Halochromatium roseum sp. nov., a non-motile phototrophic gammaproteobacterium with gas vesicles, and emended description of the genus *Halochromatium*

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A rod-shaped, marine, phototrophic, purple sulfur bacterium containing gas vesicles was isolated from a marine solar saltern at Kakinada, India. Cells of strain JA134^T are Gram-negative, non-motile rods, with vesicular intracytoplasmic membranes, and the strain has an absolute requirement for NaCl for growth. Bacteriochlorophyll *a* and the carotenoid okenone are present as photosynthetic pigments. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA134^T clusters with members of the genus *Halochromatium*, but is distinctly separated from the species reported so far. The morphological and physiological differences of strain JA134^T from other *Halochromatium* species support the description of strain JA134^T (=ATCC BAA-1363^T =DSM 18859^T =JCM 14151^T) as the type strain of a novel species, *Halochromatium roseum* sp. nov.

The genus *Halochromatium* was established to separate the halophilic species of the genus *Chromatium* from their freshwater counterparts (Imhoff *et al.*, 1998). At present, the genus *Halochromatium* contains two well-described species, *Halochromatium salexigens* (Imhoff *et al.*, 1998) (originally described as *Chromatium salexigens*; Caumette *et al.*, 1988) and *Halochromatium glycolicum* (Imhoff *et al.*, 1998) (originally described as *Chromatium glycolicum*; Caumette *et al.*, 1997). In the family *Chromatiaceae*, there are six genera that contain members that are reported to have gas vesicles, *Thiocapsa* (*Thiocapsa rosea* and *Tca. pendens*), *Thiodictyon* (*Thiodictyon elegans* and *Tdc. bacillosum*), *Thiolamprovum* (*Thiolamprovum pedioforme*), *Thiopedia* (*Thiopedia rosea*) and *Lamprocystis* (*Lamprocystis roseopersicina* and *Lpc. purpurea*), which were isolated from freshwater habitats, while *Lamprobacter* (*Lamprobacter modestohalophilus*) was isolated from H₂S-containing saline water bodies (Gorlenko & Imhoff, 2005). However, neither the type strain of *Lpb. modestohalophilus*, which represents the only species of this genus, nor its 16S rRNA gene sequence is currently available, and no neotype strain has been designated (Gorlenko & Imhoff, 2005). We

observed the presence of gas vesicles in one of our isolates from a saltpan, the 16S rRNA gene sequence of which clustered with those of members of the genus *Halochromatium*, but the phylogenetic distinctiveness and phenotypic variations observed with strain JA134^T indicate that it represents a novel species.

Strain JA134^T was isolated from photoautotrophic enrichments of a sample collected from a marine solar saltpan, which was in its initial stages of salt processing, at Kakinada, India (16° 54' N 82° 13' E). The sample was a light-pinkish-coloured mat with subsurface black sooty sediment. Purification and polyphasic taxonomic studies were carried out as described earlier (Arunasri *et al.*, 2005).

The genomic DNA was extracted and purified according to the method of Marmur (1961) and the G + C content was determined by HPLC (Mesbah *et al.*, 1989). Cell material for 16S rRNA gene sequencing was taken from 1–2 ml well-grown liquid culture. DNA was extracted and purified by using the Qiagen genomic DNA extraction kit. PCR amplification and 16S rRNA gene sequencing were performed as described previously (Imhoff *et al.*, 1998). Recombinant *Taq* polymerase was used for PCR, which was started with the primers 5'-GTTTGATCCTGGC-TCAG-3' and 5'-TACCTTGTTACGACTTCA-3' (respectively positions 11–27 and 1489–1506 according to the *Escherichia coli* 16S rRNA numbering of the International Union of Biochemistry). Sequences were obtained by cycle

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA134^T is AM283535.

A transmission electron micrograph of a cell of strain JA134^T and whole-cell and acetone-extract absorption spectra are available as supplementary material with the online version of this paper.

Fig. 1. Phase-contrast photomicrographs of cells of strain JA134^T showing gas vesicles and chain formation. Bar, 5 µm.

sequencing with the SequiTherm sequencing kit (Biozym) and the chain termination reaction (Sanger *et al.*, 1977) using an automated laser fluorescence sequencer (Pharmacia). Sequences were aligned using the CLUSTAL X program (Thompson *et al.*, 1997) and the alignment was corrected manually. The CLUSTAL X alignment file was used as the input file to the SEQBOOT program in the PHYLIP package (Felsenstein, 1993) 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 generated using 100 trees generated during maximum-likelihood analysis using the CONSENSE program. The final dendrogram with evolutionary distances was constructed by taking the alignment .phy file as the infile and the consensus tree as intree in the maximum-likelihood program of the PHYLIP package (Felsenstein, 1993).

Samples were collected on 11 January 2006. The sample that yielded strain JA134^T had a pH of 6.8, salinity of 2–3 ‰ and a temperature of about 30 °C. Individual cells of strain JA134^T were rod-shaped, 2.0–3.0 µm wide and 3.0–5.0 µm long, and non-motile, and they multiply by binary fission and occasionally form long chains (Fig. 1). Electron photomicrographs of ultrathin sections of the cells revealed a vesicular type of internal membrane structure and the presence of gas vesicles (see Supplementary Fig. S1 in IJSEM Online). Strain JA134^T was able to grow photolithoautotrophically [anaerobic, light from fluorescent lamp (30 µE m⁻² s⁻¹), Na₂S·9H₂O (1 mM), Na₂S₂O₃·5H₂O (5 mM) and NaHCO₃ (0.1 % w/v)] and photolithoheterotrophically [anaerobic, light from fluorescent lamp (30 µE m⁻² s⁻¹), Na₂S·9H₂O (1 mM), NaHCO₃ (0.1 % w/v) and pyruvate (0.3 % w/v)]. Photoorganotrophic and chemotrophic growth could not be demonstrated. In the presence of 1 mM Na₂S·9H₂O and 0.1 % (w/v) NaHCO₃, pyruvate, fumarate, succinate, malate, butanol, peptone, Casamino acids and glutamate were photoassimilated. Formate, acetate, propionate, butyrate, caproate, valerate, lactate, fructose, glucose, methanol, ethanol, propanol, glycerol, mannitol, glycolate, crotonate, ascorbic acid, 2-oxoglutarate and tartrate were not photoassimilated. Ammonium chloride and glutamine were utilized as nitrogen sources, while urea, nitrate, nitrite, glutamate and molecular nitrogen did not support growth. Sulfate assimilation was not observed. Na₂S·9H₂O and Na₂S₂O₃·5H₂O were used as sulfur sources, whereas sulfate, sulfite, sulfur, thioglycolate and cysteine did not support growth. NaCl was required for growth of strain JA134^T at 1–3 % (w/v); the optimum NaCl concentration was 1.5–2.5 % (w/v). Strain JA134^T grew at pH 7–8 with optimum growth at pH 7.5. The temperature optimum for growth was 27 °C (range 20–35 °C). The strain required

Fig. 2. Evolutionary-distance dendrogram depicting the phylogenetic relationships of strain JA134^T within the family Chromatiaceae as determined using 16S rRNA gene sequence analysis. Bar, 2 nucleotide substitutions per 100 nucleotides.

vitamin B₁₂ as a growth factor. The colour of the photosynthetically grown cell suspension was purple–pink. The whole-cell absorption spectrum of strain JA134^T gave absorption maxima at 371, 410, 515, 550, 590, 806, 836, 880 and 959 nm, confirming the presence of bacteriochlorophyll *a*, and the absorption spectrum for pigments extracted with acetone gave absorption maxima at 462, 487 and 516 nm, indicating the presence of carotenoids of the okenone group (Supplementary Fig. S2). The DNA base composition of strain JA134^T was 64 mol% G+C (by HPLC). The phylogenetic relationships of strain JA134^T to other purple sulfur bacteria were 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 *Halochromatium* and was distinct from other genera of purple sulfur bacteria. The highest sequence similarities of strain JA134^T were found with the type strains of *Hch. glycolicum* (95.6%) and *Hch. salexigens* (95.4%) (Fig. 2). Apart from the 16S rRNA gene sequence dissimilarity, strain JA134^T showed clear phenotypic

differences from other *Halochromatium* species (Table 1) that justify the description of a novel species, *Halochromatium roseum* sp. nov.

Description of *Halochromatium roseum* sp. nov.

Halochromatium roseum (ro'se.um. L. neut. adj. *roseum* rose-coloured, the colour of cell suspensions of the type strain).

Cells are rods, 2.0–3.0 µm wide and 3.0–5.0 µm long, and non-motile and cells divide by binary fission. Growth occurs under anaerobic conditions in the light (photolithoautotrophy/photoheterotrophy). Internal photosynthetic membranes are of the vesicular type. The colour of phototrophic cultures is purple–pink (rose). The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 371, 410, 515, 550, 590, 806, 836, 880 and 959 nm. Bacteriochlorophyll *a* and carotenoids of the okenone group are present. The type strain is mesophilic (optimum growth at 27 °C), with a pH optimum at 7.5,

Table 1. Differentiating characteristics of *Halochromatium* species and *Lpb. modestohalophilus*

Data for reference species were taken from Imhoff & Caumette (2005) (*Halochromatium* species) and Gorlenko & Imhoff (2005) (*Lpb. modestohalophilus*). +, Substrate utilized or present; –, substrate not utilized or absent; (+), weak growth; ND, not determined; NA, no data available. Sulfide and thiosulfate were utilized by all the strains. For all four taxa, organic substrate utilization was tested during photolithoautotrophic growth. Propionate, butyrate, glucose, fructose, crotonate and valerate were not utilized by any of the strains.

Characteristic	Strain JA134 ^T	<i>Hch. salexigens</i>	<i>Hch. glycolicum</i>	<i>Lpb. modestohalophilus</i>
Cell shape	Rod	Rod	Rod	Ovoid to rod
Motility	–	+	+	+
Cell size (µm)	2.0–3.0 × 3.0–5.0	2.0–2.5 × 4.0–7.5	0.8–1.0 × 2.0–4.0	2.0–2.5
Gas vesicles	+	–	–	+
Colour of cell suspension	Purple–pink	Pink, rose red	Pink, pinkish red	Purple–red
Carotenoid group	Okenone	Spirilloxanthin	Spirilloxanthin	Okenone
G+C content of DNA (mol%)	64	64.6	66.1–66.5	60–64
Vitamin B ₁₂ requirement	+	+	–	+
Chemolithotrophic growth	–	+	+	+
pH optimum (range)	7.5 (7–8)	7.4–7.6 (7.0–8.0)	7.2–7.4 (6.2–9.0)	7.4–7.6
Temperature optimum (°C)	27	20–30	25–35	23–27
NaCl optimum (%) (range)	1.5–2.5 (1–3)	8–11 (4–20)	4–6 (2–20)	1–4 (≤9)
Photoassimilation of:				
Formate	–	–	(+)	–
Acetate	–	+	(+)	+
Pyruvate	+	+	(+)	+
Lactate	–	–	–	+
Fumarate	+	–	+	–
Succinate	+	–	+	–
Malate	+	–	–	–
Ethanol	–	–	–	+
Propanol	–	–	NA	+
Glycerol	–	–	+	+
Glycolate	–	–	+	NA
Casamino acids	+	–	(+)	NA
Electron donors				
Hydrogen	ND	+	+	+
Sulfur	–	+	+	+

and requires NaCl (1.5–2.5 %) for optimal growth. Photolithoautotrophy is the preferred mode of growth. Organic compounds such as pyruvate, fumarate, succinate, malate, glutamate, butanol, Casamino acids and peptone are photoassimilated in the presence of 1 mM Na₂S · 9H₂O and 0.1 % (w/v) NaHCO₃. Chemotrophic growth can not be demonstrated. Vitamin B₁₂ is required as a growth factor. The DNA base composition of the type strain is 64 mol% G + C (HPLC).

The type strain, JA134^T (=ATCC BAA-1363^T =DSM 18859^T =JCM 14151^T), was isolated from a saltpan at Kakinada, India.

Emended description of the genus *Halochromatium* Imhoff et al. 1998

The description is as given previously (Imhoff et al., 1998; Imhoff & Caumette, 2005) with the following modifications. Most species are without gas vesicles and grow at elevated salt concentrations, but some species possess gas vesicles and can grow only at lower salt concentrations (1–3 % NaCl w/v).

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