3
NaCl concentration for growth (% w/v)	0.5–10	1–10	0.2–5	0.5–15	1–5	1–5
Assimilatory SO_4^{2-} reduction	+	–	–	+	–	–
Dark aerobic growth	+	μ	μ	μ	+	+
Photolithoautotrophic growth (e^- donor)	–	+ (H_2S)	+ (H_2S , S_2O_3)	+ (H_2S)	–	–
Utilization of carbon/electron source:						
Acetate	+	+	+	+	–	(+)
Aspartate	+	+	+	+	+	–
Benzoate	–	+	–	–	–	–
Butyrate	+	+	+	+	–	+
Casamino acids, yeast extract	+	+	+	ND	–	–
Citrate	–	+	–	–	–	–
Crotonate	+	+	+	ND	–	–
Cysteine	(+)	ND	ND	ND	+	–
Formate	(+)	–	–	–	–	(+)
Fructose	+	–	–	–	–	–
Glucose	–	–	+	–	–	(+)
Glutamate	+	+	+	+	–	–
Glycerol	+	+	+	+	–	+
Lactate	+	+	+	+	–	+
Malate, fumarate, succinate	+	+	–	+	–	(+), +, –
Mannitol	+	+	–	–	+	–
2-Oxoglutarate	+	+	+	ND	–	+
Peptone	+	+	+	ND	+	+
Propionate	+	+	–	+	–	–
Sulfide	–	+	+	+	–	–
Thiosulfate	–	–	+	–	–	–
Valerate	+	+	+	–	–	+

*Organic substrate utilization was tested during photoheterotrophic growth.

Fig. 1. Dendrogram constructed using the maximum-likelihood method depicting the phylogenetic relationships of strains JA131^T and JA135^T within the family *Rhodospirillaceae* determined using 16S rRNA gene sequence analysis. Bar, 1 nucleotide substitution per 100 nucleotides.

The type strain, strain JA131^T (=ATCC BAA-1365^T=JCM 14190^T), was isolated from water samples from a fishing harbour at Visakhapatnam, a city which faces into the Bay of Bengal and is on the East coast of India.

Description of *Roseospira goensis* sp. nov.

Roseospira goensis (go'en.sis. N.L. fem. adj. *goensis* pertaining to Goa, a state in India known for its beautiful beaches, from where the type strain was isolated).

Cells are vibrioid to crescent-shaped, 0.8–1.0 µm wide and 3.0–8.0 µm long. Cells are motile by means of a pair of monopolar flagella and divide by binary fission. Growth occurs under anaerobic conditions in the light (photoorganoheterotrophy) or under aerobic conditions in the dark (chemoorganoheterotrophy). Internal photosynthetic membranes are of the vesicular type. Phototrophic cultures are red–brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 377, 473, 503, 540, 590, 803 and 857 nm confirming the presence of bacteriochlorophyll *a* and most probably rhodovibrine. The type strain is mesophilic (30 °C), with a pH optimum at 7.5 and requires 1–3 % NaCl (w/v) for optimal growth. The preferred mode of growth is photoorganoheterotrophy with a few organic compounds. Butyrate, valerate, lactate, pyruvate, fumarate, glutarate, glycerol, 2-oxoglutarate and peptone are good carbon sources. Growth also occurs on formate, acetate, malate and glucose. Photoautotrophic

and chemoautotrophic growth does not take place in the presence of sulfide/thiosulfate/hydrogen as the electron donor and NaHCO₃ as the carbon source. Fermentative growth does not take place in the presence of pyruvate as the fermentable carbon source. Sulphate assimilation is absent. Sulfide, thiosulphate, thioglycolate and elemental sulfur are used as sulfur sources. Niacin, thiamine and *p*-aminobenzoic acid are required as growth factors. The DNA G+C content is 71 mol% (by HPLC). Natural habitats are marine salterns.

The type strain, JA135^T (=ATCC BAA-1364^T=JCM 14191^T) was isolated from sediment samples from a marine saltern, Goa, facing the Arabian sea on the West coast of India.

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