Rhodospirillum sulfurexigens sp. nov., a phototrophic alphaproteobacterium requiring a reduced sulfur source for growth

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A Gram-negative, spiral-shaped, phototrophic, purple non-sulfur bacterial strain, JA143T, was isolated from a freshwater habitat. Strain JA143T was motile by means of bipolar tufts of flagella. Intracellular photosynthetic membranes are of the lamellar stacked type. Bacteriochlorophyll a and carotenoids of the spirilloxanthin series with rhodovibrin are present as photosynthetic pigments. Thiamine and a reduced sulfur source are required for growth. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA143T clusters with species of the genus Rhodospirillum, belonging to the class Alphaproteobacteria. The highest sequence similarities of strain JA143T were found with the type strains of Rhodospirillum rubrum (95.6 %) and Rhodospirillum photometricum (95.7 %). Based on 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA143T was significantly different from the other two recognized species of the genus Rhodospirillum and represents a novel species, for which the name Rhodospirillum sulfurexigens sp. nov. is proposed. The type strain is JA143T (=DSM 19785T =NBRC 104433T).

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA143T is AM710622. 16S rRNA gene sequences were aligned using the CLUSTAL_X program (Thompson et al., 1997) and the alignment was corrected manually. The alignment file was saved with ‘.phy’ extension. The dendrogram was constructed using the PhyML program (Guindon & Gascuel, 2003) using 100 replicates of non-parametric bootstrap analysis, the GTR model of nucleotide substitution and four substitution rate categories.

Cells of strain JA143T are spiral shaped, 1.0–1.3 μm wide; one single turn is 8–9 μm long. Cells also form long chains, more than 30 μm long (Fig. 1) and multiply by binary fission. Strain JA143T is motile by means of bipolar tufts of flagella (Supplementary Fig S1, available in IJSEM Online). Transmission electron micrographs of ultrathin sections of the strain revealed a lamellar stacked type of internal membrane structures (Supplementary Fig S2). Strain JA143T was able to grow photoorganoheterotrophically [anaerobic, light (2400 lx), with pyruvic acid (0.3 %, w/v) as carbon source/electron donor and Na2S2O3.5H2O (6 mM) as sulfur source]. Photolithoautotrophy [anaerobic, light (2400 lx), Na2S.9H2O (2 mM)/Na2S2O3.5H2O (6 mM) and NaHCO3 (0.1 %, w/v)], chemolithoautotrophy [aerobic, dark, Na2S2O3.5H2O (2 mM) and NaHCO3 (0.1 %, w/v)], chemoorganoheterotrophy [aerobic, dark, pyruvate
(0.3 %, w/v) as carbon source/electron donor and Na$_2$S$_2$O$_3$·5H$_2$O (2 mM) as sulfur source] and fermentative growth [anaerobic, dark with pyruvate (0.3 %, w/v) as carbon source/electron donor and Na$_2$S$_2$O$_3$·5H$_2$O (2 mM) as sulfur source] could not be demonstrated. Organic substrates utilized for photoorganoheterotrophy include acetate, butyrate, caproate, crotonate, fructose, fumarate, glucose, glutamate, lactate, malate, pyruvate, succinate and valerate (Table 1). Those that could not be utilized by strain JA143$^T$ include arginine, aspartate, benzoate, caprylate, citrate, ethanol, formate, glycerol, glycolate, mannitol, methanol, propionate and tartrate. Strain JA143$^T$ could utilize ammonium chloride, aspartate, glutamate and glutamine as nitrogen sources, while molecular nitrogen, urea, nitrate and nitrite did not support growth. Strain JA143$^T$ requires thiamine as growth factor. There is no sulfate assimilation and sodium sulfide, thiosulfate, thioglycollate, cysteine and methionine are used as sulfur sources by the strain. There is no salt requirement for strain JA143$^T$, but it can tolerate up

**Table 1. Differentiating characteristics of strain JA143$^T$ and species of the genus *Rhodospirillum***

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>JA143$^T$</th>
<th>Rsp. rubrum</th>
<th>Rsp. photometricum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell width (μm)</td>
<td>1.0–1.3</td>
<td>0.8–1.0</td>
<td>1.1–1.5</td>
</tr>
<tr>
<td>Length and width of a complete spiral turn (μm)</td>
<td>8–9 × 2.5–3.0</td>
<td>7–10 × 1.5–2.5</td>
<td>4–7 × 2.5–4.0</td>
</tr>
<tr>
<td>Colour</td>
<td>Pink</td>
<td>Pink to deep red</td>
<td>Brown–orange to brown–red/dark brown</td>
</tr>
<tr>
<td>Main carotenoid(s)</td>
<td>Rhodovibrin</td>
<td>Spirilloxanthin</td>
<td>Rhodopin and lycopene</td>
</tr>
<tr>
<td>Internal membrane system</td>
<td>Stacks</td>
<td>Vesicles</td>
<td>Stacks</td>
</tr>
<tr>
<td>Assimilatory sulfate reduction</td>
<td>–</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DNA G+C content (mol%)</td>
<td>64.7</td>
<td>63.8–65.8</td>
<td>64.8–65.8</td>
</tr>
<tr>
<td>Growth factors</td>
<td>Thiamine</td>
<td>Biotin</td>
<td>Nicotinamide</td>
</tr>
<tr>
<td>Dark aerobic growth</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Photolithoautotrophic growth</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Use as carbon source/electron donor of:
- Arginine
- Aspartate
- Caproate
- Caprylate
- Ethanol
- Fructose
- Glucose
- Glutamate
- Glycerol
- Glycolate
- Lactate
- Mannitol
- Methanol
- Pelargonic acid
- Propionate
Cells are spiral shaped, 1.0–1.3 μm wide; one single turn is 8–9 μm long. Cells also form long chains of more than 30 μm long, multiply by binary fission and are motile by means of bipolar tufts of flagella. Internal photosynthetic membranes are lamellar stacks. The colour of phototrophic culture is pink. The in vivo absorption spectrum of intact cells in sucrose exhibits maxima at 374, 473, 500, 530, 590, 791 and 848 nm, indicating the presence of bacteriochlorophyll a and carotenoids of the spirilloxanthin series. Mesophilic (30 °C), with a pH optimum at 7.0 for growth. Photoorganoheterotrophy with a few organic compounds is the preferred mode of growth. Pyruvate, caproate, crotonate, malate, fumarate and lactate are good carbon sources. Growth also occurs on acetate, butyrate, succinate, glucose, fructose, glutamate and lactate. Photoautotrophic and chemotrophic growth is not possible. There is no assimilatory sulfate reduction. Requires thiosulfate, sulfide, thioglycollate, cysteine or methionine as sulfur sources. Thiamine is required as a growth factor. The DNA base composition of strain JA143T was 64.7 mol% G+C, with a pH optimum at 7.0 for growth.

The data obtained revealed that the new isolate clustered with the type strains of Rhodospirillum species, but was distinct from other genera of purple non-sulfur bacteria (Fig. 2). Apart from 16S rRNA gene sequence dissimilarity, strain JA143T also showed clear phenotypic differences from other Rhodospirillum species (Table 1) that justify the description of the strain JA143T as the type strain of a novel species, Rhodospirillum sulfurexigens sp. nov.

Description of Rhodospirillum sulfurexigens sp. nov.

Rhodospirillum sulfurexigens (sul.fu.re’xi.gen.s. L. n. sulfur -uris sulfur, L. v. exigo to demand; N.L. part. adj. sulfurexigens sulfur-demanding, referring to the requirement for a reduced sulfur source for growth).

Cells are spiral shaped, 1.0–1.3 μm wide; one single turn is 8–9 μm long. Cells also form long chains of more than 30 μm long, multiply by binary fission and are motile by means of bipolar tufts of flagella. Internal photosynthetic membranes are lamellar stacks. The colour of phototrophic culture is pink. The in vivo absorption spectrum of intact cells in sucrose exhibits maxima at 374, 473, 500, 530, 590, 791 and 848 nm, indicating the presence of bacteriochlorophyll a and carotenoids of the spirilloxanthin series. Mesophilic (30 °C), with a pH optimum at 7.0 for growth. Photoorganoheterotrophy with a few organic compounds is the preferred mode of growth. Pyruvate, caproate, crotonate, malate, fumarate and valerate are good carbon sources. Growth also occurs on acetate, butyrate, succinate, glucose, fructose, glutamate and lactate. Photoautotrophic and chemotrophic growth is not possible. There is no assimilatory sulfate reduction. Requires thiosulfate, sulfide, thiglycollate, cysteine or methionine as sulfur sources. Thiamine is required as a growth factor. The DNA base composition of the type strain is 64.7 mol% G+C (by HPLC).

The type strain, JA143T (=DSM 19785T =NBRC 104433T), was isolated from a water sample from the freshwater reservoir at Mudasarlova, Visakhapatnum, India.

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References


