Rhodobacter megalophilus sp. nov., a phototroph from the Indian Himalayas possessing a wide temperature range for growth

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Two strains of phototrophic, purple non-sulfur bacteria capable of growing at low temperatures (5 °C) were isolated from the Himalayas. The two strains showed positive phototaxis and grew over a relatively wide temperature range (5–40 °C). Phylogenetic analysis based on 16S rRNA gene sequences showed that strain JA194T clustered with members of the genus *Rhodobacter*. Strain JA194T showed highest 16S rRNA gene sequence similarity with *Rhodobacter sphaeroides* DSM 158T (99 %). However, DNA–DNA hybridization experiments between *Rba. sphaeroides* DSM 158T and strain JA194T revealed a level of relatedness of only 67 %. The DNA base composition of strain JA194T was 66.67 mol% G+C (by HPLC). Based on 16S rRNA gene sequence analysis, morphological, physiological, Fourier transform infrared fingerprinting and DNA–DNA hybridization studies, strain JA194T (=KCTC 5602T =JCM 14598T) is sufficiently different from other *Rhodobacter* species to merit its description as the type strain of a novel species, for which the name *Rhodobacter megalophilus* sp. nov. is proposed.

The genus *Rhodobacter* includes both motile and non-motile species. Motile species of the genus have a single polar flagellum and those that lack flagellar motility include *Rhodobacter veldkampii* (Imhoff, 2005), *Rba. vinayakumarii* (Srinivas et al., 2007c), *Rba. changlensis* (Anil Kumar et al., 2007b) and *Rba. ovatus* (Srinivas et al., 2008). *Rba. changlensis*, a phototrophic bacterium from the Indian Himalayan region, was recently described by our group (Anil Kumar et al., 2007b). In the present communication, we propose a novel species of this genus isolated from the Indian Himalayas, which has properties distinct from all other *Rhodobacter* species based on phenotypic and phylogenetic analysis.

Strain JA194T was isolated from a soil sample collected from Suraj tal near Sarchu [34°17'N 77°58'E, 11 975 feet (3650 m) above sea level], in the Indian Himalayas. Strain JA247 was isolated from a soil sample collected from an army camp located at Kargil, Ladakh [34°17' N 77°55'E, 18 050 feet (5502 m) above sea level], in the Himalayas. Purification of the strains and polyphasic study for their taxonomic characterization were performed as described by Srinivas et al. (2007a). For dendrogram construction, sequences were aligned by using the program CLUSTAL_X (Thompson et al., 1997) and the alignment was corrected manually. Phylogenetic analysis was carried out by using the PHYLIP package, version 3.5 (Felsenstein, 1993). The evolutionary distance matrix was calculated according to the distance model of Jukes & Cantor (1969). The resultant tree topologies were evaluated by bootstrap analysis based on 100 resamplings via the SEQBOOT and CONSENSE programs in the PHYLIP package.

For metabolome fingerprinting, exponentially growing photoheterotrophic cultures were harvested by centrifugation (15 000 r.p.m. for 15 min for reference strain *Rba. sphaeroides* DSM 158T; 35 000 r.p.m. for 30 min for strains JA194T and JA247) and the resultant pellets were washed...
twice with distilled water. The cell pellet was quenched in liquid nitrogen (−196 °C) according to the protocol of Chassagnole et al. (2002) and the pellet was freeze-dried. The freeze-dried pellet was mixed with KBr. The KBr pellets were used for recording spectra between 4000 and 450 cm⁻¹ (at a resolution of 4 cm⁻¹) by using a Fourier transform infrared (FT-IR) spectrometer (Spectrum 100; Perkin Elmer) equipped with a KBr beam splitter and a DTGS (deuterated triglycine sulfate) detector. Spectral data were processed using Spectrum One FT-IR software (Perkin Elmer). Lipid fingerprinting was performed following extraction of the lipids from the freeze-dried samples. The lipid extract was mixed with KBr and the pellets were used for FT-IR spectral analysis.

For DNA–DNA hybridization experiments, DNA was isolated by using a French pressure cell (Thermo Spectronic) and was purified by chromatography on hydroxyapatite as described by Cashion et al. (1977). DNA–DNA hybridization was carried out as described by De Ley et al. (1970), with the modifications given by Huß et al. (1983), by using a model Cary 100 Bio UV/Vis spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and temperature controller with in-situ temperature probe (Varian).

Cells of strains JA194ᵀ and JA247 were ovoid (see Supplementary Fig. S1 in IJSEM Online), measured 1.2–1.5 × 1.5–2.0 μm in size, divided by binary fission and were non-motile. Negatively stained transmission electron micrographs revealed the absence of flagella from both strains. The two strains showed positive phototaxis. Ultrathin sections when viewed under the electron microscope revealed the presence of vesicular internal membrane structures. Whole-cell absorption spectra of strains JA194ᵀ and JA247 had absorption maxima at 374, 407, 446, 476, 509, 800 and 854 nm, confirming the presence of bacteriochlorophyll a. The absorption spectrum for pigments extracted with acetone showed maxima at 455 and 488 nm, indicating the presence of carotenoids, spheroidene and spheroidenone.

Strains JA194ᵀ and JA247 grew photoheterotrophically, photoautotrophically and chemoheterotrophically (aerobically but not anaerobically). Data for strain JA194ᵀ regarding organic substrates used as carbon/electron donors, nitrogen sources metabolized and growth factor requirements are given in the species description. The two novel strains were able to grow well from 5 to 40 °C.

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Table 1. Differential characteristics between strain JA194<sup>T</sup> and recognized species of the genus Rhodobacter

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell size (μm)</td>
<td>1.2–1.5 × 1.5–2.0</td>
<td>2.0–2.5 × 2.5–3.5</td>
<td>0.5–1.2 × 0.5–1.5</td>
<td>0.6–0.8 × 1.0–1.5</td>
<td>0.6–0.8 × 1.0–1.5</td>
<td>0.6–1.0 × 0.9–1.5</td>
<td>0.9–1.2 × 1.0–2.0</td>
<td>0.8–1.0 × 2.0–4.0</td>
<td>0.8–1.2 × 1.5–3.0</td>
</tr>
<tr>
<td>Cell shape*</td>
<td>O to R</td>
<td>O to R</td>
<td>C to R</td>
<td>O to R</td>
<td>O to R</td>
<td>C to R</td>
<td>C to R</td>
<td>C to R</td>
<td>R</td>
</tr>
<tr>
<td>Flagellar motility</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Colour of cell suspension†</td>
<td>GB</td>
<td>YB</td>
<td>YB</td>
<td>OB</td>
<td>OB</td>
<td>OB</td>
<td>OB</td>
<td>OB</td>
<td>OB</td>
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<tr>
<td>Internal membrane system‡</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>L</td>
<td>L</td>
<td>V</td>
<td>V</td>
<td>V</td>
<td>V</td>
</tr>
<tr>
<td>Slime production</td>
<td>±</td>
<td>±</td>
<td>±</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Sedimentation of culture at 15 000 r.p.m.</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Requirement for NaCl</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>+ (1–4)</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>ND</td>
<td>6.0–8.5</td>
<td>6.5–7.5</td>
<td>NR</td>
<td>NR (7.5)</td>
<td>NR</td>
<td>6.0–8.0</td>
<td>6.5–8.0</td>
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<tr>
<td>Growth at 5 °C</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Sulfate assimilated</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>−</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Vitamins required§</td>
<td>t, b, t, n</td>
<td>b, n, t, B&lt;sub&gt;12&lt;/sub&gt;</td>
<td>b, p-ABA, t</td>
<td>b, n, t</td>
<td>b</td>
<td>b, t</td>
<td>b, n, t</td>
<td>b</td>
<td></td>
</tr>
<tr>
<td>DNA G + C content (mol%) (HPLC)</td>
<td>66.67</td>
<td>70.8–72.3</td>
<td>68.1–69.6</td>
<td>65.3</td>
<td>64.4–67.5</td>
<td>69.5–70.2</td>
<td>70.06</td>
<td>69.4</td>
<td>68.8</td>
</tr>
</tbody>
</table>

* C, Chains; O, ovoid; R, rod-shaped; S, spherical.
† GB, Greenish brown; OB, orange–brown; YB, yellowish brown.
‡ L, Lamellar; V, vesicular.
§ Optimal growth in the absence of NaCl but able to grow at 3% (a) or 5% (b) NaCl.
Il情報 on saline tolerance of *Rba. capsulatus* from Hiraishi et al. (1996).
¶ B, Biotin; B<sub>12</sub>, vitamin B<sub>12</sub>; n, niacin; p-ABA, p-aminobenzoic acid; t, thiamine; (b, n), a few strains require biotin and/or niacin.
(Fig. 1a), whereas Rba. sphaeroides DSM 158T was mesophilic (Fig. 1b). Strains JA194T and JA247 had no NaCl requirement for growth, but were able to tolerate up to 4 % NaCl.

16S rRNA gene sequence analysis revealed that strains JA194T and JA247 were closely related to Rba. sphaeroides DSM 158T (sequence similarity of 99 %) (Fig. 2). The level of genomic DNA–DNA hybridization between strain JA194T and Rba. sphaeroides DSM 158T was 67 %, which is very close to the boundary recommended for the delineation of species (Wayne et al., 1987).

We utilized metabolome fingerprinting (IR analysis; Mashego et al., 2007) and FT-IR analysis (Ramana et al., 2006; Srinivas et al., 2007b; Anil Kumar et al., 2007a) to delineate the strains studied here. FT-IR spectrum similarity data were obtained via the Euclidean analysis delineate the strains studied here. FT-IR spectrum

Table 1). Strain JA194T is therefore considered to represent DSM 158T (sequence similarity of 99 %) (Fig. 2). The level of genomic DNA–DNA hybridization between strain JA194T and other recognized

Growth occurs photo-organoheterotrophically with various sources, including malate, fumarate, acetate, propionate, butyrate, valerate, caproate, glutamate, aspartate, glucose, tartrate, ethanol, mannitol, sorbitol and glycerol. Photolithoautotrophic growth is possible in the presence of thiosulfate as electron donor and NaHCO₃ as carbon source. Nitrogen sources providing good growth are ammonium chloride and glutamate. Nitrate and molecular nitrogen also support growth as nitrogen sources. Thiamine is required for growth. Growth occurs from 5 to 40 °C. The DNA G+C content of the type strain is 66.67 mol% (by HPLC).

The type strain, JA194T (=KCTC 5602T =JCM 14598T), and an additional strain of the species, JA247, were isolated from soils of the Indian Himalayas.

Acknowledgements

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References


