Marichromatium indicum sp. nov., a novel purple sulfur gammaproteobacterium from mangrove soil of Goa, India

K. Arunasri,[1] C. Sasikala,[1] C. V. Ramana,[2] J. Süling[3] and J. F. Imhoff[3]

[1]Environmental Microbial Biotechnology Laboratory, Center for Environment, Institute of Science and Technology, J.N.T. University, Kukatpally, Hyderabad 500 072, India

[2]Department of Plant Sciences, School of Life Sciences, University of Hyderabad, PO Central University, Hyderabad 500 046, India

[3]Institut für Meereskunde, Abteilung Marine Mikrobiologie, Düsternbrooker Weg 20, 24105 Kiel, Germany

Correspondence
C. Sasikala
sasi449@yahoo.ie *or*
r449@sify.com

A reddish-brown bacterium was isolated from photoheterotrophic enrichments of mangrove soil from the western coast of India, in a medium that contained 10 % (w/v) NaCl. Phylogenetic analysis on the basis of 16S rRNA gene sequences showed that strain JA100^T clusters with species of the genus *Marichromatium* of the class '*Gammaproteobacteria*'. Cells of strain JA100^T are Gram-negative, motile rods with monopolar single flagella; they require NaCl, the optimum concentration being 1–4 %, and tolerate concentrations up to 13 %. The strain has vesicular internal membrane structures, bacteriochlorophyll *a* and, most probably, carotenoids of the spirilloxanthin series. No growth factors are required. A reduced sulfur source is required for growth, and, during growth on reduced sulfur sources as electron donors, sulfur is intermediately deposited as a single large granule within the cell. Strain JA100^T could not grow at the expense of other tricarboxylic acid cycle intermediates, except malate. On the basis of 16S rRNA gene sequence analysis and its morphological and physiological characteristics, strain JA100^T is sufficiently different from other *Marichromatium* species to justify its designation as a novel species, for which the name *Marichromatium indicum* sp. nov. is proposed. The type strain is JA100^T (=DSM 15907^T=ATCC BAA-741^T=JCM 12653^T).

Anoxygenic phototrophic bacteria comprise a number of physiologically and phylogenetically distinct groups, including purple sulfur, purple non-sulfur, green sulfur and green non-sulfur bacteria, heliobacteria and aerobic anoxygenic phototrophic bacteria. Although an ability to perform anoxygenic photosynthesis is shared by all these bacteria, according to 16S rRNA gene sequence data they belong to different major evolutionary lines (Madigan, 2001). On the basis of 16S rRNA gene sequence similarity, phototrophic purple bacteria and aerobic bacteria containing bacteriochlorophyll are members of the *Proteobacteria*, while green sulfur bacteria and green non-sulfur bacteria and heliobacteria represent separate major phylogenetic branches. Purple non-sulfur bacteria belong to the '*Alphaproteobacteria*' and '*Betaproteobacteria*', whereas purple sulfur bacteria are members of the '*Gammaproteobacteria*' (Imhoff, 2001a). Marine ecosystems are among the major sources of anoxygenic phototrophic bacteria (Imhoff, 2001b), and, in a reclassification of this group, salt requirements were found to be a phenotypic characteristic, in agreement with their phylogenetic relationship (Imhoff *et al.*, 1998). In this report, we describe a novel purple sulfur bacterium isolated from mangrove soils from Goa, India. On the basis of phenotypic characteristics and the results of a molecular analysis, the novel isolate is classified as a novel species of the genus *Marichromatium*, for which the name *Marichromatium indicum* is proposed.

Strain JA100^T was isolated from enrichments of mangrove soils from Goa, India. The medium of Biebl & Pfennig (1981), supplemented with sodium chloride (1 %, w/v), was used. Malate, pyruvate and succinate (each at 0·1 %, w/v) were used as the carbon source and ammonium chloride was used as the nitrogen source and the cultures were grown phototrophically under light (2400 lx) at 30±2 °C. Purification was achieved by means of repeated streaking on agar slants under an argon atmosphere. Purified cultures were grown in completely filled screw-cap test tubes

Published online ahead of print on 4 October 2004 as DOI 10.1099/ijs.0.02892-0.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA100^T is AJ543328.

(10 × 100 mm) for photoheterotrophic growth. Morphological properties (cell shape, cell division, cell size, flagella) were observed by light microscopy (with an Olympus BH-2 microscope). To study the ultrastructure of the flagella, cells were stained with 1 % phosphotungstic acid; ultrathin sections were viewed through a transmission electron microscope (H-7500; Hitachi) to examine intracytoplasmic structures such as the internal membrane system. *In vivo* absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer using sucrose solution (Trüper & Pfennig, 1981). Absorption spectra were also recorded from pigments extracted with acetone after elution of the cell suspension with acetone through a 10 × 200 mm column packed with aluminium oxide. The utilization of different carbon substrates and electron donors (0·3 %, w/v or v/v, unless otherwise mentioned) was tested in the medium of Biebl & Pfennig (1981) containing 5 mM $Na_2S.9H_2O$. Nitrogen source (0·12 %, w/v) utilization was tested by replacing ammonium chloride with different nitrogen sources. Diazotrophy of the culture was determined by growth under an N_2 atmosphere and was confirmed by repeated subculturing (four times). Growth was measured turbidometrically at 660 nm. Genomic DNA was extracted and purified according to the method of Marmur (1961) and the G + C content of the DNA (mol%) was determined by thermal denaturation (Marmur & Doty, 1962). Cell material for 16S rRNA gene sequencing was taken from 1–2 ml well-grown liquid cultures. DNA was extracted and purified by using the Qiagen genomic DNA buffer set. PCR amplification and 16S rRNA gene sequencing were done as described previously (Imhoff *et al.*, 1998; Imhoff & Pfennig, 2001). Sequences were aligned using the CLUSTAL W program (Thompson *et al.*, 1994) and the alignment was corrected manually. The distance matrix was calculated using the algorithm of Jukes & Cantor (1969) with the DNADIST program within the PHYLIP package (Felsenstein, 1989). The FITCH program in the PHYLIP package fitted a tree to the evolutionary distances.

The blackish, subsurface, moist soil sample was collected from mangroves near Goa, India, during May 2001. The area is occasionally flooded by sea water and is also washed by fresh water during the rainy season. During the summer, drying was observed in the area, which may increase the salt concentration of the soil.

Soil samples collected from mangroves were enriched at three different saline concentrations (10, 6 and 0·05 %, w/v) under photoheterotrophic conditions. Purplish-brown enrichments were observed in all the samples after 6 days. Enrichments from the 10 % saline yielded three different colonies in anaerobic agar slants. Under the microscope, cells from one of these, a brown, circular and elevated colony, showed a peculiar cell shape with a single refractile body at the centre of a bloated cell. This isolate, strain JA100^T, was used for further studies. The morphology and fine structure of strain JA100^T are given in the species description and are shown in Fig. 1 and Fig. 2. A rosette arrangement of cells is very common in both young and old cultures. Transmission electron microscopy of thin sections revealed that strain JA100^T possessed vesicular internal membrane structures extending throughout the cell (Fig. 3).

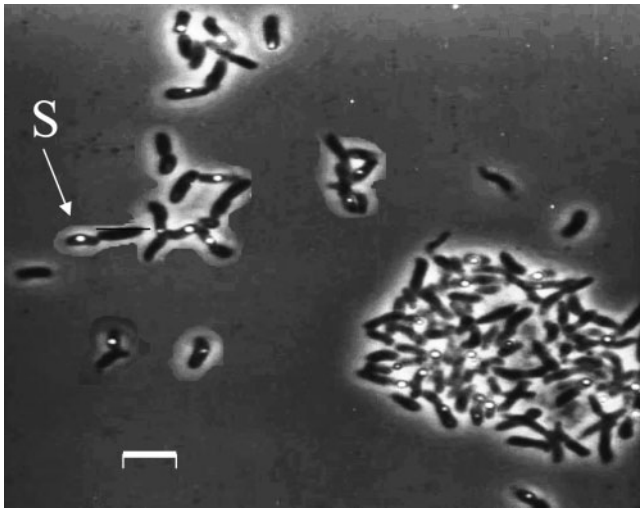

Fig. 1. Phase-contrast micrograph of strain JA100^T. Arrow indicates globule of elemental sulfur. Bar, 5 μm.

In vivo absorption maxima of intact cells (Fig. 4a) were recorded at 372, 462, 489, 522, 591, 798 and 852 nm,

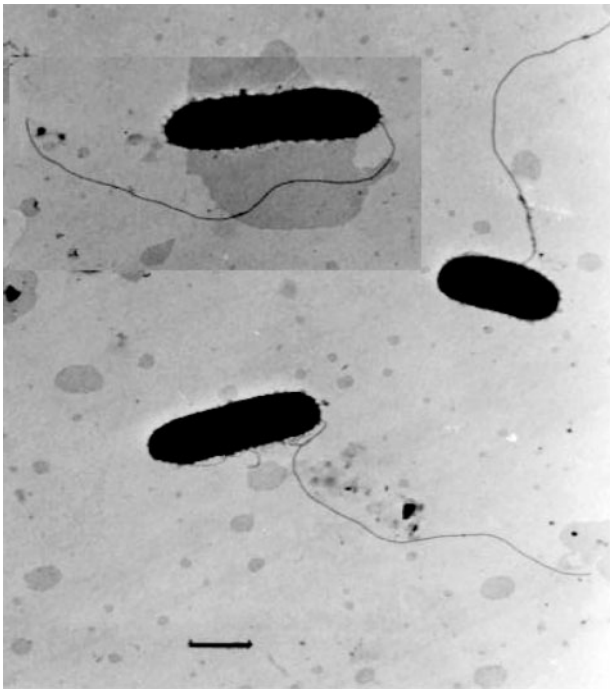

Fig. 2. Electron micrograph of negatively stained cells of strain JA100^T showing single monopolar monotrichous flagellum. Bar, 1 μm.

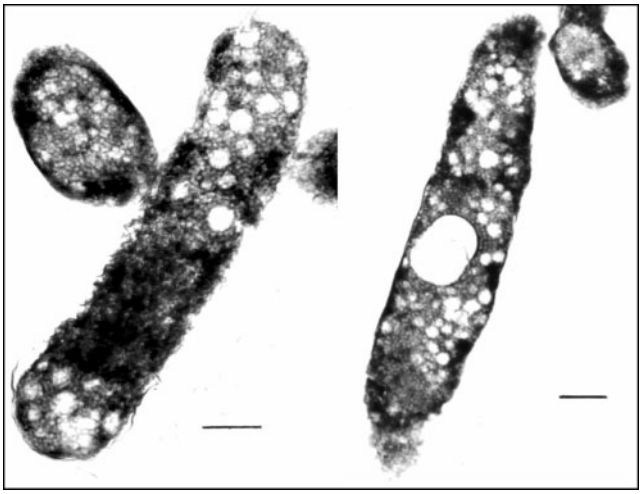

Fig. 3. Electron micrograph of ultrathin section of strain JA100^T showing vesicular nature of photosynthetic membranes extending throughout the cell. Bars, 0·5 μm.

indicating the presence of bacteriochlorophyll *a* and carotenoids. Orange/brown-coloured acetone extracts with absorption maxima at 447, 475 and 502 nm (Fig. 4b) may indicate the presence of carotenoids of the spirilloxanthin series.

Strain JA100^T was able to grow photolithoautotrophically [anaerobic conditions, in light (2400 lx), with $Na_2S.9H_2O$ (5 mM) and $NaHCO_3$ (0·1 %, w/v)], photolithoheterotrophically [anaerobic conditions, in light (2400 lx), with $Na_2S.9H_2O$ (5 mM) and pyruvate (0·3 %, w/v)], photo-organoheterotrophically [anaerobic conditions, in light (2400 lx), with pyruvate (0·3 %, w/v) and cysteine (1 mM)] and chemolithoheterotrophically [microaerobic

Table 1. Photolithohetero-/autotrophic growth of strain JA100^T on different reduced sulfur sources

Values shown are means of duplicate experiments performed after 48 h light (2400 lx) anaerobic incubation at 30±2 °C in the absence of yeast extract (unless otherwise mentioned). Concentrations used: pyruvate, 0·22 % (w/v); bicarbonate, 0·1 % (w/v); $Na_2S.9H_2O$, 5 mM; yeast extract, 1 % (w/v); cysteine, thiosulfate and thioglycollate, all 1 mM. Symbols: +, growth present; −, no growth.

Carbon source/electron donor + reduced sulfur source	Growth yield (OD_{660})	Growth
Pyruvate*	0·15	+
Pyruvate + $Na_2S.9H_2O$	0·32	+
Pyruvate + yeast extract	0·75	+
Pyruvate + cysteine	0·35	+
Pyruvate + thiosulfate	0·36	+
Pyruvate + thioglycollate	0·35	+
HCO_3^-	0·00	−
HCO_3^- + $Na_2S.9H_2O$	0·10	+
HCO_3^- + yeast extract	0·25	+
HCO_3^- + cysteine	0·00	−
HCO_3^- + thiosulfate	0·01	−
HCO_3^- + thioglycollate	0·00	−

*Photo-organoheterotrophic growth was not observed upon repeated subculturing.

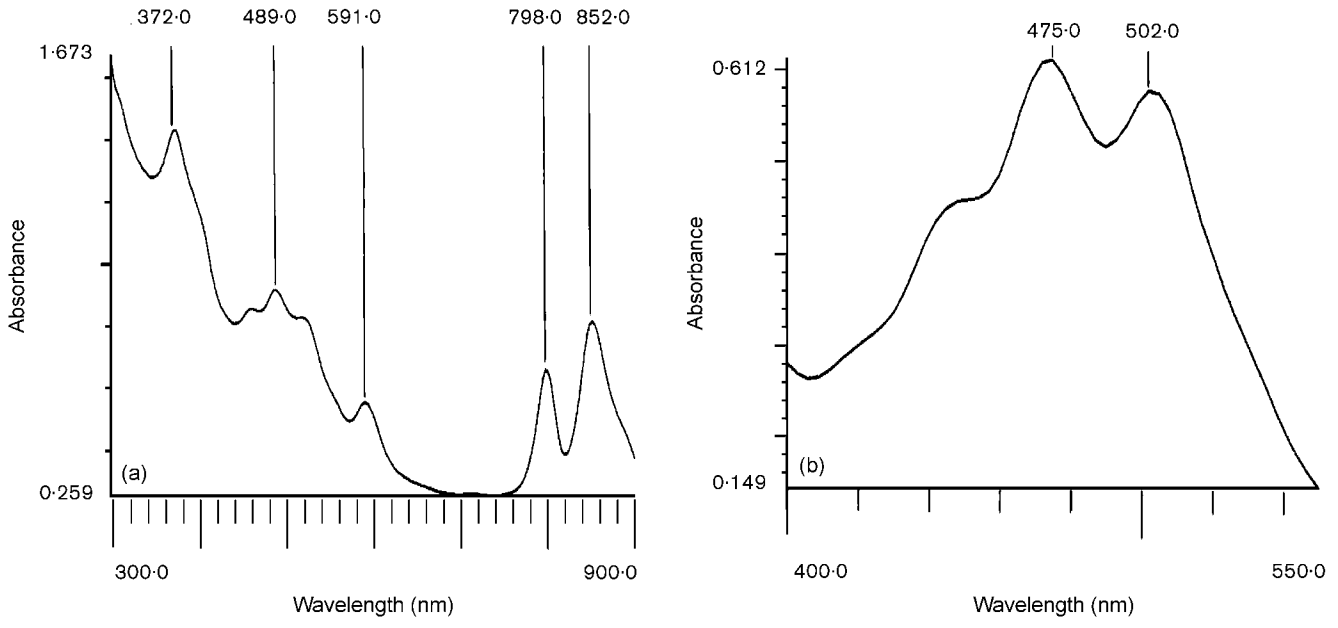

Fig. 4. Whole-cell absorption spectrum (a) of strain JA100^T and acetone spectrum (b) of extracted pigments.

conditions, in the dark, with thiosulfate (1 mM) and pyruvate (0·3 %, w/v)]. Chemolithoautotrophy could not be demonstrated. The preferred growth mode of strain JA100^T was photolithoheterotrophy and photo-organoheterotrophy (only in the presence of cysteine as sulfur source). Strain JA100^T could tolerate 8 mM $Na_2S.9H_2O$, with an optimal range of 3–6 mM. Sulfide could be replaced with other reduced sulfur compounds such as cysteine, thiosulfate and thioglycollate (Table 1). Although feeble growth could be observed in the absence of any reduced sulfur sources, such photo-organoheterotrophic growth was not observed upon repeated subculturing or from old cultures. Replacement of sulfide with cysteine resulted in good biomass yields, which indicates that reduced sulfur compounds may be required for sulfur assimilation in this strain. However, cysteine could not replace reduced inorganic sulfur compounds for photoautotrophic growth (Table 1). The organic substrates supporting growth of the strain are given in the species description. Strain JA100^T lacks the ability to liquefy gelatin and biotransform tryptophan to indole. Nitrogen sources utilized by JA100^T include ammonium chloride, urea and dinitrogen. Those which did not support growth were nitrate, nitrite and glutamate. Strain JA100^T has no growth factor requirement. The G+C content of the DNA of strain JA100^T was 67·1 mol% (T_m). The sequences of the 16S rRNA genes of strain JA100^T, *Marichromatium gracile* and *Marichromatium purpuratum* were determined and aligned. Comparative 16S rRNA gene sequence analysis revealed that strain JA100^T had the highest levels of similarity to *M. gracile* and *M. purpuratum*. These three bacteria formed a cluster separate from other purple sulfur bacteria (Fig. 5). This suggests that the novel strain JA100^T could be regarded as a representative of the genus *Marichromatium*.

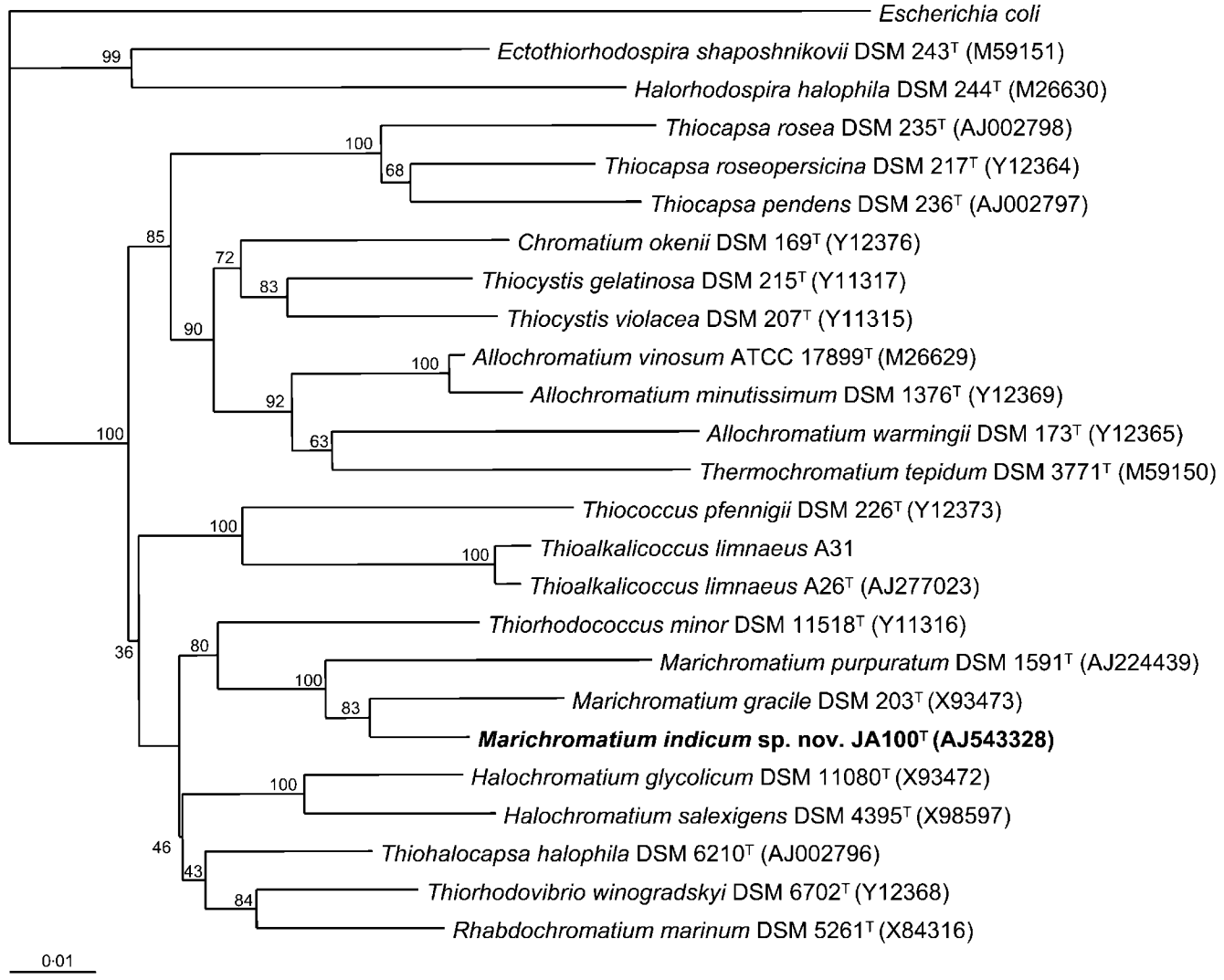

Fig. 5. Dendrogram depicting the phylogenetic relationships of strain JA100^T within the family *Chromatiaceae*, determined using 16S rRNA gene sequence analysis. Bar, 1 nucleotide substitution per 100 nucleotides.

Improving knowledge of 16S rRNA gene sequence analyses has enabled the phylogenetic relationships of the phototrophic purple sulfur bacteria to be evaluated, and these data were used to initiate a taxonomic reclassification (Imhoff & Süling, 1996; Imhoff *et al.*, 1998; Guyoneaud *et al.*, 1998). One of the proposed reclassifications was the formation of *Marichromatium* gen. nov. (Imhoff *et al.*, 1998) by reclassifying two species of marine purple sulfur bacteria of the genus *Chromatium*, *Chromatium gracile* and *Chromatium purpuratum*. The genus *Marichromatium* represents true marine purple sulfur bacteria belonging to the class '*Gammaproteobacteria*', and comprises two well-described species of this genus (the only species known to date), viz. *M. gracile* and *M. purpuratum* (Imhoff, 2001a). Although the 16S rRNA gene sequence of '*Rhodobacter marinus*' (Burgess *et al.*, 1994) also clusters with those of the genus *Marichromatium* (Imhoff *et al.*, 1998), this bacterial name was never validly published.

In an attempt to isolate marine purple non-sulfur bacteria from mangrove soils of the west coast of India, near Goa, strain JA100^T was isolated after enrichment in saline photoheterotrophic media. However, strain JA100^T failed to grow under photoheterotrophic conditions [in the absence of sulfide and in the presence of sodium ascorbate (0·05 %, w/v) as reducing agent] upon repeated subculturing in a saline (NaCl at 1 %, w/v) medium. The 16S rRNA gene sequence of strain JA100^T clustered with those of the '*Gammaproteobacteria*' and was found to be most similar to those of the genus *Marichromatium*, having sequence dissimilarity with *M. gracile* and *M. purpuratum* of 3 and 5 %, respectively. Apart from the sequence dissimilarity, strain JA100^T has phenotypic variations with respect to these two species (Table 2), which justify the description of this strain as a novel species.

Although the common habitats of purple sulfur bacteria are the top (anoxic) layers of stratified lakes and shallow aquatic sediments (Van Gemerden & Mas, 1995), these organisms are also known to occur in anaerobic and sulfide-containing parts of moist and muddy soils (Pfennig & Trüper, 1989), as observed in the case of strain JA100^T.

Table 2. Differentiating characteristics of species of the genus *Marichromatium*

Substrates that were utilized by all of the strains include acetate, propionate, butyrate, pyruvate, lactate and malate; those that could not be utilized include caproate, glutamate, glucose, mannitol, glycerol, ethanol, methanol and benzoate. Other similarities of the three strains include rod shape, cell division by binary fission, the presence of a single polar flagellum, vesicular internal membrane structures and the absence of a requirement for a vitamin source. Symbols: +, substrate utilized; −, substrate not utilized; NT, not tested.

Characteristic	*M. gracile**	*M. purpuratum**	Strain JA100^T†
Cell size			
Width (μm)	1·0–1·3	1·2–1·7	0·8–1·0
Length (μm)	2–6	3–4	2–7
Rosette formation	Absent	Absent	Present
NaCl range (%)	0·50–8·00	2·00–7·00	0·05–13·00
Optimum NaCl concentration (%)	2–3	5	1–4
Optimum growth temperature (°C)	25–30	30–35	30–35
Optimum pH	6·5–7·6	7·2–7·6	6·0–7·5
Growth modes	Photo-organoheterotrophy; photolithoautotrophy; chemo-organoheterotrophy; chemolithoautotrophy; chemolithoheterotrophy	Photo-organoheterotrophy; photolithoautotrophy; chemo-organoheterotrophy; chemolithoautotrophy; chemolithoheterotrophy	Photolithoheterotrophy; photolithoautotrophy; chemolithoheterotrophy; photo-organoheterotrophy (with cysteine)
Colour of cell suspension	Reddish-brown	Purple–red	Reddish-brown
Carotenoids	Spirilloxanthin series	Okenone series	Spirilloxanthin series
DNA G+C content (mol%) (T_m)	70·4	68·9	67·1
N sources utilized	Ammonium salts, N_2	Ammonium salts	NH_4Cl, urea, N_2
Organic substrates utilized:			
Valerate	−	+	−
Citrate	−	NT	−
Succinate	+	NT	−
Fumarate	+	+	−
Tartrate	NT	NT	−
Fructose	−	−	+

*Sources: Pfennig & Trüper (1989) and Caumette *et al.* (1997).

†Organic substrate utilization was tested during photolithoheterotrophic growth in the presence of $Na_2S.9H_2O$ (5 mM).

Strain JA100^T, like other *Marichromatium* species, has a vesicular type of internal membrane system; this is most common among the *Chromatiaceae*, with the exception of *Thiococcus pfennigii* (previously known as *Thiocapsa pfennigii*; Eimhjellen, 1970), *Thioalkalicoccus limnaeus* (Bryantseva *et al.*, 2000) and *Thioflavicoccus mobilis* (Imhoff & Pfennig, 2001), which have a tubular internal membrane system.

Unlike other purple sulfur bacteria, the cells of which usually contain multiple sulfur globules, cells of strain JA100^T were always found to contain only one large sulfur globule each. Like all the described species of *Marichromatium*, strain JA100^T also has a requirement for salinity. However, strain JA100^T has the lowest salt requirement (0·05 %), and the level of tolerance of NaCl (13 %) in this strain is much higher than that in the others. The observed growth of JA100^T in a wide range (0·05–13 %) of salinities reflects its adaptation to its natural habitat, which exhibits different salt concentrations during various seasons. The most obvious difference between strain JA100^T and other *Marichromatium* species is its inability to grow chemoautotrophically (Table 3) and its requirement for a reduced sulfur compound as a source of sulfur. Even though the ability to use organic compounds is restricted in purple sulfur bacteria, these organisms are known to metabolize tricarboxylic acid cycle intermediates as carbon sources (Imhoff, 1995). However, strain JA100^T lacks such an ability, with the exception of malate utilization (Table 3). Furthermore, JA100^T represents the only species of *Marichromatium* known to utilize urea as a nitrogen source, though urea-metabolizing representatives are known from other genera of purple sulfur bacteria, i.e. *Thiocapsa* (*Thiocapsa roseopersicina*), *Thiocystis* (*Thiocystis violacea*) and *Allochromatium* (a few strains of *Allochromatium vinosum*) (Bast, 1986). In view of the phenotypic and genetic differences between JA100^T and its closest relatives in the genus *Marichromatium*, we propose JA100^T as the type strain of a novel species, *Marichromatium indicum* sp. nov.

Table 3. Phototrophic growth of strain JA100^T on various carbon sources

Values shown are means of experiments performed in triplicate after 72 h of light (2400 lx) anaerobic incubation at 30 ± 2 °C. Organic substrates were tested in the presence of $Na_2S.9H_2O$ (5 mM). Organic substrates were utilized at a concentration of 0·3 % (w/v), except for fatty acids (propionate, butyrate, valerate, caproate), alcohols (ethanol, methanol) and glycerol, which were each used at a concentration of 0·1 % (v/v). Symbols: +, growth present; −, no growth; NA, not applicable.

Carbon source/electron donor	Growth yield (OD_{660})	Growth
Control (without carbon source)	0·04	NA
Control (HCO_3^-; 0·1 %, w/v)	0·03	NA
Acetate	0·29	+
Propionate*	0·08	+
Butyrate*	0·07	+
Valerate*	0·05	−
Caproate*	0·05	−
Lactate	0·13	+
Malate	0·10	+
Succinate	0·00	−
Fumarate	0·00	−
Tartrate	0·00	−
Citrate	0·00	−
Glutamate	0·00	−
Glucose	0·06	−
Fructose	0·09	+
Mannitol	0·04	−
Glycerol	0·04	−
Ethanol*	0·03	−
Methanol*	0·04	−
Thiosulfate*	0·00	−
Pyruvate	0·35	+
HCO_3^- (0·1 %, w/v)	0·13	+
HCO_3^- (0·1 %, w/v) + H_2 (20 %, v/v, gas phase) without 5 mM $Na_2S.9H_2O$	0·00	−
Benzoate (0·5 mM)*	0·04	−

*Supplemented with sodium bicarbonate (0·1 %, w/v).

Description of *Marichromatium indicum* sp. nov.

Marichromatium indicum (in′di.cum. L. neut. adj. *indicum* named after India, the country in which the type strain was isolated).

Cells are rod-shaped, measuring about 0·8–1·0 μm in width and 2–7 μm in length. Multiply by binary fission and are motile by means of polar flagella. Culture suspension is orange–brown in colour. Internal membranes are of the vesicular type, extending throughout the cell. Pigments include bacteriochlorophyll *a* and, most probably, carotenoids of the spirilloxanthin series. No requirement for growth factors. Reduced sulfur compounds are obligatory for sulfur assimilation. Growth modes are photolithoheterotrophy and chemolithoheterotrophy, photolithoautotrophy, and photo-organoheterotrophy with cysteine. Organic substrates metabolized include acetate, propionate, butyrate, pyruvate, lactate, malate and fructose; those which cannot be metabolized include valerate, caproate, citrate, fumarate, succinate, tartrate, glutamate, glucose, mannitol, glycerol, ethanol, methanol and benzoate. Ammonium chloride, dinitrogen and urea are the nitrogen sources metabolized. Optimum growth temperature is 30–35 °C, and temperature required for growth is in the range 25–39 °C; pH range is 6·0–7·5. Sodium chloride is required for growth, the optimum being between 1 and 4 % (w/v); 0·05–13 % (w/v) NaCl is tolerated. G+C content of the DNA is 67·1 mol% (T_m).

The habitat of the type strain, JA100^T (= DSM 15907^T = ATCC BAA-741^T = JCM 12653^T), is marine mangrove soils.

Acknowledgements

Financial assistance received from the Department of Science & Technology (FIST), the Department of Biotechnology and C.S.I.R, Government of India, is acknowledged. The skilful assistance of F. Lappe in preparing DNA, performing sequence analysis and constructing the phylogenetic trees is gratefully acknowledged. Y. Nagamalleshwari and K. Venkatesham of Ruska Labs, ANGR Agricultural University, Hyderabad, are acknowledged for their skilful help with the taking of electron micrographs.

References

Bast, E. (1986). Urease formation in purple sulfur bacteria (*Chromatiaceae*) grown on various nitrogen sources. *Arch Microbiol* **146**, 199–203.

Biebl, H. & Pfennig, N. (1981). Isolation of members of the family *Rhodospirillaceae*. In *The Prokaryotes*, pp. 167–273. Edited by M. P. Starr, H. Stolp, H. G. Truper, A. Balows & H. G. Schlegel. New York: Springer.

Bryantseva, I. A., Gorlenko, V. M., Kompantseva, E. I. & Imhoff, J. F. (2000). *Thioalkalicoccus limnaeus* gen. nov., sp. nov., a new alkaliphilic purple sulfur bacterium with bacteriochlorophyll *b*. *Int J Syst Evol Microbiol* **50**, 2157–2163.

Burgess, J. G., Kawaguchi, R., Yamada, A. & Matsunaga, T. (1994). *Rhodobacter marinus* sp. nov.: a new marine hydrogen producing photosynthetic bacterium which is sensitive to oxygen and sulphide. *Microbiology* **140**, 965–970.

Caumette, P., Imhoff, J. F., Suling, J. & Matheron, R. (1997). *Chromatium glycolicum* sp. nov., a moderately halophilic purple sulfur bacterium that uses glycolate as substrate. *Arch Microbiol* **167**, 11–18.

Eimhjellen, K. E. (1970). *Thiocapsa pfennigii* sp. nov. a new species of the phototrophic sulfur bacteria. *Arch Microbiol* **73**, 193–194.

Felsenstein, J. (1989). PHYLIP – phylogeny inference package (version 3.2). *Cladistics* **5**, 164–166.

Guyoneaud, R., Süling, J., Petri, R., Matheron, R., Caumette, P., Pfennig, N. & Imhoff, J. F. (1998). Taxonomic rearrangements of the genera *Thiocapsa* and *Amoebobacter* on the basis of 16S rDNA sequence analyses, and description of *Thiolamprovum* gen. nov. *Int J Syst Bacteriol* **48**, 957–964.

Imhoff, J. F. (1995). Taxonomy and physiology of phototrophic purple bacteria and green sulfur bacteria. In *Anoxygenic Photosynthetic Bacteria*, pp. 1–15. Edited by R. E. Blankenship, M. T. Madigan & C. E. Bauer. The Netherlands: Kluwer.

Imhoff, J. F. (2001a). The anoxygenic phototrophic purple bacteria. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 631–637. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Imhoff, J. F. (2001b). True marine and halophilic anoxygenic phototrophic bacteria. *Arch Microbiol* **176**, 243–254.

Imhoff, J. F. & Pfennig, N. (2001). *Thioflavicoccus mobilis* gen. nov., sp. nov., a novel purple sulfur bacterium with bacteriochlorophyll *b*. *Int J Syst Evol Microbiol* **51**, 105–110.

Imhoff, J. F. & Süling, J. (1996). The phylogenetic relationship among *Ectothiorhodospiraceae*: a reevaluation of their taxonomy on the basis of 16S rDNA analysis. *Arch Microbiol* **165**, 106–113.

Imhoff, J., Süling, J. & Petri, R. (1998). Phylogenetic relationships among the *Chromatiaceae*, their taxonomic reclassification and description of the new genera *Allochromatium*, *Halochromatium*, *Isochromatium*, *Marichromatium*, *Thiococcus*, *Thiohalocapsa* and *Thermochromatium*. *Int J Syst Bacteriol* **48**, 1129–1143.

Jukes, T. H. & Cantor, C. R. (1969). Evolution of protein molecules. In *Mammalian Protein Metabolism*, vol. 3, pp. 21–132. Edited by H. N. Munro. New York: Academic Press.

Madigan, M. T. (2001). Family VI. ''*Heliobacteriaceae*'' Beer-Romero and Gest 1987, 113. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 1, pp. 625–630. Edited by D. R. Boone, R. W. Castenholz & G. M. Garrity. New York: Springer.

Marmur, J. (1961). A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

Marmur, J. & Doty, P. (1962). Determination of the base composition of deoxyribonucleic acid from its thermal denaturation temperature. *J Mol Biol* **5**, 109–118.

Pfennig, N. & Trüper, H. G. (1989). I. Purple bacteria. In *Bergey's Manual of Systematic Bacteriology*, vol. 3, pp. 1637–1653. Edited by J. T. Staley, M. P. Bryant, N. Pfennig & J. G. Holt. Baltimore: Williams & Wilkins.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W : improving the sensitivity of progressive multiple sequence alignment though sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.

Trüper, H. G. & Pfennig, N. (1981). Characterization and identification of the anoxygenic phototrophic bacteria. In *The Prokaryotes*, pp. 299–312. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. New York: Springer.

Van Gemerden, H. & Mas, J. (1995). Ecology of phototrophic sulfur bacteria. In *Anoxygenic Photosynthetic Bacteria*, pp. 49–85. Edited by R. E. Blankenship, M. T. Madigan & C. E. Bauer. The Netherlands: Kluwer.