International Journal of Systematic and Evolutionary Microbiology (2008), **58**, 2660–2664 DOI 10.1099/ijs.0.65775-0

Thiophaeococcus mangrovi gen. nov., sp. nov., a photosynthetic, marine gammaproteobacterium isolated from the Bhitarkanika mangrove forest of India

P. Anil Kumar,[1] T. N. R. Srinivas,[1] Ch. Sasikala,[1] Ch. V. Ramana[2] and J. F. Imhoff[3]

Correspondence
Ch. V. Ramana
r449@sify.com
or
sasi449@yahoo.ie

[1]Bacterial Discovery Laboratory, Centre for Environment, Institute of Science and Technology, J. N. T. University, Kukatpally, Hyderabad 500 085, India

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

[3]Leibniz-Institut für Meereswissenschaften, IFM-GEOMAR, Marine Mikrobiologie, Düsternbrooker Weg 20, 24105 Kiel, Germany

A coccoid, phototrophic purple sulfur bacterium was isolated in pure culture from a mud sample collected from brackish water in the Bhitarkanika mangrove forest of Orissa, India, in a medium containing 2 % NaCl (w/v). This bacterium, strain JA304^T, was Gram-negative and had a requirement for NaCl. Intracellular photosynthetic membranes were of the vesicular type. The colour of the phototrophically grown culture was saddle-brown. Bacteriochlorophyll *a* and the carotenoid lycopene were present as photosynthetic pigments. Strain JA304^T was able to grow photolithoautotrophically and could photoassimilate a number of organic substrates. Yeast extract was required for growth of strain JA304^T. The DNA G+C content was 68.1–68.9 mol%. 16S rRNA gene sequence comparisons indicate that the isolate represents a member of the *Chromatiaceae* within the class *Gammaproteobacteria*. According to sequence comparison data, strain JA304^T is positioned distinctly outside the group formed by the four genera *Thiocystis*, *Chromatium*, *Allochromatium* and *Thermochromatium*, with only 86.7–91.0 % sequence similarity. Distinct morphological, physiological and genotypic differences from these previously described taxa support the classification of this isolate as a representative of a novel species in a new genus, for which the name *Thiophaeococcus mangrovi* gen. nov., sp. nov. is proposed. The type strain of *Thiophaeococcus mangrovi* is JA304^T (=JCM 14889^T =DSM 19863^T).

16S rRNA gene sequence comparisons have revealed genetic divergence between members of the *Chromatiaceae* that originate from freshwater sources and those of truly marine and halophilic nature (Imhoff *et al.*, 1998). The *Chromatium* clade includes representatives of the genera *Thiocystis*, *Chromatium*, *Allochromatium*, *Thermochromatium*, *Lamprocystis*, *Thiodictyon*, *Thiobaca*, *Thiocapsa* and *Thiolamprovum*, which are typically from fresh water, whereas the *Marichromatium* clade is represented by truly marine, halophilic species of the genera *Marichromatium*, *Thiorhodococcus*, *Halochromatium*, *Thiohalocapsa*, *Thiococcus*, *Thioalkalicoccus*, *Thioflavicoccus*, *Thiorhodovibrio*, *Rhabdochromatium* and *Isochromatium*. Two purple sulfur bacteria *Rhabdochromatium marinum* (Imhoff, 2005b) and *Thiospirillum jenense* (Imhoff, 2005c) have been reported to contain the carotenoid lycopene as a photosynthetic pigment and cell suspensions of these bacteria were reported to be beige to orange-brown and yellowish to orange–brown, respectively. Here, data are presented on a novel truly marine taxon that is associated with the *Chromatium* clade, has the carotenoid lycopene as photosynthetic pigment and is saddle-brown in colour.

Strain JA304^T was isolated from phototrophic enrichments of an anoxic sediment sample collected from brackish water in the Bhitarkanika mangrove forest of Orissa, India, by using media containing sulfide as reductant and photosynthetic electron donor and with CO_2 and pyruvate as organic carbon substrates. Mud samples were collected on 15 May 2007 at around midday. GPS positioning of the

Abbreviation: PABA, *para*-aminobenzoic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain JA304^T is AM748925.

A phase-contrast micrograph and whole-cell absorption spectrum and an acetone spectrum of extracted pigments of strain JA304^T are available as supplementary material with the online version of this paper.

sample collection site is 20° 49′ N 86° 47′ E. The sample that yielded strain JA304^T had a pH of 7.2, a salinity of 1–2 % NaCl (w/v) and a temperature of 28–30 °C. Purification and polyphasic taxonomic studies were carried out as described previously (Anil Kumar *et al.*, 2007). *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 the spectrum data with the absorption maxima of standards from the *Carotenoids Handbook* (Britton *et al.*, 2004). Biotin, niacin, *para*-aminobenzoic acid (PABA), pantothenate, pyridoxal phosphate, riboflavin, thiamine and vitamin B_{12} were tested independently or in combination to determine the vitamin requirement (the control lacked vitamins or yeast extract). 16S rRNA gene sequences were aligned using the program CLUSTAL_X (Thompson *et al.*, 1997) and the alignment was corrected manually. Phylogenetic analysis was carried out using the PHYLIP package, version 3.5 (Felsenstein, 1993). The evolutionary distance matrix was calculated using the distance model of Jukes & Cantor (1969). The evolutionary tree was constructed using the neighbour-joining method (Saitou & Nei, 1987) and the resultant tree topologies were evaluated by bootstrap analysis based on 100 resamplings using the SEQBOOT and CONSENSE programs in the PHYLIP package.

Individual cells of strain JA304^T were spherical (2.0–2.5 μm), multiplied by binary fission (Supplementary Fig. S1, available in IJSEM Online) and were motile with a single polar flagellum. Electron microphotographs of ultrathin sections of the cells revealed a vesicular type of internal membrane structure (Fig. 1). Strain JA304^T was able to grow photolithoautotrophically [anaerobic, light (2400 lx), $Na_2S.9H_2O/Na_2S_2O_3.5H_2O$ (1 mM/5 mM) and $NaHCO_3$ (0.1 %, w/v)] and under these conditions could photoassimilate pyruvate (0.3 %, w/v) as organic carbon source. Photoorganoheterotrophy [anaerobic, light (2400 lx), sodium pyruvate (0.3 %, w/v)], chemolithoautotrophy [aerobic, dark, thiosulfate (5 mM), $NaHCO_3$ (0.1 %, w/v)], chemoorganoheterotrophy [aerobic, dark, sodium pyruvate (0.3 %, w/v)] and fermentative growth [anaerobic, dark, glucose (0.3 %, w/v)] could not be demonstrated. The organic substrates photoassimilated by strain JA304^T included pyruvate, succinate, malate, glucose, fructose, glutamate, mannitol, sorbitol, glycerol and Casamino acids. Substrates that could not be photoassimilated by strain JA304^T included formate, acetate, propionate, butyrate, valerate, glycolate, crotonate, benzoate, lactate, fumarate, citrate, methanol, ethanol, propanol, peptone and yeast extract. $Na_2S.9H_2O$ and $Na_2S_2O_3.5H_2O$ were utilized as electron donors under photolithoautotrophic conditions, but growth could not be supported by sulfite, elemental sulfur or hydrogen. The strain grew with a minimum concentration of 0.5 mM $Na_2S.9H_2O$ and tolerated up to 4 mM $Na_2S.9H_2O$. $Na_2S.9H_2O$ and $Na_2S_2O_3.5H_2O$ were utilized as sulfur sources by strain JA304^T, whereas sulfate, sulfite, thioglycolate, cysteine and elemental sulfur did not support growth. Ammonium chloride, glutamine, glutamate (weak growth) and urea (weak growth) were utilized as nitrogen sources by strain JA304^T, but nitrate, nitrite and dinitrogen did not support growth. Salt was required for growth of strain JA304^T and growth occurred in 0.5–4.0 % NaCl (w/v) with an optimum at 1.0 % (w/v). Strain JA304^T grew at pH 6.5–7.5 (optimum pH 7.2) and 20–35 °C (optimum 30 °C). It required yeast extract as a growth factor. None of the vitamins tested (biotin, niacin, PABA, pantothenate, pyridoxal phosphate, riboflavin, thiamine and vitamin B_{12}) supported growth, either singly or in combination. The colour of the photosynthetically grown culture was saddle-brown. The whole cell absorption spectrum (Supplementary Fig. S2a) of strain JA304^T gave absorption maxima at 398, 457, 491, 524, 590, 800 and 857 nm, thus confirming the presence of bacteriochlorophyll *a*, and the absorption spectrum for pigments extracted with acetone (Supplementary Fig. S2b) gave absorption maxima at 448, 473 and 503 nm, indicating the presence of the carotenoid lycopene. The DNA G+C content of the newly isolated strain was 68.1–68.9 mol%. Characteristics that enable the new genus to be differentiated from the genera *Thiocystis*, *Thiorhodococcus*, *Thioflavicoccus*, *Lamprocystis*, *Thioalkalicoccus*, *Thiococcus*, *Thiohalocapsa*, *Thiocapsa*, *Thiolamprovum* and *Thiopedia* are shown in Table 1.

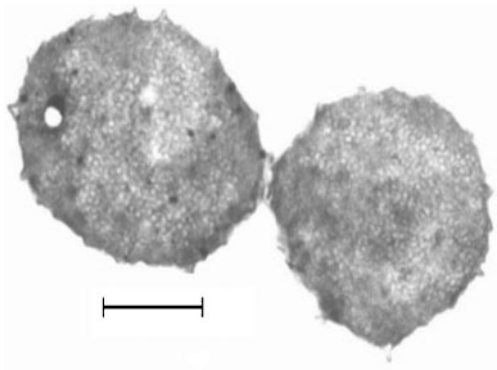

Fig. 1. Electron photomicrograph of an ultrathin section of a pair of cells of strain JA304^T showing the vesicular nature of photosynthetic membranes extending throughout the cell. Bar, 500 nm.

The phylogenetic relationship between strain JA304^T and other purple sulfur bacteria, as examined by 16S rRNA gene sequencing, revealed that the isolate belongs to the family *Chromatiaceae* of the class *Gammaproteobacteria* (Fig. 2). Highest sequence similarities were found between strain JA304^T and the type strains of *Thiocystis violacea* (91 %) and *Allochromatium vinosum* (90.5 %). The similarity to other species of the genera *Thiocystis*, *Chromatium*, *Allochromatium* and *Thermochromatium* was 86.7–89.6 %. The similarity to other species of genera of the

Table 1. Differentiation of *Thiophaeococcus* gen. nov. from other genera of the *Chromatiaceae* with coccoid cells

Genera: 1, *Thiophaeococcus* gen. nov. (strain JA304^T); 2, *Thiocystis*; 3, *Thiorhodococcus*; 4, *Thioflavicoccus*; 5, *Lamprocystis*; 6, *Thioalkalicoccus*; 7, *Thiococcus*; 8, *Thiohalocapsa*; 9, *Thiocapsa*; 10, *Thiolamprovum*; 11, *Thiopedia*. Data for reference genera were taken from Imhoff (2005a). Cells of all taxa studied divide by binary fission. Organic substrate utilization was tested in the presence of sulfide and hydrogen. Photoassimilation of organic substrates was tested in the presence of bicarbonate (0.1 %, w/v) and 0.5 mM $Na_2S \cdot 9H_2O$. Pyruvate was utilized by all taxa. +, Substrate utilized or present; −, substrate not utilized or absent; ±, variable; ND, no data available.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Cell shape	Sphere	Rod or ovoid to spherical	Spherical to ovoid	Sphere	Spherical to ovoid	Spherical to oval	Sphere	Sphere	Sphere	Spherical to oval	Spherical to ovoid
Motility	+	+	+	+	+	−	−	−	−	−	−
Cell size (μm)	2.0–2.5	2.5–3.0	1.0–2.0	0.8–1.0	2.0–3.5	1.3–1.8	1.2–1.5	1.5–2.5	1.2–3.0	2.0	2.0–2.5
Slime capsule	−	+	−	−	+	−	−	+	−	−	−
Gas vesicles	−	−	−	−	+	−	−	−	±	+	+
Aggregate formation	−	Irregular	Single, pairs, irregular	−	Branching	−	−	−	Tetrads	Platelets	Rectangular
Internal photosynthetic membranes	Vesicular	Vesicular	Vesicular	Tubular	Vesicular	Tubular	Tubular	Vesicular	Vesicular	Vesicular	Vesicular
Bacteriochlorophyll	*a*	*a*	*a*	*b*	*a*	*b*	*b*	*a*	*a*	*a*	*a*
Colour of cell suspension*	Sanb–Bakch	Puv	Bo	Yb, Ob	Pv	Yb, Ob	Ob	Pur	P, Rr	P, Rr	Pur
Carotenoid group†	LY	RA	SP	THS	RA	THS	THS	OK	SP	SP	OK
DNA G+C content (mol%)	68.5	61.3–67.9	66.8–67.0	66.5	63.4–64.1	63.6–64.8	69.4–69.9	65.9–66.6	63.3–66.3	64.5–66.5	62.5–63.5
Vitamin B_{12} requirement	−	−	−	−	ND	−	−	+	−	−	−
Sulfate assimilation	−	±	−	ND	ND	ND	−	−	+	−	−
Growth mode(s)‡	PLA	PLA, CLA, COH	PLA, CLA	PLA	PLA, CLA	PLA	PLA	PLA, POH, CLA, COH	PLA, POH, CLA, COH	PLA, CLA	PLA
pH optimum	7.5	6.5–7.6	7.0–7.2	6.5–7.5	7.0–7.3	8.8–9.2	6.5–7.5	6.5–7.5	7.3	7.4–7.6	7.3–7.5
Temperature range (°C)	20–35	25–35	30–35	20–30	20–30	20–25	20–35	20–30	20–35	37	20
Salt requirement	+	−	−	+	−	+	±	+	−§	−	−

*Bakch, Baker's chocolate; Bo, brown–orange; Ob, orange–brown; P, pink; Pur, purple–red; Puv, purple–violet; Pv, pinkish–violet; Rr, rose red; Sanb, sandy brown; Yb, yellowish brown.
†LY, Lycopene; OK, okenone; RA, rhodopinal; RN, rhodopin; THS, tetrahydrospirilloxanthin.
‡CLA, Chemolithoautotrophy; COH, chemoorganoheterotrophy; PLA, photolithoautotrophy; POH, photoorganoheterotrophy.
§Marine strains may tolerate low concentrations of NaCl.

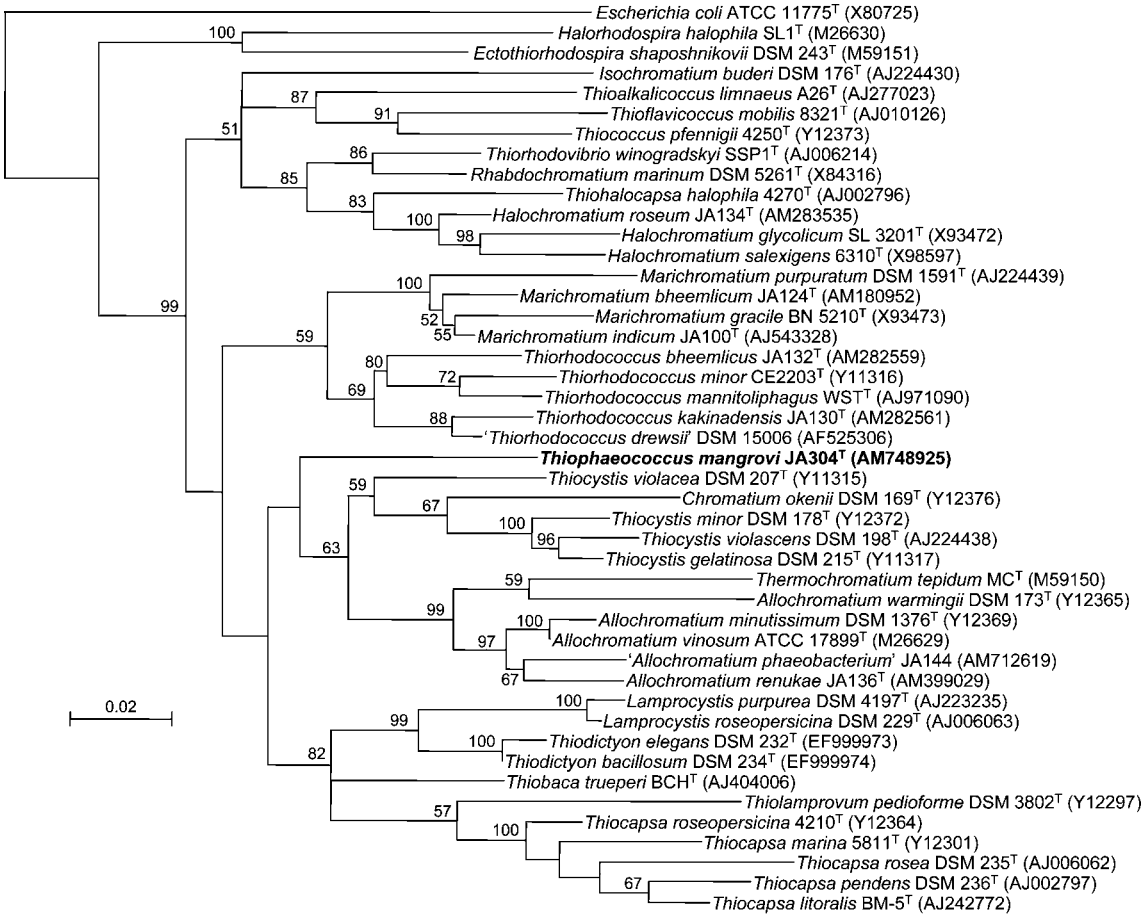

Fig. 2. Phylogenetic tree based on almost-complete 16S rRNA gene sequences showing the relationship between strain JA304^T and members of the family *Chromatiaceae*. The tree was constructed by the neighbour-joining method and rooted by using *Escherichia coli* ATCC 11775^T as the outgroup. Numbers at nodes represent bootstrap values (based on 100 resamplings). The GenBank accession numbers for 16S rRNA gene sequences are shown in parentheses. Bar, 2 substitutions per 100 nucleotide positions.

Marichromatium clade was 84.0–88.5 %. The phylogenetic distinctiveness of strain JA304^T from its closest relative, *Thiocystis violacea*, is supported by its requirement for salt for growth, the spherical shape of the cells, the brown colour of the cultures, its obligately anaerobic metabolism and the requirement for complex growth factors. Therefore, based on both phenotypic and genotypic evidence, it is proposed that strain JA304^T be classified in a new genus, *Thiophaeococcus* gen. nov., as a representative of the novel species *Thiophaeococcus mangrovi* gen. nov., sp. nov.

Description of *Thiophaeococcus* gen. nov.

Thiophaeococcus [Thi.o.phae′o.coc′cus. Gr. n. *thion* sulfur; Gr. adj. *phaeos* dark brown; N.L. masc. n. *coccus* coccus (from Gr. n. *kokkos* a grain, berry); N.L. masc. n. *Thiophaeococcus* brown sulfur(-reducing) coccus].

Cells are spherical, multiply by binary fission and are motile by means of a polar flagellum. Gram-negative. Internal membranes are of the vesicular type. Photosynthetic pigments are bacteriochlorophyll *a* and carotenoids. Metabolism is strictly anaerobic and obligately phototrophic. During growth on reduced sulfur sources as electron donors, elemental sulfur is intermediately deposited as a number of small granules within the cell. In the presence of sulfide and bicarbonate, organic substrates may be photoassimilated. Salt and growth factors are required for growth. The only known strain was isolated from an anoxic sediment that is rich in organic matter and hydrogen sulfide and exposed to light. The DNA G+C content is approximately 68–69 mol% (HPLC). The type species is *Thiophaeococcus mangrovi*.

Description of *Thiophaeococcus mangrovi* sp. nov.

Thiophaeococcus mangrovi (man.gro′vi. N.L. gen. n. *mangrovi* of a mangrove, referring to the isolation of the type strain from a mangrove forest).

Displays the following properties in addition to those given for the genus. Cells are 2.0–2.5 μm in diameter. The colour of phototrophic cultures is saddle-brown. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 398, 457, 491, 524, 590, 800 and 857 nm. Bacteriochlorophyll *a* and lycopene are the major photosynthetic pigments. Photolithoautotrophic growth occurs under anaerobic conditions in the light. In the presence of sulfide (0.5 mM) and bicarbonate (0.1 %, w/v), organic compounds are photoassimilated. Good carbon sources are pyruvate, glucose and fructose. Growth also occurs on succinate, malate, glutamate, mannitol, sorbitol, glycerol and Casamino acids. Photoorganoheterotrophy and chemotrophy do not occur. Yeast extract is required as a growth factor. Mesophilic (30 °C). Optimum growth at pH 7.5. Growth occurs in 0.5–4.0 % NaCl (w/v), with an optimum at 1.0 % (w/v).

The type strain is JA304^{T} (=JCM 14889^{T} =DSM 19863^{T}), isolated from mud from brackish water in the Bhitarkanika mangrove forest of Orissa, India.

Acknowledgements

We thank Dr J. Euzéby for his expert suggestions for the correct genus name, species epithet and Latin etymology. Financial assistance received from the Department of Biotechnology, Government of India is acknowledged. A. K. P. and T. N. R. S. acknowledge the CSIR, Government of India, for the award of SR fellowships.

References

Anil Kumar, P., Sasi Jyothsna, T. S., Srinivas, T. N. R., Sasikala, Ch., Ramana, Ch. V. & Imhoff, J. F. (2007). *Marichromatium bheemlicum* sp. nov., a non-diazotrophic, photosynthetic gammaproteobacterium from a marine aquaculture pond. *Int J Syst Evol Microbiol* **57**, 1261–1265.

Britton, G., Liaaen-Jensen, S. & Pfander, H. (2004). Carotenoids with a C_{40} skeleton. (a) Hydrocarbons. In *Carotenoids Handbook*, Main List, 31. Edited by G. Britton, S. Liaaen-Jensen & H. Pfander. Basel, Boston & Berlin: Birkhauser.

Felsenstein, J. (1993). PHYLIP (phylogeny inference package), version 3.5c. Distributed by the author. Department of Genome Sciences, University of Washington, Seattle, USA.

Imhoff, J. F. (2005a). Family I. *Chromatiaceae* Bavendamm 1924, 125^{AL} emend. Imhoff 1984b, 339. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 3–9. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Imhoff, J. F. (2005b). Genus X. *Rhabdochromatium* Dilling, Liesack and Pfennig 1996, 362^{VP} (Effective publication: Dilling, Liesack and Pfennig 1995, 130). In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 23–24. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Imhoff, J. F. (2005c). Genus XXIII. *Thiospirillum* Winogradsky 1888, 104^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 39–40. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

Imhoff, J. F., 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.

Saitou, N. & Nei, M. (1987). The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Trüper, H. G. & Pfennig, N. (1981). Isolation of members of the families *Chromatiaceae* and *Chlorobiaceae*. In *The Prokaryotes: a Handbook on Habitats, Isolation, and Identification of Bacteria*, pp. 279–289. Edited by M. P. Starr, H. Stolp, H. G. Trüper, A. Balows & H. G. Schlegel. Berlin: Springer.