Allochromatium phaeobacterium sp. nov.T. N. R. Srinivas,¹ P. Anil Kumar,¹ K. Sucharitha,¹ Ch. Sasikala¹
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A rod-shaped, phototrophic, purple sulfur bacterium was isolated in pure culture from brackish water near Bheemli, Visakhapatnam, India, in a medium that contained 2 % NaCl (w/v). Strain JA144^T was Gram-negative and motile. It did not require salt, but tolerated up to 3 % NaCl (w/v). Intracellular photosynthetic membranes were of the vesicular type. Bacteriochlorophyll *a* and carotenoids that probably belonged to the rhodopinal series were present as photosynthetic pigments. Strain JA144^T was able to grow photolithoautotrophically, photolithoheterotrophically and photoorganoheterotrophically. It did not require vitamins. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA144^T clustered with species of the genus *Allochromatium* belonging to the class *Gammaproteobacteria*. Highest sequence similarities of strain JA144^T were found with the type strains of *Allochromatium vinosum* (96.7 % similarity), *Allochromatium renukae* (96.3 %), *Allochromatium minutissimum* (95.6 %) and *Allochromatium warmingii* (89.2 %). Based on 16S rRNA gene sequence analysis and morphological and physiological characteristics, strain JA144^T is sufficiently different from other *Allochromatium* species to represent a novel species, *Allochromatium phaeobacterium* sp. nov.; the type strain is JA144^T (=JCM 14796^T=DSM 19781^T).

The genus *Allochromatium* was created to accommodate some species that had previously been classified as members of the genus *Chromatium*, based on their genetic distance from *Chromatium okenii* (Imhoff *et al.*, 1998). At present, the genus *Allochromatium* comprises four well-described species: *Allochromatium vinosum*, *Allochromatium minutissimum*, *Allochromatium warmingii* and *Allochromatium renukae*; the latter was characterized in our laboratory (Anil Kumar *et al.*, 2008). In the family *Chromatiaceae*, suspensions of cells of the following species, which belong to eight genera, are brownish in colour (Imhoff, 2005b): *A. vinosum*, *A. minutissimum* and *Marichromatium gracile* (brownish-red); *Thiococcus pfennigii* and *Thiospirillum jenense* (orange-brown); *Thioalkalicoccus limnaeus* and *Thioflavococcus mobilis* (yellowish-brown to orange-brown); *Rhabdochromatium marinum* (beige to orange-brown); and *Thiorhodococcus minor* (brown-orange). Here, a novel brown phototrophic gammaproteobacterium belonging to the genus *Allochromatium* is described.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JA144^T and JA316 are AM712619 and AM901431, respectively.

A supplementary figure showing whole-cell and pigment (acetone-extracted) absorption spectra of strain JA144^T is available with the online version of this paper.

Strain JA144^T was isolated from photolithoheterotrophic enrichments. The sample (anoxic sediment along with water) that yielded strain JA144^T was collected on 18 March 2007 from a pool of brackish water at Bheemli, Visakhapatnam, India (17° 53' N 83° 27' E). The sample had a pH of 6.8, a salinity of 10 ‰ and a temperature of 30 °C. The medium used for isolation of strain JA144^T was modified medium of Biebl & Pfennig (1981) containing (l⁻¹): KH₂PO₄, 0.5 g; MgSO₄·7H₂O, 2 g; CaCl₂·2H₂O, 0.15 g; NH₄Cl, 0.68 g; NaCl, 20 g; yeast extract, 0.5 g; sodium pyruvate, 3 g; micronutrient solution SL7, 1 ml; and ferric citrate solution (0.1 %, w/v), 5 ml. Purification and polyphasic taxonomic studies were carried out as described previously (Anil Kumar *et al.*, 2007, 2008).

Individual cells of strain JA144^T were rod-shaped (1.0–1.5 × 2.0–4.0 µm), multiplied by binary fission and were motile. Electron microphotographs of ultrathin sections of the cells revealed internal membrane structures of a vesicular type. Strain JA144^T was able to grow photolithoautotrophically [anaerobic conditions, light (2400 lx), Na₂S₂O₃·5H₂O (5 mM) and NaHCO₃ (0.1 %, w/v)], photolithoheterotrophically [anaerobic conditions, light (2400 lx), Na₂S₂O₃·5H₂O (5 mM), NaHCO₃ (0.1 %, w/v) and sodium pyruvate (0.3 %, w/v)] and photoorganoheterotrophically [anaerobic conditions, light (2400 lx), sodium pyruvate (0.3 %, w/v)]. Chemolithoautotrophy

[aerobic conditions, dark, thiosulfate (5 mM) and NaHCO₃ (0.1 %, w/v)], chemoorganoheterotrophy [aerobic conditions, dark and sodium pyruvate (0.3 %, w/v)] and fermentative growth [anaerobic conditions, dark with fructose (0.3 %, w/v)] could not be demonstrated. The organic substrates utilized for photoorganoheterotrophy (Table 1) in the presence of bicarbonate (0.1 %, w/v) and 0.5 mM Na₂S·9H₂O included lactate, pyruvate, fumarate, malate and fructose. Organic substrates that could not be utilized by strain JA144^T included formate, acetate, propionate, butyrate, valerate, succinate, glucose, ethanol, propanol, glycerol, glycollate, crotonate and Casamino acids. Na₂S₂O₃·5H₂O was utilized as electron donor (growth was feeble) under photolithoautotrophic conditions by strain JA144^T, whereas Na₂S·9H₂O, sulfite, elemental sulfur and hydrogen did not support growth. Sodium sulfide, sulfate, sulfite and thiosulfate, and elemental sulfur were utilized as sulfur sources by

strain JA144^T, but thioglycollate and cysteine did not support growth. Strain JA144^T utilized ammonium chloride, dinitrogen and glutamine as nitrogen sources, but nitrate, nitrite, urea and glutamate did not support growth. Strain JA144^T did not require salt, but tolerated up to 3 % (w/v) NaCl. The pH range for growth of strain JA144^T was 7.0–8.0 (optimum pH 7.5). The temperature optimum was 30 °C (range 25–35 °C). Strain JA144^T did not require vitamins. The colour of a suspension of photosynthetically grown cells was brown. The whole-cell absorption spectrum of strain JA144^T gave absorption maxima at 374, 460, 488, 518, 593, 800 and 857 nm, thus confirming the presence of bacteriochlorophyll *a*; the absorption spectrum for pigments extracted with acetone gave absorption maxima at 448, 473 and 506 nm, probably indicating carotenoids of the rhodospinal series (see Supplementary Fig. S1, available in IJSEM Online).

Table 1. Differential characteristics of species of the genus *Allochrochromatium*

Taxa: 1, JA144^T; 2, *A. vinosum*; 3, *A. minutissimum*; 4, *A. warmingii*; 5, *A. renukae*. Data for taxa 2–4 are from Imhoff (2005a, b); data for *A. renukae* are from Anil Kumar *et al.* (2008). Organic substrate utilization was tested during photoorganoheterotrophic growth in the presence of 0.1 % (w/v) bicarbonate and 0.5 mM Na₂S·9H₂O. Pyruvate was utilized by all taxa. Ethanol, propanol and glycerol were not utilized by any of the taxa. +, Substrate utilized or present; –, substrate not utilized or absent; ±, variable; (+), weak growth; NR, not reported. None of the strains required salt and a few marine strains tolerated low concentrations of NaCl. All strains were motile.

Characteristic	1	2	3	4	5
Cell shape	Rod	Rod	Rod	Rod	Ovoid to rod
Cell size (µm)	1.0–1.5 × 2.0–4.0	2.0 × 2.5–6.0	1.0–1.2 × 2.0	3.5–4.0 × 5.0–11.0	2.0–2.5 × 3.0–5.0
Colour of cell suspensions	Brown	Brownish-red	Brownish-red	Purple-violet	Purple-violet
Carotenoid group	Rhodospinal series	Spirilloxanthin	Spirilloxanthin	Rhodospinal	Lycopene
DNA G + C content (mol%)	59.8 (HPLC)	61.3–66.3	63.7	55.1–60.2	62.3 (HPLC)
Vitamin B ₁₂ requirement	–	–	–	+	–
Sulfate assimilation	+	+	+	–	–
Chemolithotrophic growth	–	+	+	–	–
pH optimum (range)	7.5 (7.0–8.0)	7.0–7.3 (6.5–7.6)	7.0–7.3 (6.5–7.6)	7.0 (6.5–7.3)	7.2–8.0 (6.8–8.5)
Temperature optimum (°C)	30	25–35	25–35	25–30	28–32
Substrates used:					
Hydrogen	–	+	+	–	–
Sulfide	–	+	+	+	(+)
Thiosulfate	(+)	+	+	–	–
Sulfur	–	+	+	+	–
Sulfite	–	+	+	NR	–
Formate	–	+	+	–	–
Acetate	–	+	+	+	+
Propionate	–	+	+	NR	–
Butyrate	–	±	±	–	(+)
Lactate	+	NR	NR	–	–
Fumarate	+	+	+	–	+
Succinate	–	+	+	–	+
Malate	+	+	+	–	+
Glucose	–	–	–	–	+
Fructose	(+)	–	–	–	–
Glycollate	–	+	+	–	–
Crotonate	–	–	+	–	–
Valerate	–	–	+	–	–

Genomic DNA was extracted and purified according to the method of Marmur (1961) and the DNA G + C content (mol%) was determined by HPLC (Mesbah *et al.*, 1989). The DNA G + C content of strain JA144^T was 59.8 mol%. The phylogenetic relationship of strain JA144^T to other purple sulfur bacteria was examined by 16S rRNA gene sequence analysis. 16S rRNA gene sequences were aligned by using the program CLUSTAL_X (Thompson *et al.*, 1997) and the alignment was corrected manually by using BIOEDIT software (Hall, 1999). The evolutionary tree was constructed by using the maximum-likelihood method [DNAML in the PHYLIP package (transition/transversion ratio used was 2.0000)] and the resultant tree topologies were evaluated by bootstrap analysis based on 100 resamplings using the programs SEQBOOT and CONSENSE in the PHYLIP package (Felsenstein, 1989). The data obtained revealed that the novel isolate branched separately, but clustered with type strains of species of the genus *Allochromatium*

and was distinct from members of other genera of purple sulfur bacteria (family *Chromatiaceae*). Highest sequence similarities of strain JA144^T were found with the type strains of *A. vinosum* (96.7%), *A. renukae* (96.3%), *A. minutissimum* (95.6%) and *A. warmingii* (89.2%) (Fig. 1). An unusual signature sequence (TACCTGTCCT) was found between positions 825 and 837 (based on *Escherichia coli* numbering) in the 16S rRNA gene sequence of strain JA144^T compared with all other *Allochromatium* species. In addition to 16S rRNA gene sequence dissimilarity, strain JA144^T could be differentiated clearly from other *Allochromatium* species based on phenotypic properties (Table 1), thus justifying the description of this strain as a representative of a novel species, *Allochromatium phaeobacterium* sp. nov. An additional strain, JA316, with 99.8% 16S rRNA gene sequence similarity to strain JA144^T (Fig. 1) and possessing other traits of this strain, was isolated from Chilika salt lagoon (Orissa), at Rambha, India (19° 30' N 85° 5' E).

Fig. 1. Evolutionary-distance dendrogram depicting the phylogenetic relationships of strain JA144^T within the family *Chromatiaceae* as determined by 16S rRNA gene sequence analysis. The dendrogram was constructed by using the program DNAML in the PHYLIP package. Numbers at nodes are bootstrap values; only those over 50 are shown. Bar, 2 substitutions per 100 nt.

Description of *Allochromatium phaeobacterium* sp. nov.

Allochromatium phaeobacterium [phae.o.bac.te'ri.um. Gr. adj. *phaeos* dark, brown; L. neut. n. *bacterium* a stick, staff, rod; N.L. neut. n. (nominative in apposition) *phaeobacterium* the brown rod].

Cells are rod-shaped, $1.0\text{--}1.5 \times 2.0\text{--}4.0\ \mu\text{m}$, motile and divide by binary fission. Growth occurs under anaerobic conditions in the light (photolithoautotrophy, photolithoheterotrophy and photoorganoheterotrophy). Internal photosynthetic membranes are of the vesicular type. The colour of phototrophically grown cultures is brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 374, 460, 488, 518, 593, 800 and 857 nm, thus confirming the presence of bacteriochlorophyll *a* and carotenoids that are probably of the rhodospinal series. The type strain is mesophilic (30 °C), with a pH optimum for growth of 7.5. There is no obligatory NaCl requirement, but up to 3% (w/v) NaCl is tolerated. Photoorganoheterotrophy with a few organic compounds is the preferred mode of growth. Good carbon sources for growth are pyruvate, fumarate and malate; growth also occurs on lactate and fructose. Chemotrophy is not possible. Growth factors are not required.

The type strain is JA144^T (=JCM 14796^T=DSM 19781^T), isolated from a brackish water body at Bheemli, Visakhapatnam, India. The DNA G+C content of strain JA144^T is 59.8 mol% (HPLC).

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