*International Journal of Systematic and Evolutionary Microbiology* (2007), **57**, 1984–1987 **DOI** 10.1099/ijs.0.65077-0

# *Rhodobacter vinaykumarii* sp. nov., a marine phototrophic alphaproteobacterium from tidal waters, and emended description of the genus *Rhodobacter*

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A rod-shaped, phototrophic, purple non-sulfur bacterium was isolated in pure culture from seawater collected from the seashore of Visakhapatnam, on the east coast of India, in a medium that contained 2 % NaCl (w/v). Strain JA123$^T$ was Gram-negative and non-motile and had a requirement for NaCl. Photo-organoheterotrophic and chemo-organoheterotrophic growth occurred with organic compounds as carbon sources and electron donors. Photolithoautotrophic, chemolithoautotrophic and fermentative growth could not be demonstrated. Strain JA123$^T$ contained vesicular intracellular photosynthetic membrane structures. Bacteriochlorophyll *a* and probably carotenoids of the spheroidene series were present as photosynthetic pigments. Biotin was required for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA123$^T$ clustered with species of the genus *Rhodobacter.* Based on 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA123$^T$ is sufficiently different from other *Rhodobacter* species to propose a novel species, *Rhodobacter vinaykumarii* sp. nov., to accommodate this strain; the type strain is JA123$^T$ (=DSM 18714$^T$ =JCM 14544$^T$ =CCUG 54311$^T$).

The genus *Rhodobacter* was established to separate species of purple non-sulfur bacteria with certain characteristics, i.e. those that have vesicular internal membranes and oval to rod-shaped cells, divide by binary fission, contain carotenoids of the spheroidene series, and have a number of differing molecular taxonomic characteristics, from other species of the genus *Rhodopseudomonas* (Imhoff *et al.*, 1984). At present, the genus *Rhodobacter* comprises five species [*Rhodobacter massiliensis* (Greub & Raoult, 2003) has been reclassified recently as *Haematobacter massiliensis* (Helsel *et al.*, 2007)]; the currently recognized species are *Rhodobacter capsulatus* (Imhoff *et al.*, 1984), *Rba. sphaeroides* (Imhoff *et al.*, 1984), *Rba. blasticus* (Kawasaki *et al.*, 1993), *Rba. veldkampii* (Hansen & Imhoff, 1985) and *Rba. azotoformans* (Hiraishi *et al.*, 1996). The marine representatives of the genus, *Rhodobacter adriaticus* (Imhoff *et al.*, 1984), *Rba. euryhalinus* (Kompantseva, 1985) and *Rba. sulfidophilus* (Imhoff *et al.*, 1984), have been transferred to the genus *Rhodovulum* based on their habitat, salt requirement and separate clustering from their freshwater counterparts and formation of a distinct clade based on 16S rRNA gene analysis (Hiraishi & Ueda, 1994). Six more species in the genus *Rhodovulum* have also been proposed: *Rhodovulum iodosum* (Straub *et al.*, 1999), *Rdv. imhoffii* (Srinivas *et al.*, 2007a), *Rdv. marinum* (Srinivas *et al.*, 2006), *Rdv. robiginosum* (Straub *et al.*, 1999), *Rdv. strictum* (Hiraishi & Ueda, 1995) and *Rdv. visakhapatnamense* (Srinivas *et al.*, 2007b). Other marine phototrophic members of the *Alphaproteobacteria* (growing with 1–12 % NaCl) belong to the genera *Rhodospira*, *Roseospirillum*, *Roseospira*, *Rhodobium*, *Rhodobaca*, *Rhodothalassium* and *Rhodovibrio* (Imhoff, 2001). In this study, a strain from tidal seawater

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JA123$^T$ and JA249 are AM408117 and AM600642, respectively.

A phase-contrast micrograph, an electron micrograph and whole-cell and pigment absorption spectra of strain JA123$^T$ are available as supplementary material with the online version of this paper.

collected from the seashore of Visakhapatnam, India, was isolated. This strain, JA123$^T$, required NaCl for growth and tolerated up to 10 % NaCl and clustered in the genus *Rhodobacter* based on 16S rRNA gene sequence analysis. Based on phenotypic and phylogenetic analysis, it is proposed that strain JA123$^T$ represents a novel species.

Strain JA123$^T$ was isolated from enrichments of tidal seawater collected from Ramakrishna beach, Bay of Bengal, Visakhapatnam, India, on 30 March 2004 (GPS position of the site 17° 42′ N 83° 18′ E). The sample yielding strain JA123$^T$ had a pH of 6.8 and a temperature of 30 °C. Purification and polyphasic taxonomic analyses were carried out as described previously (Srinivas *et al.*, 2007a).

Individual cells of strain JA123$^T$ were rod-shaped, 0.8–1.2 μm wide and 1.5–3.0 μm long, non-motile and multiplied by binary fission (see Supplementary Fig. S1 available in IJSEM Online). Electron microphotographs of ultrathin sections of the cells revealed vesicular internal membrane structures (Supplementary Fig. S2). Strain JA123$^T$ was able to grow photo-organoheterotrophically [anaerobic conditions, in the light (2400 lx)] and chemo-organoheterotrophically [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), $Na_2S_2O_3$ (0.5 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)] could not be demonstrated. Substrates that were utilized as carbon sources and electron donors under photo-organoheterotrophic conditions included acetate, butyrate, lactate, pyruvate, fumarate, oxoglutarate, succinate, malate, glucose, glycerol, sorbitol, cysteine, peptone and Casamino acids (Table 1). Those that could not be utilized included formate, propionate, valerate, crotonate, caproate, caprylate, glycollate, benzoate, tartrate, citrate, fructose, sucrose, mannitol, gluconate, methanol, ethanol, propanol, methionine, aspartate, glutamate, ascorbate and thioglycollate. Ammonium chloride, glutamate, glutamine and molecular nitrogen were utilized as nitrogen sources, whereas urea, nitrate and nitrite did not support growth. Salt (0.5–10 % w/v NaCl) was required for growth of strain JA123$^T$; optimum growth occurred in 1–4 % (w/v) NaCl. Strain JA123$^T$ grew at pH 6.0–8.0 and 20–30 °C; optimum growth was observed at pH 6.0–7.5 and 30 ± 2 °C. It was able to grow photo-organoheterotrophically under fluorescent light (optimum light intensity ~2400 lx; range 1000–4000 lx). Furthermore, the strain required biotin as a growth factor. The colour of photosynthetically grown cell suspension was yellowish brown. The whole-cell absorption spectrum of strain JA123$^T$ gave absorption maxima at 377, 452, 479, 512, 590, 803 and 851 nm, thus confirming the presence of bacteriochlorophyll *a* and probably carotenoids of the spheroidene series (Supplementary Fig. S3).

The DNA G+C content of strain JA123$^T$ was 68.8 mol% (by HPLC). The phylogenetic relationship between strain JA123$^T$ and other purple non-sulfur bacteria was examined by 16S rRNA gene sequencing. Sequences were aligned using the program CLUSTAL X (Thompson *et al.*, 1997) and the alignment was corrected manually. The CLUSTAL X alignment file was used as the input file to the program SEQBOOT in the PHYLIP package (Felsenstein, 1989) 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 produced using 100 trees generated during maximum-likelihood analysis using the program CONSENSE. A final dendrogram with evolutionary distances was constructed by taking the alignment .phy file as the infile and the consensus tree as the intree in the maximum-likelihood program of the PHYLIP package. Data showed 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. The highest sequence similarities of strain JA123$^T$ were found with the type strains of *Haematobacter massiliensis* (95.7 %), *Rba. veldkampii* (95.2 %), *Rba. sphaeroides* (95 %), *Rba. capsulatus* (94.7 %), *Rba. azotoformans* (94.6 %) and *Rba. blasticus* (94.2 %) (Fig. 1). Apart from 16S rRNA gene sequence dissimilarity, strain JA123$^T$ clearly differed phenotypically from other *Rhodobacter* species (Table 1), which justifies the description of a novel species to accommodate this strain; the name *Rhodobacter vinaykumarii* sp. nov. is proposed. An additional strain, JA249, which also represents *Rhodobacter vinaykumarii*, was isolated from marine water in Kiel, Germany (54° 21′ N 10° 08′ E).

## Description of *Rhodobacter vinaykumarii* sp. nov.

*Rhodobacter vinaykumarii* (vi′nay.ku.ma′ri.i. N.L. masc. gen. n. *vinaykumarii* of Vinaykumar, named after the late Dr M. Vinaykumar, an Indian microbiologist and research supervisor of Ch. V. R. and Ch. S, who initiated work on anoxygenic phototrophic bacteria in India).

Cells are rod-shaped, 0.8–1.2 × 1.5–3.0 μm, non-motile and divide by binary fission. 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 photosynthetically grown cultures is yellowish brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 377, 452, 479, 512, 590, 803 and 851 nm, thus confirming the presence of bacteriochlorophyll *a* and probably carotenoids of the spheroidene series. Mesophilic (30 °C), with optimum growth at pH 6.0–7.5. Requires NaCl for growth (optimum 1–4 %, w/v). Photoheterotrophy with organic compounds is the preferred mode of growth. Good growth is obtained on pyruvate, fumarate, oxoglutarate and malate. Growth on acetate, succinate, lactate, glucose, glycerol, sorbitol, cysteine, peptone and Casamino acids also occurs. Photolithoautotrophic and chemolithoautotrophic growth is not possible in the presence of thiosulfate/hydrogen as electron donor and $NaHCO_3$ as carbon source. Fermentative growth is not possible in the

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

Strain/species: 1, JA123$^T$; 2, *Rba. capsulatus*; 3, *Rba. azotoformans*; 4, *Rba. blasticus*; 5, *Rba. sphaeroides*; 6, *Rba. veldkampii*. Data for reference species were taken from Imhoff (2005). Cells of all taxa studied divide by binary fission. Organic substrate utilization was tested during photoheterotrophic growth for all taxa. Acetate, pyruvate, lactate, malate, succinate, fumarate and D-glucose were utilized by all the taxa. Benzoate, methanol and arginine were not utilized by any of the taxa. +, Substrate utilized or present; −, substrate not utilized or absent; ±, variable in different strains; (+), weak growth; NR, not reported.

| Characteristic | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Cell size (μm) | 0.8–1.2 × 1.5–3.0 | 0.5–1.2 × 2.0–2.5 | 0.6–1.0 × 0.9–1.5 | 0.6–0.8 × 1.0–2.5 | 2.0–2.5 × 2.5–3.5 | 0.6–0.8 × 1.0–1.3 |
| Shape | Rods | Ovoid to rods, chains | Ovoid to rods | Ovoid to rods | Spherical to ovoid | Ovoid to rods |
| Motility | − | + | + | − | + | − |
| Colour of cell suspension | Yellowish brown | Yellowish brown | Yellowish brown | Orange–brown | Greenish brown | Yellowish brown |
| Internal membrane system | Vesicular | Vesicular | Vesicular | Lamellar | Vesicular | Vesicular |
| Slime production | + | ± | + | − | ± | − |
| Requirement for NaCl (optimum, %) | + (1–4) | −* | −* | − | −* | − |
| pH range | 6.0–8.0 | 6.5–7.5 | NR | NR | 6.0–8.5 | NR |
| Optimal pH | 6.0–7.5 | 7.0 | 7.0–7.5 | 6.5–7.5 | 7.0 | 7.5 |
| Temperature optimum (°C) | 20–30 | 30–35 | 30–35 | 30–35 | 30–34 | 30–35 |
| Sulfate assimilated | + | + | + | + | + | − |
| Vitamins required† | b | t (b, n) | b, n, t | b, n, t, $B_{12}$ | b, t, n | b, *p*-ABA, t |
| DNA G+C content (mol%) | 68.8 | 68.1–69.6 | 69.5–70.2 | 65.3 | 70.8–73.2 | 64.4–67.5 |
| Electron donors | | | | | | |
| Hydrogen | − | + | NR | + | + | − |
| Sulfide | − | + | + | − | + | + |
| Thiosulfate | − | − | − | − | − | + |
| Sulfur | − | − | − | − | − | + |
| Carbon/electron donors | | | | | | |
| Formate | − | + | + | − | − | − |
| Propionate | − | + | + | + | + | + |
| Butyrate | (+) | + | + | + | + | + |
| Valerate | − | + | NR | NR | + | + |
| Caproate | − | + | NR | NR | + | + |
| Caprylate | − | + | − | NR | + | + |
| Tartrate | − | − | − | − | + | − |
| Citrate | − | ± | NR | + | + | − |
| Aspartate | − | ± | NR | NR | NR | + |
| Glutamate | − | + | + | + | + | + |
| Fructose | − | + | + | + | + | − |
| Mannitol | − | ± | + | + | + | − |
| Sorbitol | − | ± | + | + | + | − |
| Glycerol | + | − | + | + | + | − |
| Methanol | − | − | − | − | ± | − |
| Ethanol | − | − | NR | − | + | − |
| Propanol | − | + | NR | NR | NR | − |

*Grows optimally in the absence of NaCl, but able to grow in 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.

presence of pyruvate as the fermentable carbon source. Biotin is required for growth.

The type strain is JA123$^T$ (=DSM 18714$^T$ =JCM 14544$^T$ =CCUG 54311$^T$), isolated from marine tidal waters on the east coast of India. The DNA G+C content of the type strain is 68.8 mol% (by HPLC). An additional strain of this species, JA249, has been isolated from marine water in Germany.

## Emended description of the genus *Rhodobacter* Imhoff *et al.* 1984

The description is as given previously (Imhoff *et al.*, 1984; Imhoff, 2005) with the modification that most species of the genus *Rhodobacter* are freshwater bacteria and do not require salt, but some species may be adapted to the marine environment and require salt.

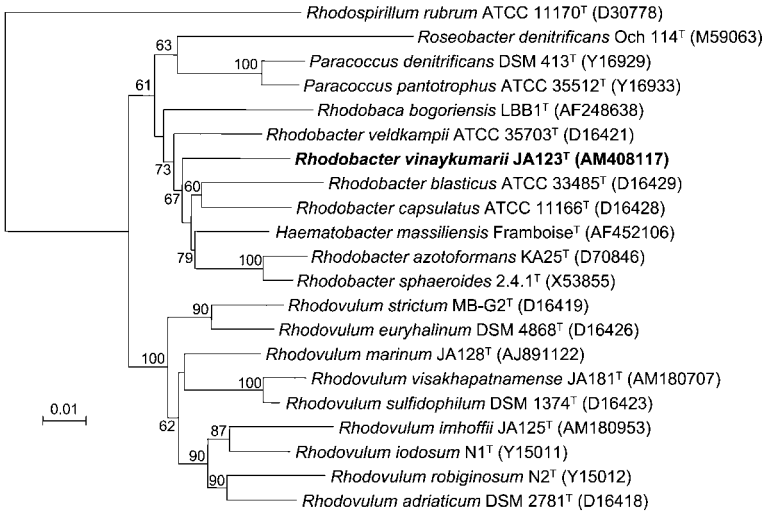


**Fig. 1.** Evolutionary-distance dendrogram depicting the phylogenetic relationships of strain JA123$^T$ within the family *Rhodobacteraceae* determined using 16S rRNA gene sequence analysis. Bar, 1 substitution per 100 nucleotide positions.

## Acknowledgements

Financial assistance received from the Department of Biotechnology and Ministry of Earth Sciences (MoES), Government of India, is acknowledged. P. A. K. and T. N. R. S. acknowledge the CSIR, Government of India, for the award of JR and SR fellowships. Financial assistance received under the DST-DAAD exchange program (grant 422-PPP-34105) is acknowledged.

## References

**Felsenstein, J. (1989).** PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

**Greub, G. & Raoult, D. (2003).** *Rhodobacter massiliensis* sp. nov., a new amoebae-resistant species isolated from the nose of a patient. *Res Microbiol* **154**, 631–635.

**Hansen, T. A. & Imhoff, J. F. (1985).** *Rhodobacter veldkampii*, a new species of phototrophic purple nonsulfur bacteria. *Int J Syst Bacteriol* **35**, 115–116.

**Helsel, L. O., Hollis, D., Steigerwalt, A. G., Morey, R. E., Jordan, J., Aye, T., Radosevic, J., Jannat-Khah, D., Thiry, D. & other authors (2007).** Identification of "*Haematobacter*", a new genus of aerobic Gram-negative rods isolated from clinical specimens, and reclassification of *Rhodobacter massiliensis* as "*Haematobacter massiliensis*" comb. nov. *J Clin Microbiol* **45**, 1238–1243.

**Hiraishi, A. & Ueda, Y. (1994).** Intrageneric structure of the genus *Rhodobacter*: transfer of *Rhodobacter sulfidophilus* and related marine species to the genus *Rhodovulum* gen. nov. *Int J Syst Bacteriol* **44**, 15–23.

**Hiraishi, A. & Ueda, Y. (1995).** Isolation and characterization of *Rhodovulum strictum* sp. nov. and some other purple nonsulfur bacteria from colored blooms in tidal and seawater pools. *Int J Syst Bacteriol* **45**, 319–326.

**Hiraishi, A., Muramatsu, K. & Ueda, Y. (1996).** Molecular genetic analyses of *Rhodobacter azotoformans* sp. nov. and related species of phototrophic bacteria. *Syst Appl Microbiol* **19**, 168–177.

**Imhoff, J. F. (2001).** Transfer of *Rhodopseudomonas acidophila* to the new genus *Rhodoblastus* as *Rhodoblastus acidophilus* gen. nov., comb. nov. *Int J Syst Evol Microbiol* **51**, 1863–1866.

**Imhoff, J. F. (2005).** Genus *Rhodobacter*. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part C, pp. 161–167. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

**Imhoff, J. F., Trüper, H. G. & Pfennig, N. (1984).** Rearrangement of the species and genera of the phototrophic 'purple nonsulfur bacteria'. *Int J Syst Bacteriol* **34**, 340–343.

**Kawasaki, H., Hoshino, Y., Hirata, A. & Yamasato, K. (1993).** Is intracytoplasmic membrane structure a generic criterion? It does not coincide with phylogenetic interrelationships among phototrophic purple nonsulfur bacteria. *Arch Microbiol* **160**, 358–362.

**Kompantseva, E. I. (1985).** *Rhodobacter euryhalinus* sp. nov., a new halophilic purple bacterial species. *Mikrobiologiia* **54**, 974–982.

**Srinivas, T. N. R., Anil Kumar, P., Sasikala, Ch., Ramana, Ch. V., Süling, J. & Imhoff, J. F. (2006).** *Rhodovulum marinum* sp. nov., a novel phototrophic purple non-sulfur alphaproteobacterium from marine tides of Visakhapatnam, India. *Int J Syst Evol Microbiol* **56**, 1651–1656.

**Srinivas, T. N. R., Anil Kumar, P., Sasikala, Ch. & Ramana, Ch. V. (2007a).** *Rhodovulum imhoffii* sp. nov. *Int J Syst Evol Microbiol* **57**, 228–232.

**Srinivas, T. N. R., Anil Kumar, P., Sasikala, Ch., Ramana, Ch. V. & Imhoff, J. F. (2007b).** *Rhodovulum visakhapatnamense* sp. nov. *Int J Syst Evol Microbiol* **57**, 1762–1764.

**Straub, K. L., Rainey, F. A. & Widdel, F. (1999).** *Rhodovulum iodosum* sp. nov. and *Rhodovulum robiginosum* sp. nov., two new marine phototrophic ferrous-iron-oxidizing purple bacteria. *Int J Syst Bacteriol* **49**, 729–735.

**Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997).** The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.