Allochrochromatium renukae sp. nov.P. Anil Kumar,¹ T. N. R. Srinivas,¹ Ch. Sasikala¹ and Ch. V. Ramana²

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An ovoid to rod-shaped, phototrophic, purple sulfur bacterium, designated strain JA136^T, was isolated in pure culture from brackish water near Kakinada, India, in a medium that contained 2 % NaCl (w/v). Cells were Gram-negative and motile by means of a single polar flagellum. Strain JA136^T had no absolute salt requirement for growth but was able to tolerate up to 4 % NaCl (w/v). Intracellular photosynthetic membranes were of the vesicular type. Bacteriochlorophyll *a* and the carotenoid lycopene of the rhodospinal series were present as photosynthetic pigments. Strain JA136^T was able to grow photolithoautotrophically and photolithoheterotrophically. There was no vitamin requirement for growth of strain JA136^T. Phylogenetic analysis on the basis of 16S rRNA gene sequences indicated that strain JA136^T clustered with species of the genus *Allochrochromatium* in the class *Gammaproteobacteria*. Strain JA136^T showed highest 16S rRNA gene sequence similarity with the type strains of *Allochrochromatium vinosum* (97.0 %), *Allochrochromatium minutissimum* (95.8 %) and *Allochrochromatium warmingii* (90.0 %). Based on 16S rRNA gene sequence similarity data and morphological and physiological characteristics, strain JA136^T was sufficiently distinct from recognized *Allochrochromatium* species to be described as representing a novel species of the genus, for which the name *Allochrochromatium renukae* sp. nov. is proposed. The type strain is JA136^T (=JCM 14262^T =DSM 18713^T).

At the time of writing, the genus *Allochrochromatium* comprises three recognized species, namely *Allochrochromatium vinosum*, *Allochrochromatium minutissimum* and *Allochrochromatium warmingii*, reclassified from *Chromatium* by Imhoff *et al.* (1998). In the present communication, we describe a novel species of the genus *Allochrochromatium*, isolated from brackish water in India.

Strain JA136^T was isolated from photolithoheterotrophic enrichments of an anoxic sediment and water sample taken from a brackish water body at Chollangi, Kakinada, India. Purification and polyphasic taxonomic studies were carried out as described previously (Anil Kumar *et al.*, 2007a, b). *In vivo* absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Absorption spectra were also recorded from pigments extracted with acetone by eluting the cell suspension with acetone through a 10 × 200-mm column packed with aluminium oxide. The carotenoid present was detected by comparing with the absorption maxima of

standards from Britton *et al.* (2004). Sequences were aligned by using the program CLUSTAL_X (Thompson *et al.*, 1997) and the alignment was corrected manually. The CLUSTAL_X alignment file was used as the input file to the program SEQBOOT in the PHYLIP package (Felsenstein, 1993) and the output file of SEQBOOT was used as the input file for maximum-likelihood analysis with 100 data sets and five times jumbling. A single tree was produced from 100 trees generated during maximum-likelihood analysis by using the program CONSENSE. 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.

The sample (anoxic sediment along with water) yielding strain JA136^T was collected on 11 January 2006 from a brackish water body at Chollangi, Kakinada, India (16° 54' N 82° 14' E). The sample had a pH of 6.8, salinity of 1 ‰ and a temperature of 30 °C. Individual cells of strain JA136^T were ovoid to rod-shaped, 2.0–2.5 × 3.0–5.0 µm in size, multiplied by binary fission (Fig. 1) and were motile by means of a single polar flagellum. Electron microscopy of ultrathin sections of cells revealed vesicular-type internal membrane structures (see Supplementary Fig. S1 in IJSEM Online). Strain JA136^T was able to grow photolithoautotrophically [anaerobically, 2400 lx, with Na₂S·9H₂O

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA136^T is AM399029.

An electron micrograph of an ultrathin section of a cell of strain JA136^T and a figure showing the whole-cell absorption spectrum and acetone spectrum of pigments of strain JA136^T are available as supplementary material with the online version of this paper.

Fig. 1. Phase-contrast micrograph of cells of strain JA136^T. Cells are 2–2.5 μm long.

(1 mM) and NaHCO₃ (0.1 %, w/v)] and photolithoheterotrophically [anaerobically, 2400 lx, with Na₂S·9H₂O

(1 mM), NaHCO₃ (0.1 %, w/v) and sodium pyruvate (0.3 %, w/v) as an organic carbon source]. Photo-organoheterotrophy [anaerobically, 2400 lx, with sodium pyruvate (0.3 %, w/v)], chemolithoautotrophy [aerobically, in the dark, with thiosulfate (5 mM) and NaHCO₃ (0.1 %, w/v)], chemo-organoheterotrophy [aerobically, in the dark, with sodium pyruvate (0.3 %, w/v)] and fermentative growth [anaerobically, in the dark, with glucose (0.3 %, w/v)] could not be demonstrated. Organic substrates utilized for photo-organoheterotrophic growth (Table 1) in the presence of bicarbonate (0.1 %, w/v) and 0.5 mM Na₂S·9H₂O were acetate, butyrate, pyruvate, fumarate, succinate, malate, glucose and Casamino acids. Substrates that could not be utilized by strain JA136^T included formate, propionate, lactate, fructose, ethanol, propanol, glycerol, glycolic acid, valerate and crotonate. Na₂S·9H₂O was required for growth of strain JA136^T, with optimum growth at a concentration of 2 mM. Na₂S·9H₂O was utilized as an electron donor (growth was very weak) under

Table 1. Differential characteristics between strain JA136^T and recognized species of the genus *Allochrodatum*

Data for reference species were taken from Imhoff (2005). +, Substrate utilized or present; –, substrate not utilized or absent; ±, variable; (+), weak growth; NR, not reported. All organisms are motile and are able to utilize acetate and pyruvate, but unable to utilize fructose, ethanol, propanol or glycerol. Organic substrate utilization was tested during photo-organoheterotrophic growth in the presence of (0.1 %, w/v) bicarbonate and 0.5 mM Na₂S·9H₂O.

Characteristic	Strain JA136 ^T	<i>A. vinosum</i>	<i>A. minutissimum</i>	<i>A. warmingii</i>
Cell shape	Ovoid to rod	Rod	Rod	Rod
Cell size (μm)	2.0–2.5 × 3.0–5.0	2.0 × 2.5–6.0	1.0–1.2 × 2.0	3.5–4.0 × 5.0–11.0
Colour of cell suspension	Purple–violet	Brownish red	Brownish red	Purple–violet
Carotenoid group	Lycopene	Spirilloxanthin	Spirilloxanthin	Rhodopinal
DNA G + C content (mol%)	62.3 (HPLC)	61.3–66.3	63.7	55.1–60.2
B ₁₂ requirement	–	–	–	+
Sulfate assimilation	–	+	+	–
Chemolithotrophic growth	–	+	+	–
pH optimum (range)	7.2–8.0 (6.8–8.5)	7.0–7.3 (6.5–7.6)	7.0–7.3 (6.5–7.6)	7.0 (6.5–7.3)
Temperature optimum (°C)	28–32	25–35	25–35	25–30
Salt requirement	None*	None*	None	None
Substrates used				
Hydrogen	–	+	+	–
Sulfide	(+)	+	+	+
Thiosulfate	–	+	+	–
Sulfur	–	+	+	+
Sulfite	–	+	+	NR
Formate	–	+	+	–
Propionate	–	+	+	NR
Butyrate	(+)	±	±	–
Lactate	–	NR	NR	–
Fumarate	+	+	+	–
Succinate	+	+	+	–
Malate	+	+	+	–
Glucose	+	–	–	–
Glycolate	–	+	+	–
Crotonate	–	–	+	–
Valerate	–	–	+	–

*Marine strains may tolerate low concentrations of NaCl.

photolithoautotrophic conditions at a minimum concentration of 0.5 mM, while $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, sulfite, elemental sulfur and hydrogen did not support growth. $\text{Na}_2\text{S} \cdot 9\text{H}_2\text{O}$ was utilized as a sulfur source by strain JA136^T, while $\text{Na}_2\text{S}_2\text{O}_3 \cdot 5\text{H}_2\text{O}$, sulfate, sulfite, thioglycolate, cysteine, elemental sulfur and methionine did not support growth. Ammonium chloride and molecular nitrogen were utilized as nitrogen sources, while nitrate, nitrite, urea, glutamate and glutamine did not support growth. There was no absolute salt requirement for growth but the strain was able to tolerate up to 4% NaCl (w/v). Strain JA136^T grew over the pH range 6.8–8.5, with optimum growth at pH 7.2–8.0. The optimum temperature for growth was 30 °C (range 25–30 °C). There was no vitamin requirement for growth.

The photosynthetically grown cell suspension was purple–violet in colour. The whole-cell absorption spectrum of strain JA136^T gave absorption maxima at 374, 460, 494, 527, 590, 809 and 857 nm, indicating the presence of bacteriochlorophyll *a*, and the absorption spectrum for pigments extracted with acetone gave absorption maxima at 448, 475 and 505 nm, indicating the presence of the carotenoid lycopene of the rhodospinal series (Supplementary Fig. S2). The DNA base composition of strain JA136^T was 62.3 mol% G+C (by HPLC). The phylogenetic relationship of strain JA136^T to other purple sulfur bacteria was examined by 16S rRNA gene sequence analysis. The data obtained revealed that the new isolate branched separately but clustered with the type strains of

recognized species of the genus *Allochromatium* and was distinct from other genera of purple sulfur bacteria (*Chromatiaceae*). Strain JA136^T showed highest 16S rRNA gene sequence similarity to the type strains of *A. vinosum* (97.0%), *A. minutissimum* (95.8%) and *A. warmingii* (90.0%) (Fig. 2). In addition to 16S rRNA gene sequence dissimilarity, strain JA136^T showed clear phenotypic differences (cell shape, colour of cell suspension, carotenoid composition, sulfate assimilation, chemolithotrophic growth, carbon and electron donor utilization) from *A. vinosum*, *A. minutissimum* and *A. warmingii* (Table 1) that justify the description of this strain as representing a novel species of the genus *Allochromatium*. The name *Allochromatium renukae* sp. nov. is proposed for this organism.

Description of *Allochromatium renukae* sp. nov.

Allochromatium renukae [re'nu.kae. N.L. gen. n. *renukae* of Renuka, named in memory of Dr (Mrs) B. Renuka, an Indian microbiologist and our (Ch. V. R., Ch. S.) research supervisor, who initiated work on purple sulfur bacteria in India].

Cells are ovoid to rod-shaped, 2.0–2.5 × 3.0–5.0 µm in size, are motile by means of a single polar flagellum and divide by binary fission. Growth occurs under anaerobic conditions in the light (photolithoautotrophy and photolithoheterotrophy). Internal photosynthetic membranes are of the vesicular type. Phototrophic cultures are purple–violet in

Fig. 2. Evolutionary-distance dendrogram showing the phylogenetic relationship of strain JA136^T within the family *Chromatiaceae* based on 16S rRNA gene sequence analysis. Bar, 2 nucleotide substitutions per 100 nucleotides.

colour. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 374, 460, 494, 527, 590, 809 and 857 nm, indicating the presence of bacteriochlorophyll *a*, and the absorption spectrum for pigments extracted with acetone has absorption maxima at 448, 475 and 505 nm, indicating the presence of the carotenoid lycopene of the rhodospinal series. Growth is mesophilic (30 °C); optimum growth at pH 7.2–8.0. There is no absolute salt requirement for growth but it tolerates up to 4 % NaCl (w/v). Photolithoheterotrophy with a few organic compounds is the preferred mode of growth. Good growth is observed with acetate, pyruvate, fumarate, succinate, malate and glucose as carbon sources. Growth also occurs with butyrate and Casamino acids. Photo-organoheterotrophy and chemotrophy are not observed. No growth factors are required. The DNA base composition is 62.3 mol% G + C (by HPLC).

The type strain, JA136^T (=JCM 14262^T =DSM 18713^T), was isolated from a brackish water body at Chollangi, Kakinada, India.

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