Rhodospirillum sulfurexigens sp. nov., a phototrophic alphaproteobacterium requiring a reduced sulfur source for growth

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A Gram-negative, spiral-shaped, phototrophic, purple non-sulfur bacterial strain, JA143^T, was isolated from a freshwater habitat. Strain JA143^T was motile by means of bipolar tufts of flagella. Intracellular photosynthetic membranes are of the lamellar stacked type. Bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series with rhodovibrin are present as photosynthetic pigments. Thiamine and a reduced sulfur source are required for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA143^T clusters with species of the genus *Rhodospirillum*, belonging to the class *Alphaproteobacteria*. The highest sequence similarities of strain JA143^T were found with the type strains of *Rhodospirillum rubrum* (95.6 %) and *Rhodospirillum photometricum* (95.7 %). Based on 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA143^T was significantly different from the other two recognized species of the genus *Rhodospirillum* and represents a novel species, for which the name *Rhodospirillum sulfurexigens* sp. nov. is proposed. The type strain is JA143^T (=DSM 19785^T =NBRC 104433^T).

The genus *Rhodospirillum* comprises nine species with validly published names; six of these have been reclassified in other genera (Imhoff *et al.*, 1998), and *Rhodospirillum centenum* is a synonym of *Rhodocista centenaria*. The remaining two species, *Rhodospirillum rubrum* and *Rhodospirillum photometricum*, can both assimilate sulfate as a source of sulfur (Imhoff, 2005). In this communication, we propose a novel species of the genus *Rhodospirillum* that requires a reduced sulfur source for growth.

Strain JA143^T was isolated from a water sample (approx. pH 7.2, 30 °C) which was collected on 18 March 2007 from the freshwater reservoir at Mudasarlova, Visakhapatnam, India (17° 45' N 83° 17' E). Original enrichment of the strain was from mineral medium (Biebl & Pfennig, 1981) [anaerobic, 1 mM Na₂S·9H₂O plus 0.3 % (w/v) pyruvate]. Subsequent culturing, purification and characterization were done in the same medium supplemented with Na₂S₂O₃·5H₂O (6 mM) in place of Na₂S·9H₂O. Purification and polyphasic taxonomic studies were carried

out as described previously (Srinivas *et al.*, 2007). 16S rRNA gene sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997) and the alignment was corrected manually. The alignment file was saved with 'phy' extension. The dendrogram was constructed using the PhyML program (Guindon & Gascuel, 2003) using 100 replicates of non-parametric bootstrap analysis, the GTR model of nucleotide substitution and four substitution rate categories.

Cells of strain JA143^T are spiral shaped, 1.0–1.3 µm wide; one single turn is 8–9 µm long. Cells also form long chains, more than 30 µm long (Fig. 1) and multiply by binary fission. Strain JA143^T is motile by means of bipolar tufts of flagella (Supplementary Fig S1, available in IJSEM Online). Transmission electron photomicrographs of ultrathin sections of the strain revealed a lamellar stacked type of internal membrane structures (Supplementary Fig. S2). Strain JA143^T was able to grow photoorganoheterotrophically [anaerobic, light (2400 lx), with pyruvic acid (0.3 %, w/v) as carbon source/electron donor and Na₂S₂O₃·5H₂O (2 mM) as sulfur source]. Photolithoautotrophy [anaerobic, light (2400 lx), Na₂S·9H₂O (2 mM)/Na₂S₂O₃·5H₂O (6 mM) and NaHCO₃ (0.1 %, w/v)], chemolithoautotrophy [aerobic, dark, Na₂S₂O₃·5H₂O (2 mM) and NaHCO₃ (0.1 %, w/v)], chemoorganoheterotrophy [aerobic, dark, pyruvate

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA143^T is AM710622.

Electron micrographs of cells of strain JA143^T are available as supplementary material with the online version of this paper.

Fig. 1. Phase-contrast micrograph of cells of strain JA143^T. Bar, 5 µm.

(0.3 %, w/v) as carbon source/electron donor and Na₂S₂O₃·5H₂O (2 mM) as sulfur source] and fermentative growth [anaerobic, dark with pyruvate (0.3 %, w/v) as carbon source/electron donor and Na₂S₂O₃·5H₂O (2 mM) as sulfur source] could not be demonstrated. Organic substrates utilized for photoorganoheterotrophy include acetate, butyrate, caproate, crotonate, fructose, fumarate, glucose, glutamate, lactate, malate, pyruvate, succinate and valerate (Table 1). Those that could not be utilized by strain JA143^T include arginine, aspartate, benzoate, caprylate, citrate, ethanol, formate, glycerol, glycolate, mannitol, methanol, propionate and tartrate. Strain JA143^T could utilize ammonium chloride, aspartate, glutamate and glutamine as nitrogen sources, while molecular nitrogen, urea, nitrate and nitrite did not support growth. Strain JA143^T requires thiamine as growth factor. There is no sulfate assimilation and sodium sulfide, thiosulfate, thioglycollate, cysteine and methionine are used as sulfur sources by the strain. There is no salt requirement for strain JA143^T, but it can tolerate up

Table 1. Differentiating characteristics of strain JA143^T and species of the genus *Rhodospirillum*

Data for reference species were taken from Imhoff (2005). +, Substrate utilized or present; –, substrate not utilized or absent; (+), weak growth; ±, strain-dependent reaction; ND, not determined; NA, no data available. All strains are spiral shaped, divide by binary fission and are motile. Organic substrate utilization was tested during photoheterotrophic growth. Acetate, butyrate, pyruvate, fumarate, malate, succinate and valerate were utilized by all the taxa. Benzoate, citrate, formate and tartrate were not utilized by any of the taxa.

Characteristic	JA143 ^T	<i>Rsp. rubrum</i>	<i>Rsp. photometricum</i>
Cell width (µm)	1.0–1.3	0.8–1.0	1.1–1.5
Length and width of a complete spiral turn (µm)	8–9 × 2.5–3.0	7–10 × 1.5–2.5	4–7 × 2.5–4.0
Colour	Pink	Pink to deep red	Brown–orange to brown–red/dark brown
Main carotenoid(s)	Rhodovibrin	Spirilloxanthin	Rhodopin and lycopene
Internal membrane system	Stacks	Vesicles	Stacks
Assimilatory sulfate reduction	–	+	+
DNA G + C content (mol%)	64.7	63.8–65.8	64.8–65.8
Growth factors	Thiamine	Biotin	Nicotinamide
Dark aerobic growth	–	+	–
Photolithoautotrophic growth	–	+	–
Use as carbon source/electron donor of:			
Arginine	–	+	–
Aspartate	–	+	±
Caproate	+	+	–
Caprylate	–	NA	–
Ethanol	–	+	+
Fructose	(+)	±	+
Glucose	(+)	–	+
Glutamate	(+)	+	–
Glycerol	–	–	+
Glycolate	–	NA	+
Lactate	(+)	+	+
Mannitol	–	–	+
Methanol	–	±	–
Pelargonate	ND	NA	±
Propionate	–	+	±

Fig. 2. Dendrogram depicting the phylogenetic relationships of strain JA143^T within the family *Rhodospirillaceae* determined using 16S rRNA gene sequence analysis. The dendrogram was constructed using the PhyML method. Numbers at nodes are bootstrap values; values below 50 were removed from the dendrogram. Bar, 2 substitutions per 100 nucleotide positions.

to 1.5% (w/v) NaCl. The pH range for strain JA143^T is 6.5–7.5, with an optimum at pH 7.0. The temperature range is 25–35 °C and the optimum is 30 °C. Photosynthetically grown cultures are pink. The whole-cell absorption spectrum of strain JA143^T showed absorption maxima at 374, 473, 500, 530, 590, 791 and 848 nm, confirming the presence of bacteriochlorophyll *a*, and the absorption spectrum for pigments extracted with ethanol gave absorption maxima at 454, 482 and 515 nm, indicating the presence of carotenoids of the spirilloxanthin series. Two peaks at 791 and 884 nm (shoulder peak) were observed.

The DNA base composition of strain JA143^T was 64.7 mol% G + C (by HPLC). The phylogenetic relationship of strain JA143^T to other purple non-sulfur bacteria was examined on the basis of 16S rRNA gene sequences. The data obtained revealed that the new isolate clustered with the type strains of *Rhodospirillum* species but was distinct from other genera of purple non-sulfur bacteria (Fig. 2). The highest sequence similarities of strain JA143^T was found with the type strains of *Rhodospirillum photometricum* (95.72%) and *Rhodospirillum rubrum* (95.58%) (Fig. 2). Apart from 16S rRNA gene sequence dissimilarity, strain JA143^T also showed clear phenotypic differences from other *Rhodospirillum* species (Table 1) that justify the description of the strain JA143^T as the type strain of a novel species, *Rhodospirillum sulfurexigens* sp. nov.

Description of *Rhodospirillum sulfurexigens* sp. nov.

Rhodospirillum sulfurexigens (sul.fu.re'xi.gens. L. n. *sulfur* -uris sulfur, L. v. *exigo* to demand; N.L. part. adj. *sulfurexigens* sulfur-demanding, referring to the requirement for a reduced sulfur source for growth).

Cells are spiral shaped, 1.0–1.3 µm wide; one single turn is 8–9 µm long. Cells also form long chains of more than 30 µm long, multiply by binary fission and are motile by means of bipolar tufts of flagella. Internal photosynthetic membranes are lamellar stacks. The colour of phototrophic culture is pink. The *in vivo* absorption spectrum of intact

cells in sucrose exhibits maxima at 374, 473, 500, 530, 590, 791 and 848 nm, indicating the presence of bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. Mesophilic (30 °C), with a pH optimum at 7.0 for growth. Photoorganoheterotrophy with a few organic compounds is the preferred mode of growth. Pyruvate, caproate, crotonate, malate, fumarate and valerate are good carbon sources. Growth also occurs on acetate, butyrate, succinate, glucose, fructose, glutamate and lactate. Photoautotrophic and chemotrophic growth is not possible. There is no assimilatory sulfate reduction. Requires thiosulfate, sulfide, thioglycollate, cysteine or methionine as sulfur sources. Thiamine is required as a growth factor. The DNA base composition of the type strain is 64.7 mol% G + C (by HPLC).

The type strain, JA143^T (=DSM 19785^T =NBRC 104433^T), was isolated from a water sample from the freshwater reservoir at Mudasarlova, Visakhapatnam, India.

Acknowledgements

We thank Dr J. Euzéby for his expert suggestion for the correct species epithet and Latin etymology. Financial assistance received from the Ministry of Earth Sciences, Government of India, is acknowledged. P. A. K. and T. N. R. S. acknowledge the CSIR, Government of India, for the award of SR fellowships. P. A. acknowledges APNL Biotech Programme for the award of a JR fellowship.

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