Rhodobacter changlensis sp. nov., a psychrotolerant, phototrophic alphaproteobacterium from the Himalayas of India

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A Gram-negative, non-motile, oval to rod-shaped, psychrotolerant, phototrophic, purple non-sulfur bacterium (designated strain JA139^T) was isolated from a snow sample from Changla Pass in the Indian Himalayas. Strain JA139^T had vesicular-type intracytoplasmic membrane structures and contained bacteriochlorophyll *a* and most probably spheroidene-like carotenoids. Biotin, niacin and thiamine were required for growth of strain JA139^T. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that the strain clustered with species of the genus *Rhodobacter* but was distinctly separate from all recognized members of the family *Rhodobacteraceae*. Based on the genotypic and phenotypic differences observed between strain JA139^T and recognized *Rhodobacter* species, strain JA139^T is considered to represent a novel species of the genus, for which the name *Rhodobacter changlensis* sp. nov. is proposed. The type strain is JA139^T (=DSM 18774^T =CCUG 53722^T =JCM 14338^T).

Anoxygenic phototrophic bacteria are found widely distributed in various habitats including those from the extreme cold. Among such species reported from cold environments are *Rhodoferrax antarcticus* (Madigan *et al.*, 2000) isolated from an Antarctic microbial mat and *Rhodoferrax ferrireducens* (Finneran *et al.*, 2003) isolated from subsurface marine sediments of Oyster Bay, USA, both of which represent phototrophic members of the class *Betaproteobacteria*. Here we describe a novel psychrotolerant phototroph belonging to the genus *Rhodobacter* (class *Alphaproteobacteria*).

Strain JA139^T was isolated from photoheterotrophic enrichments of a snow sample from Changla Pass in the Himalayas of India. Purification and polyphasic taxonomic studies were carried out as described by Srinivas *et al.* (2006). Sequences were aligned by using the CLUSTAL X program (Thompson *et al.*, 1997) and the alignment was corrected manually. The CLUSTAL X 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 based on 100 datasets and

five times jumbling. One single tree was produced by using 100 trees generated during maximum-likelihood analysis with the CONSENSE program. 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 within the PHYLIP package (Felsenstein, 1989).

A yellowish-brown-coloured snow sample (see Supplementary Fig. S1 in IJSEM Online) was collected on 22 May 2006 from Changla Pass (~5640 m above sea level) in the Indian Himalayas (34° 10' N 78° 16' E). Individual cells of strain JA139^T were oval to rod shaped, 0.8–1.0 µm wide and 2–4 µm long, and usually formed chains; they were non-motile and multiplied by binary fission (Supplementary Fig. S2). Electron micrographs of ultrathin sections of the cells revealed vesicular-type intracytoplasmic membrane structures.

Strain JA139^T was able to grow photo-organoheterotrophically [anaerobically in the light (2400 lx)] and chemo-organoheterotrophically [aerobically in the dark, with pyruvate (0.3 %, w/v)]. We were unable to demonstrate photolithoautotrophy [anaerobically in the light (2400 lx), with Na₂S (0.5 mM), Na₂S₂O₃·5H₂O (0.5 mM) or NaHCO₃ (0.1 %, w/v)], chemolithoautotrophy [aerobically in the dark, with thiosulfate (0.5 mM) or NaHCO₃ (0.1 %, w/v)] or fermentative growth [anaerobically in the dark, with pyruvate (0.3 % w/v)] for strain JA139^T. Substrates that could be utilized as carbon/electron donors under

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA139^T is AM399030.

A photograph showing the yellowish-brown-coloured snow sample collected from Changla Pass, Indian Himalayas, from where strain JA139^T was isolated, a phase-contrast micrograph of cells of strain JA139^T and the whole-cell absorption spectrum of strain JA139^T are available as supplementary material with the online version of this paper.

Table 1. Differential phenotypic characteristics among members of the genus *Rhodobacter*

Symbols: +, substrate utilized or present; –, substrate not utilized or absent; ±, variable in different strains; (+), weak growth; NR, not reported. Organic substrate utilization was tested during photoheterotrophic growth. Cells of all taxa divide by binary fission. Pyruvate, succinate and D-glucose were utilized by all taxa. Benzoate and arginine were not utilized by any of the taxa.

Characteristic	Strain JA139 ^T	<i>Rba.</i> <i>vinaykumarii</i> *	<i>Rba. capsulatus</i>	<i>Rba.</i> <i>azotoformans</i>	<i>Rba. blasticus</i>	<i>Rba.</i> <i>sphaeroides</i>	<i>Rba.</i> <i>veldkampii</i>
Cell size (µm)	0.8–1.0 × 2.0–4.0	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	Ovoid to rods, chains	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	Yellowish brown	Orange– brown	Greenish brown	Yellowish brown
Internal membrane system	Vesicular	Vesicular	Vesicular	Vesicular	Lamellar	Vesicular	Vesicular
Slime production	+	+	±	+	–	±	–
NaCl requirement (optimum)	–	+ (1–4)	–†	–†	–	–†	–
Optimum pH (range)	6.5–7.5 (6.5–8.0)	6.0–7.5 (6.0–8.0)	7.0 (6.5–7.5)	7.0–7.5	6.5–7.5	7.0 (6.0–8.5)	7.5
Optimum temperature (range) (°C)	28–30 (5–32)	28–32 (20–30)	30–35	30–35	30–35	30–34	30–35
Sulfate assimilation	+	+	+	+	+	+	–
Vitamins required‡	b, n, t	b	t, (b, n)	b, n, t	b, n, t, B ₁₂	b, t, n	b, <i>p</i> -ABA, t
DNA G + C content (mol%)	69.4	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	–	–	+	+	–	–	–
Acetate	–	+	+	+	+	+	+
Propionate	–	–	+	+	+	+	+
Butyrate	–	(+)	+	+	+	+	+
Valerate	–	–	+	NR	NR	+	+
Caproate	–	–	+	NR	NR	+	+
Caprylate	–	–	+	–	NR	+	+
Tartrate	–	–	–	–	–	+	–
Lactate	–	+	+	+	+	+	+
Malate	–	+	+	+	+	+	+
Fumarate	–	+	+	+	+	+	+
Citrate	–	–	±	NR	+	+	–
Aspartate	–	–	±	NR	NR	NR	+
Glutamate	+	–	+	+	+	+	+
Fructose	–	–	+	+	+	+	–
Mannitol	+	–	±	+	+	+	–
Sorbitol	+	–	±	+	+	+	–
Glycerol	–	+	–	+	+	+	–
Methanol	–	–	–	–	–	±	–
Ethanol	–	–	–	NR	–	+	–
Propanol	–	–	+	NR	NR	NR	–

*Source data updated from Srinivas *et al.* (2007).

†Optimal growth in the absence of NaCl but able to grow at 3 % NaCl.

‡b, Biotin; B₁₂, vitamin B₁₂; n, niacin; *p*-ABA, *p*-aminobenzoic acid; t, thiamine; (b, n), a few strains require biotin and/or niacin.

photo-organoheterotrophic conditions at 28 ± 2 °C included pyruvate, 2-oxoglutarate, succinate, glucose, mannitol, sorbitol, glutamate, casein hydrolysate, yeast extract and peptone (see Table 1). Formate, acetate, propionate, butyrate, valerate, crotonate, caproate, caprylate, lactate, gluconate, glycolate, benzoate, tartrate, malate, fumarate, citrate, fructose, sucrose, lactose, starch, glycerol, aspartate, methionine, cysteine, ascorbate, methanol, ethanol, propanol and butanol could not be utilized. Strain JA139^T was able to use ammonium chloride, glutamine and N₂ as nitrogen sources, but not urea, nitrate or nitrite. Sulfate and thioglycolate were utilized as sulfur sources, but not sulfite, sulfur, sulfide, thiosulfate or cysteine. Although NaCl was not obligatory for growth of strain JA139^T, optimum growth occurred at 0.1 % (w/v). The pH range for growth of strain JA139^T was 6.5–9 with an optimum at pH 6.5–7.5. The optimum temperature for growth was 28 ± 2 °C, but growth was observed from 5 to 32 °C. The biomass yield of strain JA139^T grown at 5 °C was about 50 % of that grown at the optimum temperature. Strain JA139^T grew well in the presence of fluorescent light (optimum light intensity 2000 lx; range 1000–3000 lx). Furthermore, strain JA139^T required biotin, niacin and thiamine as growth factors. The photosynthetically grown cell suspension was yellowish brown. The whole-cell absorption spectrum of strain JA139^T gave absorption maxima at 380, 407, 450, 470, 512, 590, 806 and 851 nm, confirming the presence of bacteriochlorophyll *a*, and most probably carotenoids of the spheroidene series (Supplementary Fig. S3). The DNA base composition of strain JA139^T was 69.4 mol% G+C (by HPLC). The phylogenetic relationship of strain JA139^T to other purple non-sulfur bacteria was examined by 16S rRNA gene sequencing. The data obtained revealed that the new isolate branched separately but clustered with the type strains of species of the genus *Rhodobacter* and was distinct from other genera of purple non-sulfur bacteria. Strain JA139^T showed highest 16S rRNA gene sequence similarities with

the type strains of *Rhodobacter sphaeroides* (94.6 %), *Rhodobacter azotoformans* (94.4 %), *Rhodobacter veldkampii* (93.6 %), *Rhodobacter blasticus* (92.7 %), *Rhodobacter capsulatus* (92.5 %) and *Rhodobacter vinaykumarii* (93.1 %; Srinivas *et al.*, 2007) (Fig. 1). In addition to showing 16S rRNA gene sequence divergence, strain JA139^T showed clear phenotypic differences from recognized *Rhodobacter* species (Table 1) that justify the description of this strain as representing a novel species, for which the name *Rhodobacter changlensis* sp. nov. is proposed.

Description of *Rhodobacter changlensis* sp. nov.

Rhodobacter changlensis (chang.len'sis. N.L. masc. adj. *chaglensis* pertaining to Changla Pass, the location from where the type strain was isolated).

Cells are oval to rod-shaped, 0.8–1.0 µm wide and 2–4 µm long, are non-motile and divide by binary fission, and occasionally form chains. 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. Phototrophic cultures are yellowish brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 380, 407, 450, 470, 512, 590, 806 and 851 nm, confirming the presence of bacteriochlorophyll *a* and most probably carotenoids of the spheroidene series. The organism is psychrotolerant (range 5–32 °C; optimum at 28 ± 2 °C), has a pH optimum at 6.5–7.5 and does not require NaCl for growth. Photo-organoheterotrophy with a few organic compounds is the preferred mode of growth. Grows well on pyruvate, 2-oxoglutarate, mannitol and sorbitol as carbon sources. Growth is also observed on succinate, glucose, glutamate, casein hydrolysate, yeast extract and peptone. Chemoautotrophic growth is not possible in the presence of thiosulfate/hydrogen as electron donor and NaHCO₃ as carbon source. Fermentative growth is not possible in the presence of pyruvate as

Fig. 1. Dendrogram depicting the phylogenetic relationships of strain JA139^T within the family *Rhodobacteraceae* determined by using 16S rRNA gene sequence analysis. Bar, 1 substitution per 100 nucleotide positions.

fermentable carbon source. Biotin, niacin and thiamine are required as growth factors. DNA base composition is 69.4 mol% G + C (by HPLC).

The type strain, JA139^T (=DSM 18774^T =CCUG 53722^T =JCM 14338^T), was isolated from a snow sample collected from Changla Pass in the Indian Himalayas.

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