Rhodovulum imhoffii sp. nov.

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A yellowish-brown bacterium was isolated from photoheterotrophic enrichment cultures obtained from water samples of an aquaculture pond at Bhimunipatnam, India. Enrichment and isolation in a medium containing 2% NaCl (w/v) yielded strain JA125^T, the cells of which were rod-shaped and non-motile. On the basis of the 16S rRNA gene sequence, strain JA125^T belongs to the class *Alphaproteobacteria* and is closely related to the type strains of *Rhodovulum iodolum* (96%), *Rhodovulum adriaticum* (95%), *Rhodovulum robiginosum* (95%), *Rhodovulum sulfidophilum* (94%) and *Rhodovulum marinum* (94%). The cells of strain JA125^T contained vesicular internal membranes and bacteriochlorophyll *a* and carotenoids of the spheroidene series. Strain JA125^T grew optimally at 28 °C and at pH 7.0–8.0. The best growth occurred photoheterotrophically with a number of organic compounds serving as carbon sources and electron donors. The strain grew photoautotrophically, but chemoautotrophic growth did not occur. Strain JA125^T was able to utilize sulfide, sulfate, thiosulfate and thioglycolate as sulfur sources. Biotin was required as a growth factor. The DNA G + C content was 58 mol%. On the basis of 16S rRNA gene sequence analysis and the morphological and physiological data, strain JA125^T is significantly different from other species of the genus *Rhodovulum* and represents a novel species, for which the name *Rhodovulum imhoffii* sp. nov. is proposed. The type strain is JA125^T (=JCM 13589^T =DSM 18064^T).

The genus *Rhodovulum* was created to separate the marine species of the genus *Rhodobacter* from their freshwater counterparts (Hiraishi & Ueda, 1994). The genus *Rhodovulum* currently comprises seven species: *Rhodovulum sulfidophilum* (originally described as *Rhodopseudomonas sulfidophila*; Hansen & Veldkamp, 1973), *Rhodovulum adriaticum* (originally described as *Rhodopseudomonas adriatica*; Neutzling *et al.*, 1984), *Rhodovulum euryhalinum* (originally described as *Rhodobacter euryhalinus*; Kompantseva, 1985), *Rhodovulum strictum* (Hiraishi & Ueda, 1995), *Rhodovulum iodolum* and *Rhodovulum robiginosum* (Straub *et al.*, 1999) and *Rhodovulum marinum* (Srinivas *et al.*, 2006). In this communication, we propose a novel species of the genus *Rhodovulum*.

Water samples from an aquaculture pond near the sea (Bay of Bengal) at Bhimunipatnam, India, were collected in a sterile polypropylene bottle. The medium of Pfennig (Pfennig & Trüper, 1992), supplemented with NaCl (2%, w/v), pyruvate (0.3%, w/v) as a carbon source and ammonium chloride (0.12%) as a nitrogen source, was used for photoheterotrophic growth in the light (2400 lx) at

30 ± 2 °C. Purification was achieved by using the repeated agar shake dilution method (Pfennig & Trüper, 1992; Imhoff, 1988). Purified cultures were grown in completely filled screw-capped test tubes (10 × 100 mm) for photoheterotrophic growth.

Morphological properties (cell shape, cell division, cell size) were observed by using phase-contrast light microscopy (BH-2; Olympus), and intracytoplasmic structures such as the internal membrane system were viewed from ultrathin sections through a transmission electron microscope (H-7500; Hitachi). *In vivo* absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Absorption spectra were also recorded from pigments extracted with acetone after elution of the cell suspension with acetone through a 10 × 200 mm column packed with aluminium oxide. The utilization of organic compounds as carbon sources/electron donors for phototrophic growth was tested in the presence of yeast extract (0.03%, w/v) without any additional carbon source/electron donor. The concentrations of these compounds were 0.1% (v/v) (for formic acid, propionate, butyrate, caproate, valerate, lactate, glycerol, methanol and ethanol) and 0.3% (w/v) (for the other organic compounds tested), 1 mM benzoate and 0.1% NaHCO₃. To test sulfur-source utilization, MgSO₄ was replaced by MgCl₂ (0.01%) and then various sulfur sources

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA125^T is AM180953.

Phase-contrast/electron photomicrographs and absorption spectra of strain JA125^T are available as supplementary figures in IJSEM Online.

[Na₂S, Na₂S₂O₃, sodium thioglycolate, cysteine, MgSO₄ (0.5 mM) or FeSO₄ (10 mM)] were added to the medium. Nitrogen-source utilization was tested by replacing ammonium chloride with different nitrogen sources (0.12 %, w/v). Vitamin requirements were tested by replacing the yeast extract with single vitamins and combinations of vitamins as growth factors. Chemotrophy was assessed by growing the cultures at 30 °C in the dark in Erlenmeyer flasks placed in an orbital shaker. Diazotrophy was assessed by determining growth in an N₂ atmosphere in medium devoid of a combined nitrogen source, and was confirmed by repeated subculturing (four times). Growth was measured turbidometrically at 660 nm (using a Systronics colorimeter).

The genomic DNA was extracted and purified according to the method of Marmur (1961) and the G+C content (mol%) was determined by HPLC (Mesbah *et al.*, 1989). 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.*, 1998; Imhoff & Pfennig, 2001). Sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994) and the alignment was corrected manually. The CLUSTAL W alignment file was used as input file to the SEQBOOT program in the PHYLIP package and the output file of SEQBOOT was used as the input file for maximum-likelihood analysis with 100 datasets and five times jumbling. One single tree was generated using 100 trees generated during maximum-likelihood analysis using the CONSENSE program. The distance matrix was calculated on the basis of the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1989). The FITCH program in the PHYLIP package was employed to fit a tree to the evolutionary distances using the DIST file as infile and the CONSENSE file as intree.

Samples were collected on 24 February 2005 at around midday from an aquaculture pond near the sea (Bay of Bengal) at Bhimunipatnam, India; the GPS positioning of the sample collection site was 17° 51' 41.03" N 83° 25' 16.81" E. The sample that yielded strain JA125^T had a pH of 6.8, a salinity of 1–2 % NaCl and a temperature of 30–35 °C. The sample was inoculated into a sterile screw-capped tube filled with the medium for photoheterotrophic growth; incubation was for 1 week in the light, with intermittent shaking. Yellowish-brown enrichments were obtained and subcultured into the same medium. This culture was used for purification in a subsequent agar shake dilution series. After 4 days incubation, small, convex, yellowish-brown colonies at the bottom and pinkish colonies at the top (due to the diffusion of oxygen) were observed. The colony and cell morphologies were the same in all dilution series. The culture was maintained by repeated subculturing in the same medium and also in stab cultures. Stab cultures were preserved in a refrigerator at 4 °C. Individual cells of strain JA125^T were rod-shaped (see Supplementary Fig. S1

available in IJSEM Online), 0.5 µm wide and 1 µm long. The cells were non-motile and multiplied by binary fission. Electron microphotographs of ultrathin sections of the cells revealed vesicular-type internal membranes (Supplementary Fig. S2).

Strain JA125^T grows well photoorganoheterotrophically (optimum light intensity is 2400 lx) and chemoorganoheterotrophically (aerobically in the dark). It tolerates atmospheric oxygen levels. Strain JA125^T was able to grow photolithoautotrophically [anaerobically in the light (2400 lx) with Na₂S·9H₂O or Na₂S₂O₃ (0.5 mM) as the electron donor and NaHCO₃ (0.1 %, w/v)]. Chemolithoautotrophy [aerobically in the dark with thiosulfate (0.5 mM) as electron donor and NaHCO₃ (0.1 % w/v)] could not be demonstrated. The substrates that were utilized (Table 1) as carbon/electron donors under photoorganoheterotrophic conditions included acetate, pyruvate, succinate, fumarate, glycerol, glutamate, α-ketoglutaric acid, malate, caproate and Casamino acids. Those which could not be utilized included oleic acid, citrate, glucose, sucrose, lactose, maltose, starch, mannitol, sorbitol, ethanol, formic acid, lactic acid, citrate, sodium potassium tartrate, benzoate and cysteine. Thiosulfate, sulfate, thioglycolate and sodium sulfide were utilized as sulfur sources under photoheterotrophic conditions. Ammonium chloride, molecular nitrogen, glutamate, glutamine and urea were utilized as nitrogen sources, but nitrate and nitrite did not support growth. Salt is obligatory for the growth of strain JA125^T: growth occurs at 0.05–7 % NaCl (w/v), with an optimum at 0.5–4 % (w/v). This situation is similar to that observed for the growth of other marine isolates from India, *Marichromatium indicum* JA100^T (Arunasri *et al.*, 2005) and *Rhodovulum marinum* JA128^T (Srinivas *et al.*, 2006), which also grow with NaCl concentrations in the range 0.05–8 % NaCl, reflecting adaptation to the natural habitat (which can exhibit large variations in salt concentration over various seasons). The pH range for strain JA125^T is 6.0–9.0, with an optimum at pH 7.0–8.0. The temperature range is 20–35 °C and the optimum is at 28 °C. Biotin is required as a growth factor. The colour of photosynthetically grown cultures is yellowish brown; when exposed to air, they turn brown, and when they are grown chemoheterotrophically (aerobically in the dark), they appear pink in colour. The whole-cell absorption spectrum (Supplementary Fig. S3a) of strain JA125^T showed absorption maxima at 377, 467, 520, 590, 802 and 857 nm, confirming the presence of bacteriochlorophyll *a*. The absorption spectrum for pigments extracted with acetone (Supplementary Fig. S3b) gave maxima at 454 and 484 nm, indicating the presence of the carotenoids spheroidene and spheroidenone.

The DNA G+C content of strain JA125^T was 58 mol% (by HPLC). The phylogenetic relationship between strain JA125^T and purple non-sulfur bacteria was examined by means of 16S rRNA gene sequencing: the sequence of the novel isolate formed a separate branch in the cluster of the genus *Rhodovulum* (Fig. 1) and was distinct from those of

Table 1. Differentiating characteristics of strain JA125^T and species of the genus *Rhodovulum*

Taxa: 1, strain JA125^T; 2, *Rhodovulum marinum* (data from Srinivas *et al.*, 2006); 3, *Rhodovulum euryhalinum* (Kompantseva, 1985; Imhoff & Trüper, 1992); 4, *Rhodovulum strictum* (Hiraishi & Ueda, 1995); 5, *Rhodovulum iodosum* (Straub *et al.*, 1999); 6, *Rhodovulum robiginosum* (Straub *et al.*, 1999); 7, *Rhodovulum sulfidophilum* (Hansen & Veldkamp, 1973; Imhoff & Trüper, 1992; Heising *et al.*, 1996); 8, *Rhodovulum adriaticum* (Neutzing *et al.*, 1984). 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. Symbols: +, 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
Cell diameter (µm)	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*	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 (%)	0.05–7	0.05–8	0.5–10	0.25–3	2.5–5	2.5–5	0–10	1–10
pH for growth								
Range	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
Optimum	7.0–8.0	6.0–6.8	NR	NR	6.5	6.5	NR	NR
Chemoorganotrophy	+	+	–	+	+	+	+	–
Temperature for growth (°C)								
Range	20–30	25–35	NR	30–35	20–25	25–28	NR	25–30
Optimum	28	30	NR	NR	NR	NR	25	NR
Colour of cell suspension†	YB	YB	YB	YB	YB	YB	B	B
Vitamin requirement(s)‡	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%)	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; B₁₂, vitamin B₁₂; n, nicotinic acid; paba, *p*-aminobenzoic acid; t, thiamine.

other genera of purple non-sulfur bacteria. The highest sequence similarities of strain JA125^T were found with respect to the type strains of *Rhodovulum iodosum* (96%), *Rhodovulum adriaticum* (95%), *Rhodovulum robiginosum*

(95%), *Rhodovulum sulfidophilum* (94%) and *Rhodovulum marinum* (94%). Apart from the 16S rRNA gene sequence dissimilarity, strain JA125^T showed clear phenotypic differences with respect to *Rhodovulum* species (Table 1)

Fig. 1. Dendrogram depicting the phylogenetic relationships of strain JA125^T within the family *Rhodobacteraceae*, determined using 16S rRNA gene sequence analysis using the maximum-likelihood method. Bootstrap values below 50 are not shown. Bar, 2 nucleotide substitutions per 100 nucleotides.

that justify the description of this strain within a novel species, for which the name *Rhodovulum imhoffii* sp. nov. is proposed.

Description of *Rhodovulum imhoffii* sp. nov.

Rhodovulum imhoffii (im.hof'fi.i. N.L. gen. n. *imhoffii* of Imhoff, named after Johannes F. Imhoff, a German microbiologist who has made a significant contribution to our knowledge of anoxygenic phototrophic bacteria).

Cells are rod-shaped, 0.5 µm wide and 1 µm long, non-motile and multiply by binary fission. Gram-negative. Growth occurs under anaerobic conditions in the light (photoorganoheterotrophy) or under aerobic conditions in the dark (chemoorganoheterotrophy). The internal photosynthetic membranes are of the vesicular type. The colour of phototrophic cultures is yellow to brown, whereas 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. The photosynthetic pigments are bacteriochlorophyll *a* and, probably, carotenoids of the spheroidene series. The type strain is mesophilic (temperature range, 20–35 °C; optimum, 28 °C), with a pH optimum at 7.0–8.0 (range, pH 6.0–9.0) and a requirement for NaCl concentrations of 0.5–4.0 % for optimal growth. Photoorganotrophy with various organic compounds is the preferred mode of growth. Acetate, pyruvate and intermediates of the citric acid cycle (except citrate) are good carbon sources. Growth also occurs on glycerol, glutamate, caproate and Casamino acids. Photoautotrophic growth is possible in the presence of sulfide or thiosulfate as the electron donor and NaHCO₃ as the carbon source. Biotin is required as a growth factor.

The DNA G+C content is 58 mol% (by HPLC). The natural habitat of the known strain is a marine aquaculture pond exposed to light.

The type strain, JA125^T (=JCM 13589^T=DSM 18064^T), was isolated from water samples from an aquaculture pond near the sea (Bay of Bengal) at Bhimunipatnam, India.

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