Rhodovulum visakhapatnamense sp. nov.

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A Gram-negative, rod-shaped, phototrophic bacterium (JA181^T) was isolated from a tidal water sample. On the basis of 16S rRNA gene sequence similarity, strain JA181^T was shown to belong to the class *Alphaproteobacteria*, most closely related to *Rhodovulum sulfidophilum* (97.8 % similarity to the type strain), *Rhodovulum adriaticum* (93 %), *Rhodovulum robiginosum* (93 %), *Rhodovulum iodosum* (94 %), *Rhodovulum imhoffii* (94 %), *Rhodovulum strictum* (95 %), *Rhodovulum euryhalinum* (94.6 %) and *Rhodovulum marinum* (94.6 %). DNA–DNA hybridization with *Rdv. sulfidophilum* DSM 1374^T (relatedness of 39 % with strain JA181^T) and physiological and biochemical tests allowed genotypic and phenotypic differentiation of strain JA181^T from the eight *Rhodovulum* species with validly published names. Strain JA181^T therefore represents a novel species, for which the name *Rhodovulum visakhapatnamense* sp. nov. is proposed (type strain JA181^T =JCM 13531^T =ATCC BAA-1274^T =DSM 17937^T).

The genus *Rhodovulum* currently comprises eight species with validly published names, which include two described by our group, *Rhodovulum marinum* (Srinivas *et al.*, 2006) and *Rhodovulum imhoffii* (Srinivas *et al.*, 2007). In this communication, we propose a novel species of this genus for a strain that was isolated from tidal seawater.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA181^T is AM180707.

During the characterization of anoxygenic phototrophic bacteria isolated from marine habitats, strain JA181^T was recovered from phototrophic enrichments (with 2 % NaCl) of a tidal water sample collected on 13 March 2005 from Ramakrishna beach at Visakhapatnam on the Bay of Bengal, on the eastern coast of India. The GPS position of the collection site was 17° 42′ N 83° 18′ E. The sample that yielded strain JA181^T had a pH of 6.8, salinity 3 % and a temperature of 30 °C. Purification and polyphasic taxonomic studies were carried out as described earlier (Srinivas

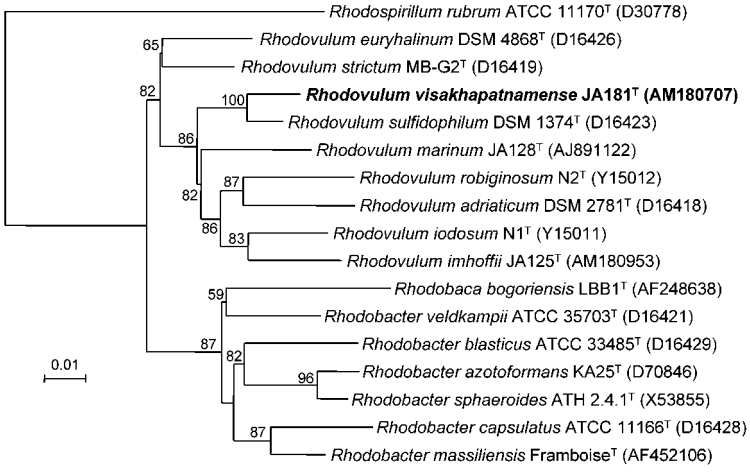

Fig. 1. Dendrogram depicting the phylogenetic relationships of strain JA181^T within the family *Rhodobacteraceae* determined using 16S rRNA gene sequence analysis and generated with the FITCH program. Bar, 1 nucleotide substitution per 100 nucleotides. Bootstrap values below 50 were omitted from the dendrogram.

Table 1. Differentiating characteristics of strain JA181^T and species of the genus *Rhodovulum*

Taxa: 1, strain JA181^T; 2, *Rdv. imhoffii*; 3, *Rdv. marinum*; 4, *Rdv. euryhalinum*; 5, *Rdv. strictum*; 6, *Rdv. iodosum*; 7, *Rdv. robiginosum*; 8, *Rdv. sulfidophilum*; 9, *Rdv. adriaticum* (source data updated from Srinivas *et al.*, 2007). Organic substrate utilization was tested during photoheterotrophic growth. Acetate, pyruvate and succinate were utilized by all of the taxa. Methanol and benzoate were not utilized by any of the taxa. Motile species exhibit polar flagella. +, Substrate utilized/present; (+), some strains utilize the substrate and some do not; −, substrate not utilized/absent; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8	9
Cell diameter (μm)	0.9–1.2	0.5–0.6	0.6–0.8	0.7–1.0	0.6–1.0	0.5–0.8	0.5–0.8	0.6–1.0	0.5–0.8
Cell shape*	O to R	R	O to R, C	O to R	O to R	O to R	O to R	O to R	R, C
Motility	−	−	−	+	+	−	−	+	−
NaCl range (%)	0–10	0.05–7	0.05–8	0.5–10	0.25–3	2.5–5	2.5–5	0–10	1–10
pH range	4.0–9.0	6.0–9.0	5.5–7.5	6.0–8.5	7.5–9.0	NR	NR	5.0–9.0	6.0–8.5
pH optimum	6.0–8.0	7.0–8.0	6.0–6.8	NR	NR	6.5	6.5	NR	NR
Chemo-organotrophy	+	+	+	−	+	+	+	+	−
Temperature range (°C)	20–35	20–30	25–35	NR	30–35	20–25	25–28	NR	25–30
Temperature optimum (°C)	30	28	30	NR	NR	NR	NR	25	NR
Colour of cell suspension†	YB	YB	YB	YB	YB	YB	YB	B	B
Vitamin requirement‡	b, n, t, paba	b	t	b, n, paba, t	b, paba, t	b, n	b, B_{12}, n	b, n, paba, t	b, t
DNA G+C content (mol%)	61.2	58	62	62.1–68.6	67.3–67.7	66	69	66.3–66.6	64.9–66.7
Carbon/electron donors									
Hydrogen	−	−	−	NR	NR	+	+	+	−
Sulfide	−	+	−	+	+	+	+	+	+
Thiosulfate	+	+	−	+	+	+	+	+	+
Sulfur	−	−	−	NR	NR	+	+	+	NR
Ferrous iron	−	−	−	−	NR	+	+	+	−
Formate	−	−	−	+	+	−	−	+	+
Propionate	+	−	−	+	+	+	+	+	+
Butyrate	+	−	−	+	+	+	−	+	−
Valerate	+	−	−	+	+	+	−	+	−
Caproate	+	+	−	NR	+	−	−	+	+
Ethanol	−	−	+	+	−	−	−	−	+
Lactate	+	−	+	+	+	+	+	+	+
Fumarate	+	+	+	+	+	+	−	+	+
Malate	+	+	−	+	+	+	+	+	+
Citrate	−	−	−	−	(+)	−	−	−	−
Aspartate	−	+	−	+	−	−	−	−	NR
Cysteine	−	−	−	NR	NR	+	−	NR	NR
Glutamate	+	+	−	+	−	+	+	+	NR
Glucose	+	−	+	+	+	−	−	+	+
Fructose	+	−	+	+	+	−	−	−	−
Glycerol	+	+	+	+	−	−	+	+	+
Mannitol	−	−	+	+	−	+	+	−	−

*C, Chains; O, ovoid; R, rod-shaped.

†B, Brown; YB, yellowish brown.

‡b, Biotin; B_{12}, vitamin B_{12}; n, nicotinic acid; paba, *p*-aminobenzoic acid; t, thiamine.

et al., 2006). Results of the physiological characterization are given in the species description. The DNA base composition of strain JA181^T was 61.2 mol% G+C (by HPLC). The phylogenetic relationship of strain JA181^T to other purple non-sulfur bacteria was examined by 16S rRNA gene sequencing. The data obtained revealed that the sequence of the new isolate formed a separate branch in the cluster of the genus *Rhodovulum* (Fig. 1) and was distinct from other genera of purple non-sulfur bacteria. The highest 16S rRNA gene sequence similarities of strain JA181^T were found with the type strains of *Rhodovulum sulfidophilum* (97.8 %), *Rhodovulum adriaticum* (93 %), *Rhodovulum robiginosum* (93 %), *Rhodovulum iodosum* (94 %), *Rdv. imhoffii* (94 %), *Rhodovulum strictum* (95 %), *Rhodovulum euryhalinum* (94.6 %) and *Rdv. marinum* (94.6 %). DNA–DNA hybridization with *Rdv. sulfidophilum* DSM 1374^T revealed a relatedness of only 39 % with strain JA181^T. In addition to the 16S rRNA gene sequence dissimilarity and

DNA–DNA hybridization studies, strain JA181^T showed clear phenotypic differences from other *Rhodovulum* species (Table 1) that justify the description of a novel species, *Rhodovulum visakhapatnamense* sp. nov.

Description of *Rhodovulum visakhapatnamense* sp. nov.

Rhodovulum visakhapatnamense (vi.sa.kha.pat.na.men′se. N.L. neut. adj. *visakhapatnamense* pertaining to Visakhapatnam, the place from which the type strain was isolated).

Cells are rods, 0.9 μm wide and 1.2 μm long, non-motile and multiply by binary fission. Gram-negative. Growth occurs under anaerobic conditions in the light (photo-organoheterotrophy) or under aerobic conditions in the dark (chemo-organoheterotrophy). Internal photosynthetic membranes are of the vesicular type. The colour of phototrophic cultures is yellowish-brown, while aerobic cultures are pink. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 377, 467, 520, 590, 802 and 857 nm. Photosynthetic pigments are bacteriochlorophyll *a* and most probably carotenoids of the spheroidene series. The type strain is mesophilic (temperature range 20–35 °C, optimum at 28 °C), with a pH optimum at pH 6.0–8.0 (range pH 4.0–9.0). Sodium chloride is not required but the type strain tolerates up to 10 % NaCl. Photo-organoheterotrophy with various organic compounds is the preferred mode of growth. The substrates which are utilized as carbon sources and electron donors under photo-organoheterotrophic conditions include acetate, propionate, butyrate, valerate, caproate, lactate, pyruvate, 2-oxoglutaric acid, malate, succinate, fumarate, glucose, fructose, sucrose, glycerol, glutamate, ascorbate, peptone and Casamino acids. Substrates that can not be utilized include formate, benzoate, tartrate, citrate, lactose, mannitol, sorbitol, methanol, ethanol, propanol, thioglycolate, methionine, aspartate and cysteine. Sulfate, cysteine, thiosulfate and thioglycolate are utilized as sulfur sources under photoheterotrophic conditions. Ammonium chloride, molecular nitrogen, glutamate and glutamine are utilized as nitrogen sources, while nitrate, nitrite, aspartate and urea do not support growth. Photoautotrophic growth is possible in the presence of thiosulfate as electron donor and $NaHCO_3$ as carbon source. Biotin, niacin, thiamine and *p*-aminobenzoic acid are required as growth factors. The DNA base composition of the type strain is 61.2 mol% G+C (by HPLC).

The type strain, JA181^T (=JCM 13531^T =ATCC BAA-1274^T =DSM 17937^T), was isolated from marine surface and tidal waters exposed to light.

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