Rhodobacter maris sp. nov., a phototrophic alphaproteobacterium isolated from a marine habitat of India

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During investigations into the diversity of anoxygenic phototrophic bacteria in marine habitats, an ovoid to rod-shaped purple non-sulfur bacterium, designated strain JA276^T, was isolated from enrichments under photoheterotrophic conditions from a marine sediment sampled from the seashore of Cochin, India. Strain JA276^T is a Gram-negative, motile, chain-forming bacterium that shows optimum growth under photoheterotrophic conditions and is also able to grow chemoorganotrophically. Thiamine is required as a growth factor. Strain JA276^T contains vesicular intracytoplasmic membranes, bacteriochlorophyll *a* and the carotenoids spheroidene and spheroidenone. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA276^T belongs to the genus *Rhodobacter* and is closely related to the type strain of *Rhodobacter capsulatus* (96.2 % sequence similarity). On the basis of the results of 16S rRNA gene sequence analysis and morphological and physiological data, strain JA276^T is significantly different from other species of the genus *Rhodobacter* and represents a novel species of the genus, for which the name *Rhodobacter maris* sp. nov. is proposed. The type strain is JA276^T (=JCM 14794^T =ATCC BAA-1549^T =CCUG 55129^T).

The members of the genus *Rhodobacter* are widely distributed and have been isolated mostly from freshwater habitats, with the exception of the recently reported *Rhodobacter vinaykumarii* (Srinivas *et al.*, 2007a), which represents a true marine species. During efforts to isolate purple phototrophic bacteria from marine habitats in India, we isolated strain JA276^T from the waters of the Arabian Sea. We propose that this strain represents a novel species of the genus *Rhodobacter*.

Strain JA276^T was isolated from enrichments of a marine sediment collected from the seashore of Cochin, Kerala State, India (GPS position 9° 58′ N 76° 14′ E). The sample that yielded strain JA276^T had a pH of 7.0, a temperature of 30 °C and a salinity of 2.0 % (w/v). Purification and polyphasic taxonomic analyses were carried out as described previously (Srinivas *et al.*, 2007b). 16S rRNA gene sequences were aligned using the CLUSTAL_X program (Thompson *et al.*, 1997) and the alignment was corrected manually. The dendrogram was constructed using the PhyML program (Guindon & Gascuel, 2003) with 100 replicates in a non-parametric bootstrap analysis, the

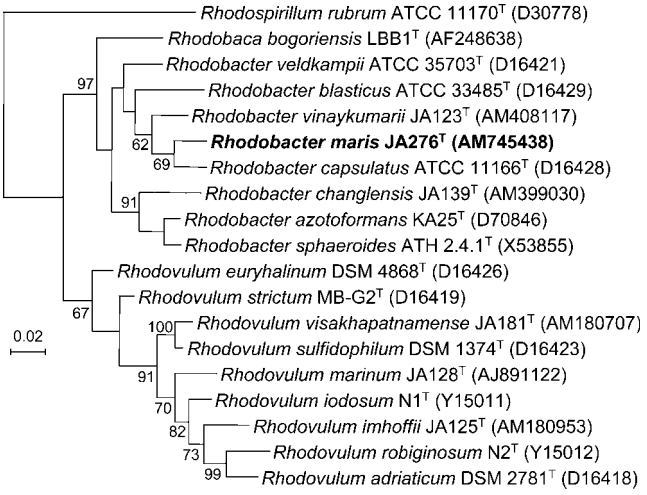

Fig. 1. Evolutionary distance dendrogram, based on 16S rRNA gene sequence analysis, depicting the phylogenetic relationships of strain JA276^T within the family *Rhodobacteraceae*. Bar, 2 substitutions per 100 nucleotide positions.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA276^T is AM745438.

A phase-contrast micrograph of strain JA276^T and spectra for pigments extracted from the strain are available as supplementary material with the online version of this paper.

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

Taxa: 1, strain JA276^T; 2, *Rba. azotoformans*; 3, *Rba. sphaeroides*; 4, *Rba. capsulatus*; 5, *Rba. blasticus*; 6, *Rba. veldkampii* (data in columns 2–6 from Imhoff, 2005); 7, *Rba. changlensis* (Anil Kumar *et al.*, 2007); 8, *Rba. vinaykumarii* (Srinivas *et al.*, 2007b). For all taxa, organic substrate utilization was tested during photoheterotrophic growth. Pyruvate and succinate were utilized by all taxa. Benzoate and arginine were not utilized by any of the taxa. Motile species exhibit polar flagella. +, Substrate utilized or present; −, substrate not utilized or absent; ±, variable across different strains; (+), weak growth; NR, not reported.

Characteristic	1	2	3	4	5	6	7	8
Cell size (μm)	0.6–1.0 × 1.0–1.5	0.6–1.0 × 0.9–1.5	2.0–2.5 × 2.5–3.5	0.5–1.2 × 2.0–2.5	0.6–0.8 × 1.0–2.5	0.6–0.8 × 1.0–1.3	0.8–1.0 × 2.0–4.0	0.8–1.2 × 1.5–3.0
Cell shape*	O to R, C	O to R	S to O	O to R, C	O to R	O to R	O to R, C	R
Motility	+	+	+	+	−	−	−	−
Colour of cell suspension†	YB	YB	GB	YB	OB	YB	YB	YB
Internal membrane system‡	V	V	V	V	L	V	V	V
Slime production	−	+	±	±	−	−	+	+
NaCl required	−§	−§	−§	−§	−	−	−	+ (1–4 %)
pH range (optimum)	5.0–8.0 (6.5–7.0)	NR (7.0–7.5)	6.0–8.5 (7.0)	6.5–7.5 (7.0)	NR (6.5–7.5)	NR (7.5)	6.5–8.0 (6.5–7.5)	6.0–8.0 (6.0–7.5)
Temperature optimum (°C)	25–30	30–35	30–34	30–35	30–35	30–35	20–30	20–30
Sulfate assimilation	+	+	+	+	+	−	+	+
Denitrification	−	+	±	−	−	−	−	−
Vitamin(s) required‖	t	b, n, t	b, t, n	t, (b, n)	b, n, t, B_{12}	b, *p*-ABA, t	b, n, t	b
DNA G+C content (mol%)	62.85	69.5–70.2	70.8–73.2	68.1–69.6	65.3	64.4–67.5	69.4	68.8
Carbon source/electron donor								
Hydrogen	−	NR	+	+	+	−	−	−
Sulfide	−	+	+	+	−	+	−	−
Thiosulfate	−	−	−	−	−	+	−	−
Sulfur	−	−	−	−	−	+	−	−
Formate	−	+	−	+	−	−	−	−
Acetate	(+)	+	+	+	+	+	−	+
Propionate	(+)	+	+	+	+	+	−	−
Butyrate	(+)	+	+	+	+	+	−	(+)
Valerate	(+)	NR	+	+	NR	+	−	−
Caproate	(+)	NR	+	+	NR	+	−	−
Caprylate	−	−	+	+	NR	+	−	−
Tartrate	−	−	+	−	−	−	−	−
Lactate	(+)	+	+	+	+	+	−	−
Malate	+	+	+	+	+	+	−	−
Fumarate	(+)	+	+	+	+	+	−	−
Citrate	−	NR	+	±	+	−	−	−
Aspartate	−	NR	NR	±	NR	+	−	−
Glutamate	−	+	+	+	+	+	+	+
Gluconate	−	NR	+	−	NR	NR	−	−
D-Glucose	−	+	+	+	+	+	+	+
Fructose	−	+	+	+	+	−	−	−
Mannitol	−	+	+	±	+	−	+	+
Sorbitol	−	+	+	±	+	−	+	−
Glycerol	(+)	+	+	−	+	−	−	−
Methanol	−	−	±	−	−	−	−	−
Ethanol	−	NR	+	−	−	−	−	−
Propanol	−	NR	NR	+	NR	−	−	+

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

†GB, Greenish brown; OB, orange–brown; YB, yellowish brown.

‡V, Vesicular; L, lamellar.

§Optimal growth occurs in the absence of NaCl, but growth occurs at 3 % NaCl.

‖b, Biotin; B_{12}, vitamin B_{12}; n, niacin; *p*-ABA, *p*-aminobenzoic acid; t, thiamine; (b, n), a few strains require biotin and/or niacin.

general time-reversible model of nucleotide substitution and four substitution-rate categories.

Individual cells of strain JA276^T were ovoid to rod-shaped, 0.6–1.0 μm wide and 1.0–1.5 μm long, motile, multiplied by binary fission and could form chains of four to six cells (see Supplementary Fig. S1, available in IJSEM Online). Electron microphotographs of ultrathin sections of the cells revealed the presence of vesicular internal membrane structures. Strain JA276^T was able to grow photoorganoheterotrophically [anaerobic conditions, in the light (2500 lx) and in the presence of pyruvate (0.3 %, w/v)] and chemoorganoheterotrophically [aerobic conditions, in the dark and in the presence of pyruvate (0.3 %, w/v)]. Photolithoautotrophic growth [anaerobic, light (2400 lx), Na_2S (0.5 mM), $Na_2S_2O_3$ (2 mM) and $NaHCO_3$ (0.1 %, w/v)], chemolithoautotrophic growth [aerobic, dark, $Na_2S_2O_3$ (0.5 mM) and $NaHCO_3$ (0.1 %, w/v)] and fermentative growth [anaerobic, dark, pyruvate (0.3 %, w/v), glucose (0.3 %, w/v)] could not be demonstrated. The colour of phototrophic cultures was yellowish brown, turning to reddish brown when exposed to air. The *in vivo* absorption spectrum of intact cells in sucrose exhibited maxima at 377, 476, 509, 590, 803 and 860 nm, confirming the presence of bacteriochlorophyll *a* (Supplementary Fig. S2a). The absorption spectrum for pigments extracted with acetone (Supplementary Fig. S2b) gave maxima at 455 and 488 nm, indicating the presence of the carotenoids spheroidene and spheroidenone. The DNA G+C content of strain JA276^T was 62.85 mol% (by HPLC).

The phylogenetic relationships between strain JA276^T and other purple non-sulfur bacteria were examined by means of 16S rRNA gene sequence analysis. The data obtained revealed that the novel isolate branched separately, but clustered with type strains of species of the genus *Rhodobacter*, and was distinct from other genera of purple non-sulfur bacteria (Fig. 1). The highest sequence similarities for strain JA276^T were found with respect to the type strains of *Rhodobacter capsulatus* (96.2 %), *Rhodobacter vinaykumarii* (94.7 %), *Rhodobacter veldkampii* (92.8 %), *Rhodobacter blasticus* (91.8 %), *Rhodobacter sphaeroides* (90.4 %), *Rhodobacter azotoformans* (90.4 %) and *Rhodobacter changlensis* (89.2 %). Apart from showing 16S rRNA gene sequence dissimilarity, strain JA276^T showed clear phenotypic differences with respect to species of the genus *Rhodobacter* (Table 1), justifying the description of a novel species of the genus, for which the name *Rhodobacter maris* sp. nov. is proposed.

Description of *Rhodobacter maris* sp. nov.

Rhodobacter maris (ma′ris. L. gen. n. *maris* of the sea, pertaining to the habitat from which the type strain was isolated).

Cells are ovoid to rod-shaped, 0.6–1.0 μm wide and 1.0–1.5 μm long, non-motile, divide by binary fission and can from chains of four to six cells. 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. The colour of phototrophic cultures is yellowish brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 377, 476, 509, 590, 803, 860 nm, confirming the presence of bacteriochlorophyll *a*. Carotenoids include spheroidene and spheroidenone. Substrates utilized by the type strain as carbon sources and electron donors under photoorganoheterotrophic conditions include acetate, fumarate, pyruvate, malate, glycerol, valerate, lactate, caproate, propionate and butyrate. The type strain cannot utilize formate, caprylate, gluconate, succinate, thiosulfate, aspartate, ascorbate, benzoate, glutamate, sulfur, propanol, glucose, fructose, mannitol, peptone, sucrose, Casamino acids, sorbitol, ethanol, tartrate, sulfite, citrate, oxaloacetate, 2-ketoglutarate, lactose, maltose, starch, sulfide, bicarbonate, pelargonate, arginine, yeast extract or oleic acid. Ammonium chloride, glutamate, glutamine and molecular nitrogen are utilized as nitrogen sources, but urea, aspartate, nitrate and nitrite do not support growth. Magnesium sulfate, thiosulfate, sulfite, thioglycolate and sulfide are utilized as sulfur sources, but elemental sulfur, methionine and cysteine do not support growth. NaCl is not required for growth of the type strain; NaCl concentrations up to 3 % (w/v) are tolerated. Strain JA276^T grows at pH 6.0–8.0 (optimally at pH 6.5–7.0) and at 25–35 °C (optimally at 30 °C). Thiamine is required as a growth factor. Autotrophic and fermentative growth is not possible. The G+C content of the type strain is 62.85 mol% (by HPLC).

The type strain, JA276^T (=JCM 14794^T =ATCC BAA-1549^T =CCUG 55129^T), was isolated from samples of a sediment collected from the seashore of Cochin, Kerala State, India.

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