Rhodobium gokarnense sp. nov., a novel phototrophic alphaproteobacterium from a saltern

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A pink-pigmented, phototrophic, purple nonsulfur bacterium, strain JA173^T, was isolated in pure culture from a saltern in Gokarna, India, in a medium containing 2 % (w/v) NaCl. Strain JA173^T was a non-motile Gram-negative rod that multiplied by budding. Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA173^T clusters with the class *Alphaproteobacteria*; highest sequence similarity (98 %) was to the type strain of *Rhodobium orientis* and 94 % similarity was observed to the 16S rRNA gene sequence of the type strain of *Rhodobium marinum*. However, DNA–DNA hybridization with *R. orientis* DSM 11290^T revealed a relatedness value of only 35.1 % with strain JA173^T. Strain JA173^T contained lamellar internal membranes, bacteriochlorophyll *a* and carotenoids of the spirilloxanthin series. Strain JA173^T had an obligate requirement for NaCl (optimum growth at 2–6 %, w/v) and grew photoheterotrophically with a number of organic compounds as carbon source or electron donor. Photoautotrophic, chemoautotrophic and fermentative growth could not be demonstrated. Yeast extract was required for growth. Based on 16S rRNA gene sequence analysis, DNA–DNA hybridization data and morphological and physiological characteristics, strain JA173^T is sufficiently different from other species of the genus *Rhodobium* to be recognized as a representative of a novel species, *Rhodobium gokarnense* sp. nov. The type strain is JA173^T (=ATCC BAA-1215^T=DSM 17935^T=JCM 13532^T).

The genus *Rhodobium* is composed of marine species that are capable of photosynthesis, multiply by budding and possess lamellar internal membrane structures (Hiraishi *et al.*, 1995). At present, the genus *Rhodobium* comprises two species, both of which were isolated from marine sediments: *Rhodobium marinum* (originally described as *Rhodopseudomonas marina*, Imhoff, 1983); and *Rhodobium orientis* (Hiraishi *et al.*, 1995). Strain JA173^T, which was isolated from a saltern, is described in this report and, based on its phylogenetic and phenotypic properties, which are distinct from those of the other *Rhodobium* species, it is proposed that this strain represents a novel species in this genus.

Abbreviation: FTIR, Fourier-transform infrared.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA173^T is AM180706.

A phase contrast micrograph, an electron micrograph, absorption spectra and FTIR spectra of strain JA173^T are available as supplementary material in IJSEM Online.

Soil and water, including salt crystals, were collected on 27 December 2003 at around midday from a saltern located in Gokarna, India. GPS positioning of the sample collection site was 14° 32′ N 74° 19′ E. The sample yielding strain JA173^T had a pH of 6.8 and a temperature of 30 °C. Strain JA173^T was isolated from photoheterotrophic enrichments of this soil sample. Purification and polyphasic taxonomic studies were carried out as described previously (Srinivas *et al.*, 2006). Fourier-transform infrared (FTIR) spectroscopic analysis data (Ramana *et al.*, 2006) of strain JA173^T were compared with those of cells of *Rhodobium orientis* DSM 11290^T. DNA–DNA hybridization was carried out at the DSMZ (Braunschweig, Germany) using a spectrophotometric method (De Ley *et al.*, 1970; Huß *et al.*, 1983) after chromatographic (hydroxyapatite) purification of DNA (Cashion *et al.*, 1977).

Individual cells of strain JA173^T were rod-shaped, 0.5–0.6 μm wide and 1.0–2.0 μm long, non-motile and multiplied by budding (see Supplementary Fig. S1 in IJSEM Online). An electron micrograph of ultrathin sections of the

cells revealed lamellar internal membrane structures (see Supplementary Fig. S2 in IJSEM Online). Strain JA173^T was able to grow photoorganoheterotrophically in the presence of incandescent light (optimum light intensity, 1000–4000 lux) (anaerobic; light, 2400 lux) and chemoorganoheterotrophically and aerobically in the dark with pyruvate (0.3 %, w/v). Photolithoautotrophic growth (anaerobic; light, 2400 lux; 20 % H_2, v/v; 0.5 mM Na_2S; 0.5 mM $Na_2S_2O_3$; and 0.1 % $NaHCO_3$, w/v), chemolithoautotrophy (aerobic; dark; 0.5 mM thiosulfate; and 0.1 % $NaHCO_3$, w/v) and fermentative growth (anaerobic; dark; 0.3 % glucose, w/v; 0.3 % fructose, w/v) could not be demonstrated. Substrates that were utilized as carbon sources/electron donors under photoorganoheterotrophic conditions included acetate, butyrate, pyruvate, citrate, succinate, fumarate, malate, glucose, mannitol, sorbitol and Casamino acids (Table 1). Formate, propionate, valerate, caproate, caprylate, lactate, tartrate, benzoate, fructose, glycerol, methanol, ethanol, glutamate, peptone and yeast extract could not be utilized. Thiosulfate, sodium sulfide and H_2 (with 0.1 % $NaHCO_3$) were not utilized as electron donors under photolithoautotrophic conditions. Ammonium chloride, molecular nitrogen and glutamine were utilized as nitrogen sources, whereas urea, glutamate, nitrate and nitrite did not

Table 1. Characteristics that differentiate species of the genus *Rhodobium*

Data for *Rhodobium orientis* are from Hiraishi *et al.* (1995). Data for *Rhodobium marinum* are from Imhoff (1983) and Imhoff & Hiraishi (2005); organic substrate utilization for this species was tested during photoheterotrophic growth. Acetate, butyrate, fumarate, D-glucose, malate, pyruvate, sorbitol and succinate are utilized by all taxa, whereas benzoate, methanol and tartrate are not utilized. All taxa exhibited sessile budding and are pink–red in culture. +, Substrate utilized or present; −, substrate not utilized or absent; +/−, variable reaction in different strains; (+), weak growth or microaerobic growth only; [+], weak growth; ND, not determined; NI, no information.

Characteristic	*Rhodobium orientis*	*Rhodobium marinum*	Strain JA173^T
Cell size (μm)	1.5–3.2 × 0.7–0.9	1.0–2.5 × 0.7–0.9	1.0–2.0 × 0.5–0.6
Rosette formation	+/−	−	+
Motility	+	+	−
Low absorption maximum at ca. 800 nm	−	+	−
Whole cell absorption maxima (nm)	377, 468, 500, 530, 591, 802, 870	375, 483, 516, 533, 590, 803, 883	370, 402, 488, 530, 590, 803, 872
Whole cell FTIR transmission maxima (cm^{-1})	3445, 2969, 2926, 2857, 1655, 1588, 1460, 1707, 621	NI	3420, 2969, 2926, 2857, 1728, 1651, 1541, 1404, 1231, 1059, 575
NaCl range (optimum) (% w/v)	2–8 (4–5)	1–5 (1–3)	0.5–10.0 (2.0–6.0)
pH range (optimum)	6.0–8.5 (7.0–7.5)	NI (6.9–7.1)	5.0–9.0 (6.5–8.0)
Aerobic dark growth	+	(+)	(+)
Denitrification	+	−	−
Fermentative growth on fructose	−	+	−
Photolithoautotrophic growth	+	+	−
Vitamin(s) required	Biotin, *p*-aminobenzoic acid	ND	Yeast extract
Utilization of carbon source/electron donor:			
Caproate, D-fructose, valerate	+	+	−
Caprylate, ethanol, glycerol, propanol, propionate	−	+/−	−
Citrate	−	−	[+]
Formate	−	+	−
Glutamate	+/−	NI	−
Lactate	+	+/−	−
Mannitol	+/−	+	+
Electron donor:			
Sulfide	−	+/−	−
Thiosulfate	+	−	−
Major quinone(s)*	Q-10, MK-10	Q-10, MK-10	ND
DNA G+C content (mol%)	65.2–65.7	61.5–64.1	72.4
Habitat	Seawater	Seawater	Saltern

*Q-10, Ubiquinone 10; MK-10, menaquinone 10.

support growth. Strain JA173^T required yeast extract as a growth factor. Salt (NaCl) was obligatory for the growth of strain JA173^T; this strain grew in 0.5–10.0 % (w/v) NaCl, with optimum growth at 2.0–6.0 % (w/v) NaCl. Strain JA173^T grew at pH 5.0–9.0, with optimum growth at pH 6.5–8.0. The temperature optimum for growth was $30 \pm 2\,^{\circ}C$.

The colour of the phototrophically grown cell suspension was pink to pink–red. The whole cell absorption spectrum of strain JA173^T showed absorption maxima at 370, 402, 488, 530, 590, 803 and 872 nm, thus confirming the presence of bacteriochlorophyll *a* and most probably carotenoids of the spirilloxanthin series (see Supplementary Fig. S3a, b in IJSEM Online). The cellular components (metabolomes) of strain JA173^T were compared with those of *Rhodobium orientis* DSM 11290^T under identical growth conditions using whole cell FTIR spectra (see Supplementary Fig. S4 in IJSEM Online), which clearly indicated differences between the two strains. Variations were recorded in the proteins (1650–1580 cm^{-1}), carbohydrates (1200–900 cm^{-1}) and aromatic compounds (870–675 cm^{-1}). No major differences were observed in lipids and fatty acids (3100–2800 cm^{-1}). An ester peak (1728 cm^{-1}) was observed in strain JA173^T alone, which is similar to that observed in *Rubrivivax gelatinosus* DSM 17011^T (Ramana *et al.*, 2006). The DNA G+C content of strain JA173^T was 72.4 mol% (determined by HPLC). The phylogenetic relationship of strain JA173^T to other purple nonsulfur bacteria was examined by 16S rRNA gene sequencing (Fig. 1). The data obtained revealed that the novel isolate branched separately, but was affiliated with the type strains of *Rhodobium* species. The highest sequence similarities to strain JA173^T were found with the type strains of *Rhodobium orientis* (98 %) and *Rhodobium marinum* (94 %). However, DNA–DNA hybridization with *Rhodobium orientis* DSM 11290^T revealed a relatedness value of only 35.1 % with strain JA173^T. Apart from 16S rRNA gene sequence dissimilarity and DNA–DNA hybridization studies, strain JA173^T clearly differed from other *Rhodobium* species in its phenotypic properties (Table 1); these results justify the description of this strain as a representative of a novel species, *Rhodobium gokarnense* sp. nov.

Description of *Rhodobium gokarnense* sp. nov.

Rhodobium gokarnense (go′kar.nense. L. neut. adj. *gokarnense* pertaining to Gokarna, the place from which the type strain was isolated).

Cells are rod-shaped, 0.5–0.6 × 1.0–2.0 μm, non-motile and divide by budding. Growth occurs under anaerobic conditions in the light (photoorganoheterotrophy) or under aerobic conditions in the dark (chemoorganoheterotrophy). Internal photosynthetic membranes have a lamellar structure. Phototrophic cultures are pink to pinkish-red. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 370, 402, 488, 530, 590, 803 and 872 nm,

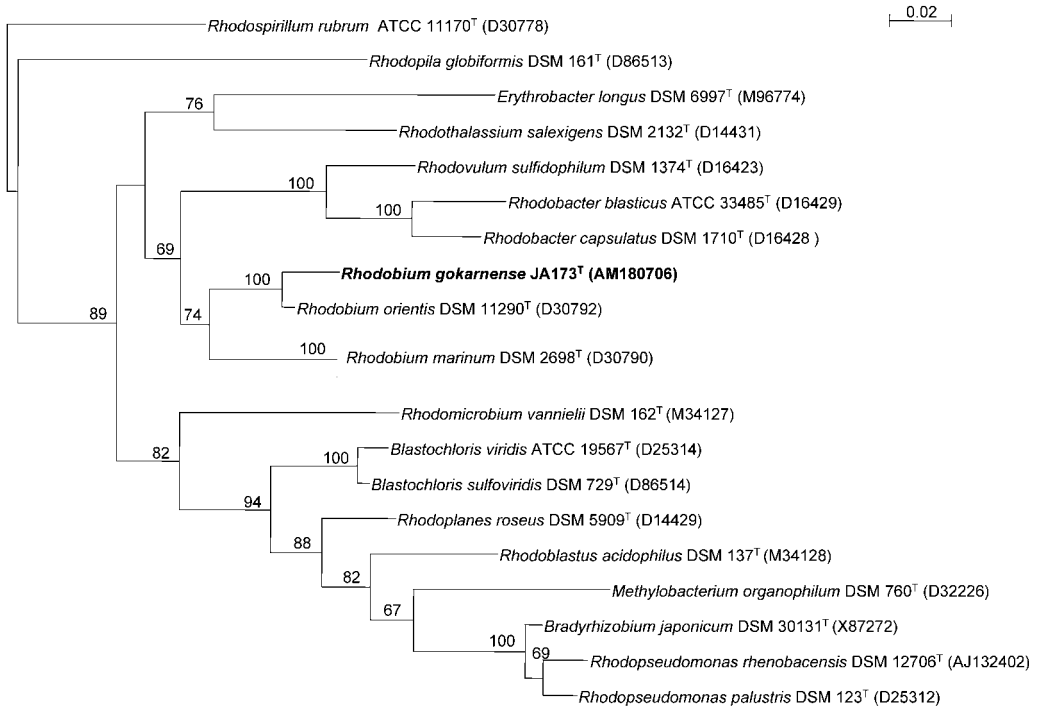

Fig. 1. Dendrogram depicting the phylogenetic relationships of strain JA173^T within the family *Rhodobiaceae* determined using 16S rRNA gene sequence analysis. Bar, 2 substitutions per 100 nucleotides.

confirming the presence of bacteriochlorophyll *a* and probably the spirilloxanthin series. Mesophilic (30 °C), with optimum growth at pH 6.5–8.0. Requires 2.0–6.0 % (w/v) NaCl for optimal growth. Photoorganoheterotrophy with various organic compounds is the preferred mode of growth. Good carbon sources are pyruvate and fumarate. Growth also occurs on acetate, butyrate, citrate, succinate, malate, glucose, mannitol, sorbitol and Casamino acids. Photoautotrophic and chemoautotrophic growth is not possible in the presence of sulfide/thiosulfate/hydrogen as electron donor and $NaHCO_3$ as carbon source. Yeast extract is required as a growth factor. The DNA G + C content of the type strain is 72.4 mol% (by HPLC).

The type strain, JA173^T (= ATCC BAA-1215^T = DSM 17935^T = JCM 13532^T), was isolated from a saltern in Gokarna, India.

Acknowledgements

Financial assistance received from Department of Biotechnology and Department of Ocean Development, Government of India is acknowledged. P. A. K. and T. N. R. S. acknowledge the CSIR, Government of India, for the award of JR & SR fellowships. The skilful assistance of F. Lappe (IFM-GEOMAR, Kiel) in molecular analysis is kindly acknowledged. Financial assistance received under DST-DAAD exchange program (grant 422-PPP-34105) is acknowledged.

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