

Author version: *Int. J. Systemat. Evolut. Microbiol.*, vol.65; 2015; 2357-2364

***Phaeobacterium nitrareducens* gen. nov., sp. nov., a phototrophic gammaproteobacterium isolated from a sediment sample of Coringa mangrove forest, India**

Nupur¹, Srinivas, T.N.R.², Takaichi, S.³ and Anil Kumar, P.^{1*}

¹Microbial Type Culture Collection and Gene Bank, CSIR-Institute of Microbial Technology, Sector 39A, Chandigarh – 160 036, INDIA

²CSIR-National Institute of Oceanography, Regional Centre, 176, Lawsons Bay Colony, Visakhapatnam-530017, INDIA

³Nippon Medical School, Department of Biology, Kosugi-cho, Nakahara, Kawasaki 211-0063, Japan

Address for correspondence* Dr. P. Anil Kumar

Microbial Type Culture Collection and Gene Bank, Institute of Microbial Technology (CSIR), Sector 39A, Chandigarh – 160 036, INDIA;

Email: apinnaka@imtech.res.in ; Telephone: 00-91-172-6665170

Subject category New taxa (*Gammaproteobacteria*)

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain AK40^T is HF968497.

All authors contributed equally for the present work.

The novel, brown coloured, Gram-negative-staining, rod shaped, motile phototrophic purple sulphur bacterium designated strain AK40^T, was isolated in pure culture from a sediment sample collected from Coringa mangrove forest. Strain AK40^T, containing bacteriochlorophyll *a* and carotenoid of rhodopin as major photosynthetic pigments. Strain AK40^T was able to grow photoheterotrophically and could utilize a number of organic substrates. It was unable to grow photoautotrophically and did not utilize sulfide and thiosulfate as electron donors. Thiamine and riboflavin were required for growth of strain AK40^T. The dominant fatty acids were C_{12:0}, C_{16:0}, C_{18:1 ω7c} and C_{16:1 ω7c} and/or iso-C_{15:0} 2OH (summed feature 3). Polar lipid content of strain AK40^T was found to be diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and eight unidentified lipids. Q-10 was the predominant respiratory quinone. The G + C content of DNA of the strain AK40^T was 65.5 mol%. The 16S rRNA gene sequence comparisons indicated that the isolate represented a member of the family *Chromatiaceae* within the class *Gammaproteobacteria*. The 16S rRNA gene sequence analysis indicated that strain AK40^T was closely related to *Phaeochromatium fluminis* with 95.2% pair-wise sequence similarity and with other species of the family, sequence similarity between 90.8 to 94.8%. Based on the sequence comparison data, strain AK40^T was positioned distinctly outside the group formed by the 6 genera *Phaeochromatium*, *Marichromatium*, *Halochromatium*, *Thiohalocapsa*, *Rhabdochromatium* and *Thiorhodovibrio*. Distinct morphological, physiological and genotypic differences from previously described taxa supported the classification of this isolate as a representative of a novel species in a new genus, for which the name *Phaeobacterium nitrareducens* gen. nov., sp. nov. is proposed. The type strain of *Phaeobacterium nitrareducens* is AK40^T (= JCM 19219^T = MTCC 11824^T).

Key words: *Phaeobacterium nitrareducens*, Coringa mangrove forest, 16S rRNA gene based phylogeny, chemotaxonomy, *Gammaproteobacteria*

Mangrove habitats are vital to humankind by virtue of their use and aesthetic value. Mangroves serve as nursery, feeding and spawning grounds for commercial fin and shell fishes. Mangrove habitats are found in locations where a river enters the sea and these dynamic ecosystems support numerous diversified soil microbes. Mangroves are found in different states of India such as: Andhra Pradesh, Andaman and Nicobar Islands, Goa, Gujarat, Karnataka, Kerala, Maharashtra, Tamil Nadu and West Bengal. The significance of microbes in aquatic ecosystems is recognized well by many pioneer studies (Longhurst and Harrison, 1989; Wheeler & Kirchman, 1986). Microbes play different roles such as: primary producers, secondary producers, consumers and decomposers. The activities of these microbes (photosynthetic, lithotrophic and organotrophic) are important for maintaining balance in the environment; which in turn, is very vital for the maintenance of other life forms. A number of novel bacterial taxa are identified from mangrove habitats. The family *Chromatiaceae* was proposed by Bavendamm (1924), with the type genus *Chromatium* and later emended by Imhoff (1984). Members of the *Chromatiaceae* are Gram negative, ovoid, rod, spherical, spiral, or vibrioid shaped, motile by flagella or non-motile, multiply by binary fission, may contain gas vesicles and consist of internal photosynthetic membranes which are either tubular or vesicular type. Cells in majority of the taxa contain photosynthetic pigments such as: bacteriochlorophyll *a* or *b* and carotenoids of the okenone, rhodopinal, or spirilloxanthin groups (Takaichi, 1999). Cultures appear in different colours like orange-brown to brownish red or pink, purple-red, and purple-violet depending on the type of carotenoids present. Cells grow under anoxic conditions in the presence of light (photolithoautotrophically) with sulfide or sulfur as electron donor and deposit sulfur granules within the cells as an intermediate oxidation product and may convert to sulfate; and can use hydrogen or reduced iron as an electron donor and also grow mixotrophically and photoassimilate a number of organic substrates. Some taxa are also capable to grow photoorganoheterotrophically. Most of the taxa are strictly anaerobic and obligately phototrophic but others grow chemolithoautotrophically or chemoorganoheterotrophically under microoxic to oxic conditions in the dark. Carbon fixation was by reductive pentose phosphate cycle in all species tested so far. Several taxa can fix N₂ and store elemental sulfur, poly-β-hydroxybutyrate, polysaccharides and polyphosphate. Some taxa may require different growth factors. *Chromatiaceae* members commonly dwell in the areas where anoxic conditions prevail with sulfide or sulfur sources with illumination, of diverse aquatic habitats and sediments like ditches, estuaries, lakes, marine habitats, moist and muddy soils, ponds, rivers, salt and soda lakes and sulfur springs. The G + C content of the DNA among the species varies between 45.5-70.4 mol% (Imhoff, 2005a). In the course of bacterial diversity studies of mangrove samples, a bacterial strain, AK40^T, was isolated from a mangrove sediment sample collected near Coringa, Andhra Pradesh, India. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain AK40^T was closely related to the genus *Phaeochromatium*

of the family *Chromatiaceae*. In this study, a polyphasic approach, including genotypic, phenotypic and chemotaxonomic characterizations, was used to determine the taxonomic position of strain AK40^T.

Strain AK40^T was isolated from the sediment sample collected from Coringa mangrove forest, India. The temperature and pH of the sediment sample was 30 °C and 7.5, respectively. Anoxygenic phototrophic bacterium AK40^T was isolated using photolithoheterotrophic enrichments of the sediment sample, and subsequent purification was performed as previously described (Anil Kumar *et al.*, 2008a, b) in modified Pfennig medium (Pfennig & Trüper, 1992) supplemented with NaCl (2 % w/v), pyruvate (0.3 % w/v), sodium thiosulfate (2 mM) and ammonium chloride (0.12 % w/v). Modified Pfennig medium was used throughout the study unless mentioned otherwise. Preservation was done at -80°C in modified Pfennig broth with 20% glycerol.

Colony morphology was studied after 72 hrs growth of the strain on modified Pfennig medium at 30 °C under 2000 lux illumination, anaerobically. The isolated pure colony was checked for cell morphology and motility by using phase contrast microscopy (Olympus, USA) at 1000X magnification and also by transmission electron microscope (Jeol JEM 2100) at operating voltage of 200 kV. Gram reaction was determined by using the Gram staining kit from HIMEDIA as described by manufacturer's protocol.

Physiological and biochemical characteristics were determined as previously described (Anil Kumar *et al.*, 2008a, b; Srinivas *et al.*, 2006). *In vivo* absorption spectrum was measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Absorption spectrum of pigments extracted with acetone was also recorded. Carotenoid composition was analysed by using C₁₈-HPLC (Takaichi & Shimada, 1992).

Strains AK40^T and *Phaeochromatium fluminis* JA418^T were grown on modified Pfennig medium with 2% NaCl at 30 °C under 2000 lux illumination, anaerobically for 3 days to prepare fatty acid methyl esters (classical methods) and were analysed using Sherlock Microbial Identification System (MIDI-6890 with database TSBA50) by the protocol described by the manufacturer. At the time of harvesting the cells are at logarithmic phase of growth. Freeze-dried cells following growth were analysed for polar lipids and quinones. Cells were extracted for the polar lipid analysis (Bligh & Dyer, 1959) and analyzed by 2D thin-layer chromatography followed by spraying with appropriate detection reagents (5% ethanolic molybdatophosphoric acid, molybdenum blue, ninhydrin and molisch reagents) (Komagata & Suzuki, 1987). Genomic DNA was isolated by using the procedure of Marmur (1961) and the DNA G + C content was determined from melting point (T_m) curves (Sly *et al.*, 1986) obtained by using Lambda 35; Perkin Elmer spectrophotometer equipped with Templab 2.0 software package.

For 16S rRNA gene sequencing, DNA was prepared using a bacterial DNA isolation kit (Qiagen). The 16S rRNA gene was amplified by PCR using universal bacterial primers 27f (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492r (5'-TAC GGY TAC CTT GTT ACG ACT T-3'). The PCR product was purified using QIA quick PCR purification kit (Qiagen) and sequenced using an ABI PRISM model 3700 automatic DNA sequencer and Big Dye Terminator cycle sequencing kit (Applied Biosystems). The 16S rRNA gene sequence of strain AK40^T was subjected to BLAST sequence similarity search (Altschul *et al.*, 1990) and EzTaxon-e server (Kim *et al.*, 2012) to identify the nearest taxa. Based on BLAST results all 16S rRNA gene sequences of type species of phototrophic members of the genera belonging to the family *Chromatiaceae* were downloaded from the NCBI database (<http://www.ncbi.nlm.nih.gov>) and aligned using the CLUSTAL_W program in MEGA5 (Tamura *et al.*, 2011). The evolutionary history was inferred by using the maximum-likelihood method (Tamura & Nei, 1993), neighbor-joining (NJ) method (Saitou & Nei, 1987) and maximum-parsimony method (Nei & Kumar, 2000) using the MEGA5 package (Tamura *et al.*, 2011). The percentage of trees in which the associated taxa clustered together is shown next to the branches. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There were a total of 1355 positions in the final dataset. Evolutionary analyses were conducted in MEGA5 (Tamura *et al.*, 2011). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) is shown next to the branches (Felsenstein, 1985). Fast DNAML tree was constructed using ARB software package (Ludwig *et al.*, 2004).

Cells of strain AK40^T were Gram-negative-staining, rod-shaped, brown pigmented, motile and multiplied by binary fission. Cells were 1.0–4.0 µm in long and 0.9–1.3 µm in wide (Fig. 1). Colonies were 0.5-1.5 mm in diameter, circular, smooth, brown, opaque, convex and raised with entire margin on modified Pfennig medium. Strain AK40^T grow photoorganoheterotrophically (optimum light intensity is 2000 lux). Photolithoautotrophic growth [anaerobic, light (2000 lux), Na₂S/Na₂S₂O₃ (0.5 mM), NaHCO₃ (0.1%, w/v)], chemolithoautotrophic growth [aerobic, dark, Na₂S₂O₃ (0.5 mM), NaHCO₃ (0.1%, w/v)], chemoorganoheterotrophic growth [aerobic, dark with glucose/pyruvate (0.3%, w/v)] and fermentative growth [anaerobic, dark with glucose/pyruvate (0.3%, w/v)] were not detected. Salt (NaCl) was not required for growth, but tolerated up to 7% (w/v, NaCl), the optimum NaCl concentration being 1.5–3% (w/v). The pH range for growth of strain AK40^T was 5–9 with the optimum at 7.5. The temperature range was from 22 to 37 °C and the optimum temperature for growth was 30 °C. Growth factors such as thiamine and riboflavin were required for growth. The colour of photosynthetically grown cell suspensions was brown. The whole-cell absorption spectrum (Supplementary Fig. S2a) of strain AK40^T in sucrose solution gave

maxima at 376, 488, 592, 702, 800, 836 nm, confirming the presence of bacteriochlorophyll *a* and carotenoids. The absorption spectrum of acetone extracted pigments (Supplementary Fig. S2b) gave maxima at 446, 476, 506 nm, indicating the presence of carotenoids. The carotenoid composition of strain AK40^T, as determined by using C₁₈-HPLC analysis, was lycopene (5%), rhodopin (71%), anhydrorhodovibrin (15%), rhodovibrin (3%) and spirilloxanthin (6%) (Supplementary Fig. S4). They were identified based on absorption spectra in the C₁₈-HPLC eluent of methanol, and the retention times on the C₁₈-HPLC (Takaichi & Shimada, 1992). Other physiological characteristics are given in the species description, Tables 1 and 2.

The cellular fatty acid composition of the strain AK40^T showed a profile of eleven fatty acids of C_{12:0} (5.6%), C_{14:0} (0.6%), C_{16:0} (22.5%), antesisio-C_{16:0} (1.1%), antesisio-C_{17:0} (1.0%), C_{18:0} (0.7%), C_{18:1} 2OH (1.9%), C_{18:1} *ω7c* (32.0%), C_{18:3} *ω6,9,12c* (2.8%), C_{12:0} aldehyde/iso-C_{16:1}I/C_{14:0}3OH (summed feature 2) (0.9%), and C_{16:1} *ω7c*/iso-C_{15:0} 2OH (summed feature 3) (30.9%). Unsaturated fatty acids constituted 65.7%. Major polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and eight unidentified lipids (L) (Supplementary Fig. S3). Q-10 was the predominant respiratory quinone. The G + C content of DNA of strain AK40^T was 65.5 mol% (*Tm*).

For phylogenetic assessment of strain AK40^T, 1464 bp of 16S rRNA gene sequence was amplified and sequenced. The sequence was compared with the database of type strains with validly published prokaryotic names [Available online <http://eztaxon-e.ezbiocloud.net/> (Kim *et al.*, 2012)], which revealed its closeness to *Phaeochromatium fluminis* of the class *Gammaproteobacteria*. Based on pair-wise sequence similarity, strain AK40^T was closely related to *Phaeochromatium fluminis* with 95.2% and to other members of the family *Chromatiaceae* with 90.8 to 94.8% sequence similarities. Phylogenetic tree based on maximum-likelihood method showed the clustering of strain AK40^T with *Phaeochromatium fluminis* and a clade comprising *Marichromatium gracile*, *Halochromatium salexigens*, *Thiohalocapsa halophila*, *Rhabdochromatium marinum* and *Thiorhodovibrio winogradskyi* (Fig. 2). In neighbour joining phylogenetic tree, strain AK40^T clustered with all six taxa together (Supplementary Fig. S1). In ARB fast DNAML tree, strain AK40^T clustered with all six taxa together (Supplementary Fig. S5).

Based on blast result strain AK40^T was compared with *Phaeochromatium fluminis* JA418^T, which is the nearest neighbor and a number of differences were observed (Table 1). For instance, strain AK40^T differed from the *Phaeochromatium fluminis* JA418^T with respect to cell size, sulfur granules per cell, colour of cell suspension, NaCl range, temperature growth range, pH growth range and optimum, growth modes, vitamin requirement, sulfide tolerance, carbon substrate utilization, nitrogen and sulfur sources, major fatty acids, polar lipids and DNA G + C content (mol%) (Table

1). Based on the phylogenetic analysis a comparison was made between the characteristics of strain AK40^T and all the genera of the family *Chromatiaceae*, *Allochromatium*, *Halochromatium*, *Isochromatium*, *Lamprocystis*, *Marichromatium*, *Nitrosococcus*, *Phaeochromatium*, *Rhabdochromatium*, *Rheinheimera*, *Thermochromatium*, *Thioalkalicoccus*, *Thiobaca*, *Thiocapsa*, *Thiococcus*, *Thiocystis*, *Thiodictyon*, *Thioflavicoccus*, *Thiohalocapsa*, *Thiolamproyum*, *Thiopedia*, *Thiophageococcus*, *Thiorhodococcus*, *Thiorhodovibrio* and *Thiospirillum* (Table 2) and a number of differences were observed. Thus, the cumulative differences that strain AK40^T exhibited with the above closely related type strains unambiguously supported the creation of a new *genus Phaeobacterium*, and a new type species of this genus, for which the name *Phaeobacterium nitratireducens* sp. nov., was proposed.

Description of *Phaeobacterium* gen. nov.

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

Cells are Gram negative-staining, rod shaped, oxidase positive, catalase negative, brown pigmented, motile and anaerobic. Cells grow photoorganoheterotrophically. The major fatty acids are C_{12:0}, C_{16:0} and iso-C_{15:0} 2OH/C_{16:1}ω7c (summed in feature 3). The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and eight unidentified lipids. Q-10 is the predominant respiratory quinone. The DNA G + C content is 65.5 mol%. The genus is a member of the family *Chromatiaceae* of the class *Gammaproteobacteria* in the phylum *Proteobacteria*. The type species is *Phaeobacterium nitratireducens*.

Description of *Phaeobacterium nitratireducens* sp. nov.

Phaeobacterium nitratireducens (ni.trati.re.du'cens. N.L. n. *nitras* -atis, nitrate; L. part. adj. *reducens*, leading back, bringing back and in chemistry converting to a different oxidation state; N.L. part. adj. *nitratireducens*, reducing nitrate).

The species exhibits the following properties in addition to those given in the genus description. Cells are 1.0–4.0 μm in long and 0.9–1.3 μm in wide, and they occur singly. Internal photosynthetic membranes are of vesicular type. Colonies grown for 3 days at 30 °C on modified Pfennig medium are circular, 0.5–1.5 mm in diameter, smooth, brown, opaque and raised with entire margin. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 376, 488, 592, 702, 800, 836 nm. The absorption spectrum in acetone extracted pigments gives absorption maxima at 446, 476, 506 nm. Major photosynthetic pigments are bacteriochlorophyll *a* and rhodopin. The type strain is mesophilic (range 22–37 °C, optimum 30 °C), can grow both in acidic and alkaline conditions (pH range 5.0–9.0, optimum 7.5) and does not require NaCl for growth but can tolerate

concentrations of NaCl up to 7% w/v with optimum growth between 1.5-3% NaCl. Growth occurs under anaerobic conditions in the light (photoorganoheterotrophy). Photoautotrophic and chemoautotrophic growth is not possible in the presence of sulfide and thiosulfate as electron donor and NaHCO₃ as carbon source. Chemoorganoheterotrophic growth is not possible in presence of pyruvate as carbon source under aerobic in dark conditions and fermentative growth is also not observed under anaerobic dark conditions in the presence of pyruvate. Photoorganoheterotrophy with various organic compounds is the preferred growth mode. Substrates that are utilized as carbon source/electron donors under photoheterotrophic condition include acetate, casamino acids, fumarate, glycolate, malate, propionate, pyruvate, succinate, valerate and yeast extract but not butyrate, citrate, crotonate, ethanol, fructose, glucose, glycerol, methanol, sucrose and trehalose. Ammonium chloride, glutamine and yeast extract are utilized as nitrogen source but not nitrate, nitrite and glutamate. Sulfate (2 mM), thiosulfate (2 mM) and sulfide (0.5 mM) are utilized as sulfur sources under photoheterotrophic condition but not sulfur. Ferrous iron cannot act as electron donor. Thiamine and riboflavin are required for growth.

The type strain AK40^T (= JCM 19219^T = MTCC 11824^T) was isolated from a sediment sample collected from Coringa mangrove forest, Andhra Pradesh, India.

Acknowledgements

We thank Council of Scientific and Industrial Research (CSIR), Directors of IMTECH, Chandigarh and NIO, Goa and Department of Biotechnology, Government of India for encouragement and financial assistance. We would like to thank Mr. Deepak for his help in sequencing 16S rRNA gene and Dr. Subash and Mr. Anil Theophilus for help in transmission electron microscopy. TNRS is thankful to project PSC0105 for providing facilities and funding respectively. IMTECH communication number is XXX/2013.

References

- Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. (1990).** Basic local alignment search tool. *J Mol Biol* **215**, 403–410.
- Anil Kumar, P., Aparna, P., Srinivas, T. N. R., Sasikala, Ch. & Ramana, Ch. V. (2008a).** *Rhodovulum kholense* sp. nov. *Int J Syst Evol Microbiol* **58**, 1723–1726.
- Anil Kumar, P., Srinivas, T. N. R., Sasikala, Ch., Ramana, Ch. V. & Imhoff, J. F. (2008b).** *Thiophaeococcus mangrovi* gen. nov., sp. nov., a photosynthetic, marine gammaproteobacterium isolated from the Bhitarkanika mangrove forest of India. *Int J Syst Evol Microbiol* **58**, 2660–2664.
- Bavendamm, W. (1924).** Die Farblosen und Roten Schwefelbakterien des Süß-und Salzwassers, Pflanzenforschung (edited by Kolkwitz), Berlin-Dahlem, pp. 1–156.

- Bligh, E. G. & Dyer, W. J. (1959).** A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* **37**, 911–917.
- Brettar, I., Christen, R. & Höfle, M. G. (2002).** *Rheinheimera baltica* gen. nov., sp. nov., a blue-coloured bacterium isolated from the central Baltic Sea. *Int J Syst Evol Microbiol* **52**, 1851–1857.
- Felsenstein, J. (1985).** Confidence limits on phylogenies: An approach using the *bootstrap*. *Evolution* **39**, 783–791.
- Guyoneaud, R., Matheron, R., Liesack, W., Imhoff, J. F. & Caumette, P. (1997).** *Thiorhodococcus minus*, gen. nov., sp. nov., A new purple sulfur bacterium isolated from coastal lagoon sediments. *Arch Microbiol* **168**, 16–23.
- Guyoneaud, R., Suling, 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. (1984).** Reassignment of the genus *Ectothiorhodospira* Pelsh 1936 to a new family, *Ectothiorhodospiraceae* fam. nov., and emended description of the *Chromatiaceae* Bavendamm 1924. *Int J Syst Bacteriol* **34**, 338–339.
- Imhoff, J. F. (2001).** Transfer of *Pfennigia purpurea* Tindall 1999 (*Amoebobacter purpureus* Eichler and Pfennig 1988) to the genus *Lamprocystis* as *Lamprocystis purpurea* comb. nov. *Int J Syst Evol Microbiol* **51**, 1699–1701.
- 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 II. *Allochromatium* Imhoff, Suling and Petri 1998b, 1140^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 12–14. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005c).** Genus IV. *Isochromatium* Imhoff, Suling and Petri 1998b, 1141^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 15–16. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005d).** Genus VII. *Marichromatium* Imhoff, Suling and Petri 1998b, 1140^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 20–21. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005e).** Genus X. *Rhabdochromatium* Dilling, Liesack and Pfennig 1996, 362^{VP}. 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. (2005f).** Genus XII. *Thioalkalicoccus* Bryantseva, Gorlenko and Imhoff 2000b, 2161^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 25–26. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005g).** Genus XIV. *Thiococcus* Imhoff, Suling and Petri 1998b, 1139^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 28–29. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005h).** Genus XV. *Thiocystis* Windogradsky 1888, 60^{AL} emend. Imhoff, Suling and Petri 1998b, 1138. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp.

29–31. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.

- Imhoff, J. F. (2005i).** Genus XVI. *Thiodictyon* Windogradsky 1888, 80^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 31–32. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005j).** Genus XX. *Thiopedia* Windogradsky 1888, 85^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 36–37. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. (2005k).** Genus XXIII. *Thiospirillum* Windogradsky 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. & Caumette P. (2005a).** Genus III. *Halochromatium* Imhoff, Süling and Petri 1998b, 1139^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 14–15. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. & Caumette P. (2005b).** Genus XIII. *Thiocapsa* Windogradsky 1888, 84^{AL} emend Guyoneaud, Süling, Petri, Matheron, Caumette, Pfennig and Imhoff 1998b, 962. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 26–28. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. & Caumette P. (2005c).** Genus XVIII. *Thiohalocapsa* Imhoff, Süling and Petri 1998b, 1139^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 34–35. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Imhoff, J. F. & Madigan, M. T. (2005).** Genus XI. *Thermochromatium* Imhoff, Süling and Petri 1998b, 1140^{VP}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 24–25. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- 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.
- Kim, O. S., Cho, Y. J., Lee, K. et al. (2012).** Introducing EzTaxon-e: a prokaryotic 16S rRNA Gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol Microbiol* **62**, 716–721.
- Komagata, K. & Suzuki, K. (1987).** Lipid and cell wall analysis in bacterial systematics. *Methods Microbiol* **19**, 161–206.
- Koops, H. P. & Roser, A. P. (2005).** Genus VIII. *Nitrosococcus* Windogradsky 1892, 127^{AL}. In *Bergey's Manual of Systematic Bacteriology*, 2nd edn, vol. 2, part B, pp. 21–22. Edited by D. J. Brenner, N. R. Krieg, J. T. Staley & G. M. Garrity. New York: Springer.
- Longhurst, A. R. & Harrison, W. G. (1989).** The biological pump: profiles of plankton production and consumption in the open ocean. *Prog Oceanogr* **22**, 47–123.
- Ludwig, W., Strunk, O., Westram, R., Richter, L., Meier, H., Yadhukumar., Buchner, A., Lai, T., Steppi, S. & other authors (2004)** ARB: a software environment for sequence data. *Nucl. Acids Res.* **32**, 1363–1371.
- Marmur, J. (1961).** A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* **3**, 208–218.

- Nei, M. & Kumar, S. (2000).** Molecular Evolution and Phylogenetics. Oxford University Press, New York 333 pp.
- Overmann, J., Fischer, U. & Pfennig, N. (1992).** A new purple sulfur bacterium from saline littoral sediments, *Thiorhodovibrio winogradskyi* gen. nov. and sp. nov. *Arch Microbiol* **157**, 329–335.
- Pfennig, N. & Trüper, H. G. (1992).** The family *Chromatiaceae*. In the Prokaryotes. A Handbook on the Biology of Bacteria. Ecophysiology, Isolation, Identification, Applications, 2nd edn, pp. 3200–3221. Edited by A. Balows, H. G. Trüper, M. Dworkin, W. Harder & K.-H. Schleifer. Berlin, Heidelberg & New York: Springer.
- Rees, G. N., Harfoot, C. G., Janssen, P. H., Schoenborn, L., Kuever, J. & Lünsdorf, H. (2002).** *Thiobaca trueperi* gen. nov., sp. nov., a phototrophic purple sulfur bacterium isolated from freshwater lake sediment. *Int J Syst Evol Microbiol* **52**, 671–678.
- Saitou, N. & Nei, M. (1987).** The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* **4**, 406–425.
- Shivali, K., Sasikala, Ch. & Ramana, Ch. V. (2012).** MLSA barcoding of *Marichromatium* spp. and reclassification of *Marichromatium fluminis* (Sucharita et al., 2010) as *Phaeochromatium fluminis* gen. nov. comb. nov. *Syst Appl Microbiol* **35**, 221–225.
- Sly, L. I., Blackall, L. L., Kraat, P. C., Tao, T.-S. & Sangkhobol, V. (1986).** The use of second derivative plots for the determination of mol% guanine plus cytosine of DNA by the thermal denaturation method. *J Microbiol Methods* **5**, 139–156.
- Srinivas, T. N. R., Anil Kumar, P., Sasikala, Ch., Ramana, Ch. V. Süling, J. & Imhoff, J. F. (2006).** *Rhodovulum marinum* sp. nov., a novel phototrophic purple non-sulfur alphaproteobacterium from marine tides of Visakhapatnam, India. *Int J Syst Evol Microbiol* **56**, 1651–1656.
- Sucharita, K., Shiva Kumar, E., Sasikala, Ch., Panda, B. B., Takaichi, S. & Ramana, Ch. V. (2010).** *Marichromatium fluminis* sp. nov., a slightly alkaliphilic, phototrophic gammaproteobacterium isolated from river sediment. *Int J Syst Evol Microbiol* **60**, 1103–1107.
- Tamura, K. & Nei, M. (1993).** Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol Bio Evol* **10**, 512–526.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011).** MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* **28**, 2731–2739.
- Takaichi, S. (1999).** Carotenoids and carotenogenesis in anoxygenic photosynthetic bacteria. In: Frank HA, Young AJ, Britton G and Cogdell RJ (eds) *The Photochemistry of Carotenoids (Advances in Photosynthesis and Respiration, Vol 8)*, pp 39–69. Kluwer Academic Publishers, Dordrecht.
- Takaichi, S. & Shimada, K. (1992).** Characterization of carotenoids in photosynthetic bacteria. *Methods Enzymol.* **213**, 374–385.
- 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.
- Wheeler, P. A. & Kirchman, D. L. (1986).** Utilization of inorganic and organic nitrogen by bacteria in marine systems. *Limnol. Oceanogr* **31**, 998–1009.

Figure Legends:

Fig. 1. Electron micrograph of negatively stained cells of strain AK40^T. Bar, 0.5 μm.

Fig. 2. Maximum-likelihood tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK40^T and the type species of the genera belongs to phototrophic members of the family *Chromatiaceae*. Numbers at the nodes are bootstrap values >50%. *Escherichia coli* ATCC 11775^T (X80725) was taken as the out group organism. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. S1. Whole-cell absorption spectrum (a) of strain AK40^T and absorption spectrum (b) of acetone extracted pigments.

Supplementary Fig. S2. The HPLC profile of AK40^T carotenoids. Peak 1-4, bacteriochlorophylls; peak 5, rhodovibrin; peak 6, rhodopin; peak 7, spirilloxanthin; peak 8, anhydrorhodovibrin; peak 9, lycopene; resolved by using a C-18 reverse-phase column.

Supplementary Fig. S3. The polar lipid profile of strain AK40^T. The polar lipids were identified as follows: diphosphatidylglycerol (DPG) phosphatidylethanolamine (PE), phosphatidylglycerol (PG), unidentified lipid (L).

Supplementary Fig. S4. Neighbor-joining tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK40^T and the type species of the genera belongs to phototrophic members of the family *Chromatiaceae*. Numbers at the nodes are bootstrap values >50%. *Escherichia coli* ATCC 11775^T (X80725) was taken as the out group organism. Bar, 0.02 substitutions per nucleotide position.

Supplementary Fig. S5. Fast DNAML ARB tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK40^T and the type species of the genera belongs to phototrophic members of the family *Chromatiaceae*. Numbers at the nodes are bootstrap values >50%. *Escherichia coli* ATCC 11775^T (X80725) was taken as the out group organism. Bar, 1.0 substitution per nucleotide position.

Table 1. Phenotypic features that distinguish strain AK40^T with the closely related type strain *Phaeochromatium fluminis* JA418^T

Data for strains AK40^T and *Phaeochromatium fluminis* JA418^T are from the present study. Both strains were rod-shaped, Gram-negative-staining and motile, optimally grew at 30 °C and 1.5-3 % NaCl (w/v), utilize acetate, fumarate, propionate, pyruvate, succinate and valerate as carbon sources; ammonium chloride and nitrate as nitrogen sources; sulfate, sulfide and thiosulfate as sulfur sources. Both strains cannot grow under aerobic condition, does not utilize ethanol, and citrate as carbon sources. +, Substrate utilized/present; (+), weak positive; -, substrate not utilized/absent; PLA, photolithoautotrophic; PLH, photolithoheterotrophic; POH, photoorganoheterotrophic; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; AL, unidentified aminolipid; L, unidentified lipid. Organic substrate utilization was tested during photoheterotrophic growth. For testing carbon sources, minimal media supplemented with respective carbon sources (5mM) were used. For Nitrogen sources, NH₄Cl was replaced by different nitrogen sources with final concentration 0.12% (w/v). For sulfur sources, MgSO₄.7H₂O was replaced by respective sulfur sources like sodium thiosulfate, sodium sulfide, sulfur of concentration (2mM) was added in media.

Characteristic	<i>Phaebacterium nitrireducens</i> AK40 ^T	<i>Phaeochromatium fluminis</i> JA418 ^T
Cell diameter (µm)	2-4 x 0.9-1.3	4-6 x 0.4-0.5
Sulfur granules per cell (n)	No	1-3
NaCl range (%)	0-7	0.5-6.5
pH range	5-9	6.5-10
pH optimum	7.5	8-8.5
Temperature range (°C)	22-37	25-30
Growth modes	PLH, POH	PLH, PLA, POH
Colour of cell suspension	Brown	Reddish-brown
Carotenoids	Spirilloxanthin group	Spirilloxanthin group
Vitamin requirement	Thiamine and riboflavin	None
Other growth factor requirement	None	Yeast extract
Sulfide tolerance (mM)	0.5	4
Carbon/electron donors		
Oxoglutarate	-	(+)
Glycerol	+	-
Crotonate	+	-
Glucose	(+)	-
Malate	(+)	-
Sucrose	-	(+)
Trehalose	+	-
Nitrogen sources		
Glutamate	-	+
Nitrate	+	-
Sulfur Sources		
Sulfur	-	+
Chemotaxonomy		
Major fatty acids	C _{12:0} , C _{16:0} , C _{18:1 ω7c} and C _{16:1 ω7c/iso-C_{15:0} 2OH}	C _{16:0} , C _{16:1 ω7c} / C _{16:1 ω6c} and C _{18:1 ω7c}
Polar lipids	DPG, PE, PG, L1-8	*DPG, PE, PG, AL1-2
DNA G + C content (mol%)	65.5	*71.4

*Data taken from Shivali *et al.*, 2012.

Table 2. Phenotypic features that distinguish strain AK40^T with the type species of the genera, *Chromatiaceae*

1. Strain AK40^T; 2. *Allochromatium* (Imhoff, 2005b); 3. *Halochromatium* (Imhoff & Caumette, 2005a); 4. *Isochromatium* (Imhoff, 2005c); 5. *Lamprocystis* (Imhoff, 2001); 6. *Marichromatium* (Imhoff, 2005d); 7. *Nitrosococcus* (Koops & Roser, 2005); 8. *Phaeochromatium* (Sucharita *et al.*, 2010 & Shivali *et al.*, 2012); 9. *Rhabdochromatium* (Imhoff, 2005e); 10. *Rheinheimera* (Brettar *et al.*, 2002); 11. *Thermochromatium* (Imhoff & Madigan, 2005); 12. *Thioalkalicoccus* (Imhoff, 2005f); 13. *Thiobaca* (Rees *et al.*, 2002); 14. *Thiocapsa* (Imhoff & Caumette, 2005b); 15. *Thiococcus* (Imhoff, 2005g); 16. *Thiocystis* (Imhoff, 2005h); 17. *Thiodictyon* (Imhoff, 2005i); 18. *Thioflavicoccus* (Imhoff & Pfennig, 2001); 19. *Thiohalocapsa* (Imhoff & Caumette, 2005c); 20. *Thiolamproovum* (Guyoneaud *et al.*, 1998); 21. *Thiopedia* (Imhoff, 2005j); 22. *Thiophaeococcus* (Anil Kumar *et al.*, 2008b); 23. *Thiorhodococcus* (Guyoneaud *et al.*, 1997); 24. *Thiorhodovibrio* (Overmann *et al.*, 1992); 25. *Thiospirillum* (Imhoff, 2005k).

Characteristics	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Cell shape	R	R	RO	R	SO	R	SE	R	R	RC	R	SO	R	S	S	R, SO	R	Co	S	SO	SO	S	SO	VS	RS
Cell size (µm)	2-4	1-4	2-4	3.5-4.5	2-3.5	1-1.7	1.5-2.5	0.4-0.5	1.5-1.7	0.5-1.5	1-2	1.3-1.8	1.6	1.2-3	1.2-1.5	2.5-3	1.5-2	0.8-1	1.5-2.5	2	2-2.5	2-2.5	1-2.0	1.2-1.6	2.5-4
Motility	+	+	+	+	+	+	+	+	+	+	+	-	+	-	-	+	-	+	-	-	-	+	+	+	+
Slime capsule	-	-	-	-	+	-	NA	NA	NA	NA	NA	-	NA	-	-	+	NA	-	+	-	-	-	-	NA	NA
Gas vesicles	-	-	-	-	+	-	NA	NA	NA	NA	-	-	-	±	-	-	+	-	-	+	+	-	-	NA	NA
Aggregate formation	-	-	-	-	B	Spc	Si pi	-	-	-	-	-	Si/Pi	tet	-	Irr	Irr	-	-	Plt	Rec	-	Si, Pi, irr	-	-
Internal photosynthetic Membranes	V	V	V	V	V	V	EI	V	V	-	V	T	V	V	T	V	V	T	V	V	V	V	V	V	V
Colour of cell suspension	Rb	Ob	Pr	Pv-Puv	Pv	Br Pur	NA	Db	BOB	Dbl-Lbl	NA	Ybr, Ob	Br	P, Rr	Ob	Puv	Puv	Ybg-Ob	Pur	P, Rr	Pur	S-B	Bo	P	Y-Ob
Bacteriochlorophyll	a	a	a	a	a	a	-	a	a	-	a	b	a	a	b	a	a	b	a	a	a	a	a	a	a
Carotenoid group	RN	RA	SP	RA	RA	SP Ok	NA	RN	LY	NA	SP	THS	LY	SP	THS	RA	RA	THS	Ok	SP	OK	LY	SP	SP	LY, RN
pH optimum	7.5	6.5-7.6	7.2-7.6	6.5-7.6	7.0-7.3	6.5-7.6	NA	6.5-7.5	7.0	NA	7.0	8.8-9.2	NA	7.3	6.5-7.5	6.5-7.6	6.7-7.3	6.5-7.5	6.5-7.5	7.4-7.6	7.3-7.5	7.5	7.0-7.2	7.0-7.4	7.0
Temperature range (°C)	22-37	25-35	20-35	25-30	20-30	25-37	NA	NA	20-35	4-30	48-50	25	25-30	20-35	20-35	25-35	20-30	20-30	20-30	37	20	20-35	30-35	14-37	20-25
Salt requirement	-	-	+	+	-	+	+	+	+	-	-	+	-	-§	±	-	-	+	+	-	-	+	-	+	-
Growth mode(s)	POH	PLA,	PLA, CLA, COH	PLA	PLA, CLA	PLA, CLA, COH	CLH	PLH	PLA	COH	PLA	PLA	PL H, PO H	PLA, POH, CLA, COH	PLA	PLA, CLA, COH	PLA	PLA	PLA, POH, CLA, COH	PLA, CLA	PLA	PLA	PLA CLA	PLA	PLA
Sulfate assimilation	-	+	+	+	NA	+	NA	NA	+	-	+	NA	-	+	-	±	NA	+	-	-	-	-	-	-	+
Vitamin B ₁₂ requirement	-	±	±	+	NA	-	NA	-	NA	NA	-	-	NA	-	-	-	NA	-	+	-	-	-	-	-	+
DNA G+C content (mol%)	65.5	55.1-66.3	64.6-66.3	62.2-62.8	63.4-64.1	68.9-70.4	50.5	68-69	60.4	47.8-48.9	60-63	63.6-64.8	63	63.3-66.3	69.4-69.9	61.3-67.9	65.3-66.3	66.5	65.9-66.6	64.5-66.5	62.5-63.5	68.5	66.8-67.0	61.0	45.5

Co, coccoid; R, rod; S, sphere; RO, rod or ovoid to spherical; SO, spherical to oval; RC, Rod to coccoid; SE, spherical to ellipsoidal; VS, vibroid to spirilloid; RS, rod to spiral; B, branching; Irr, Irregular; Plt, platelet; Rec, rectangular; Spc, Sin pairs clumps; Si, sin; pi, pairs; tet, tetrad; V, vesicular; T, Tubular; EI- extensive intracytoplasmic membrane systems arranged centrally in the protoplasm; Ob, orange brownish; Pv, pinkish-violet; Dbl, dark blue; Lbl, light blue; Ybr, yellowish brown; Puv, purple-violet; Ybg, yellow-beige; Pr, pinkish-red; Pur, purple-red; S-B, sandy brown-Baker's chocolate; Bo, brown-orange; Rb, reddish-brown; Db, dark brown; Br, brownish; BOB, beige to orange-brown; P, pinkish; Rr, rose-red; RA, rhodopinal; SP, spirilloxanthin; RN, rhodopin; RA, rhodopinal; THS, tetrahydrospirilloxanthin; Ok, okenone; LY, lycopenal; §, marine strains may tolerate low concentrations of NaCl; PLA, photolithoautotrophic; PLH, photolithoheterotrophic; POH, photoorganoheterotrophic; CLA, chemolithoautotrophic; CLH, chemolithoheterotrophic; COH, chemoorganoheterotrophic; +, present; -, absent; ±, variable; NA, no data available.

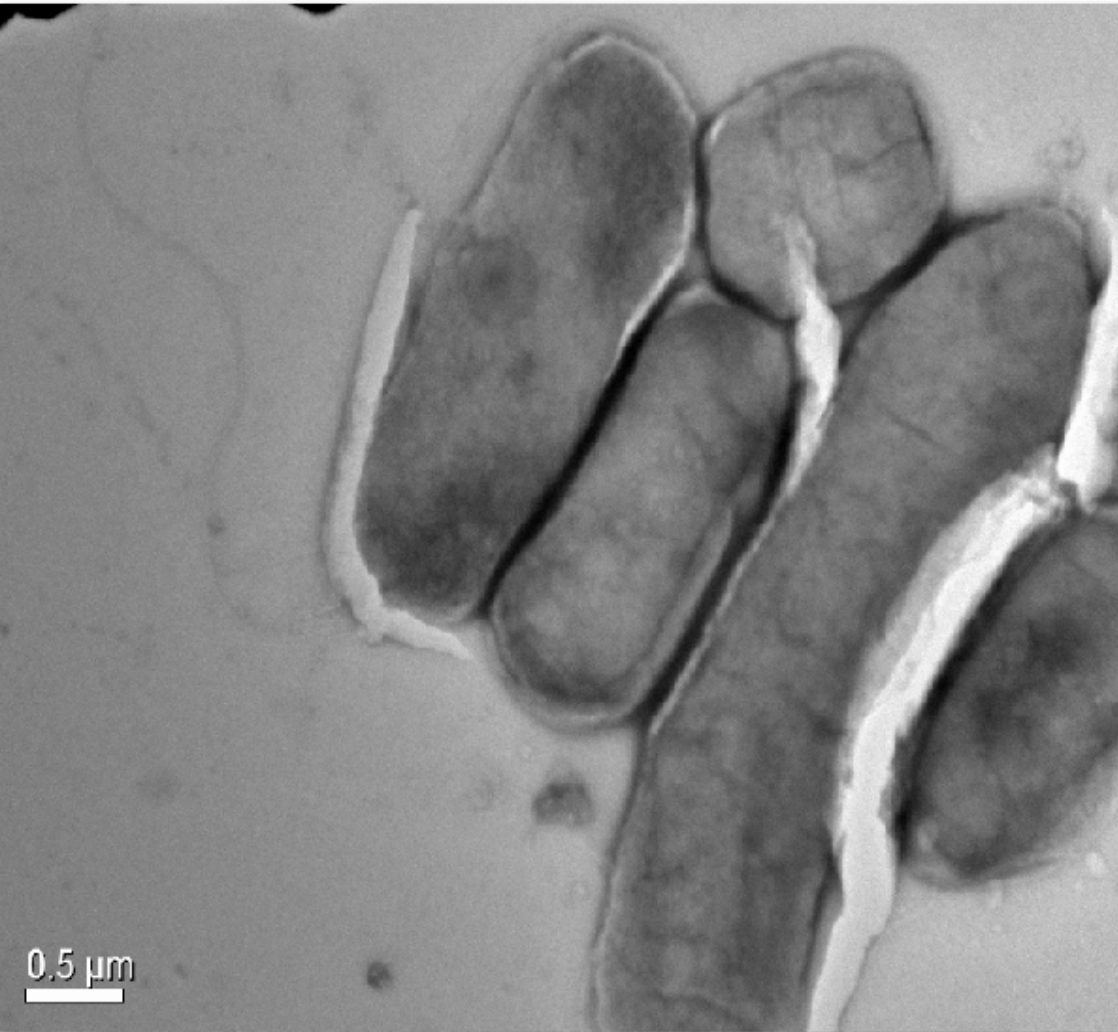


Fig. 1

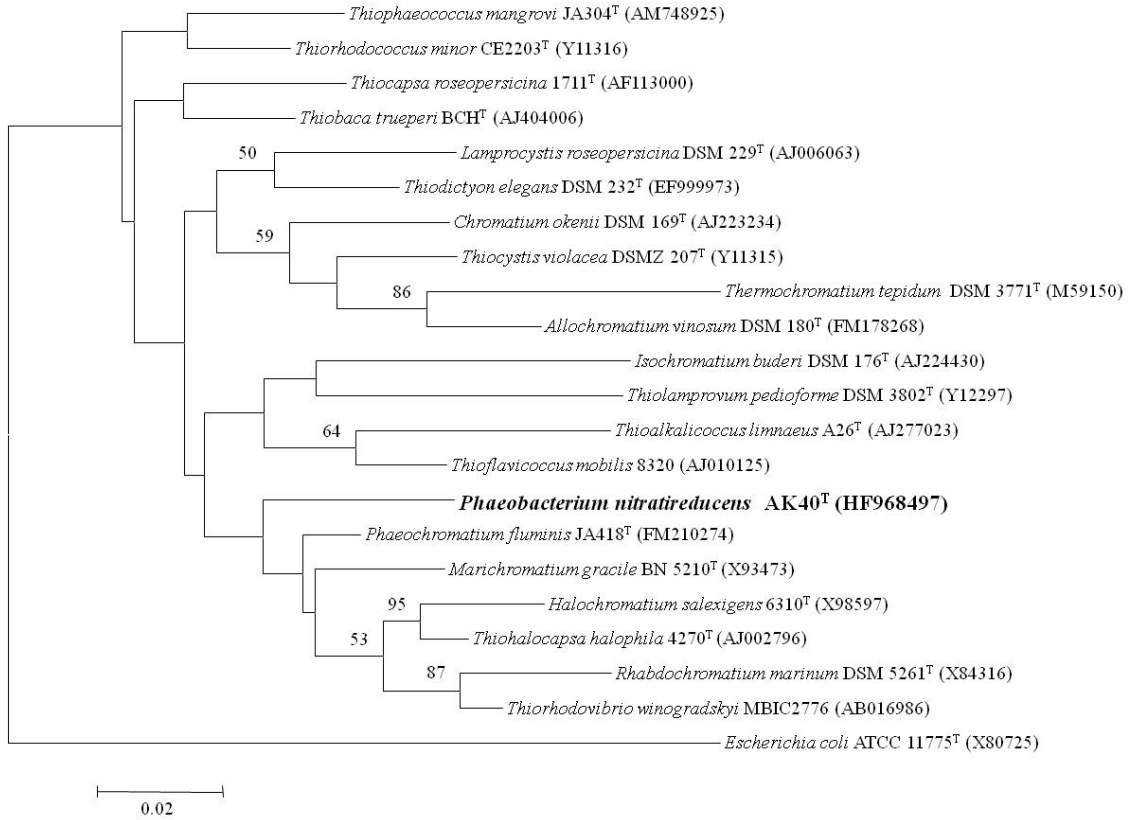
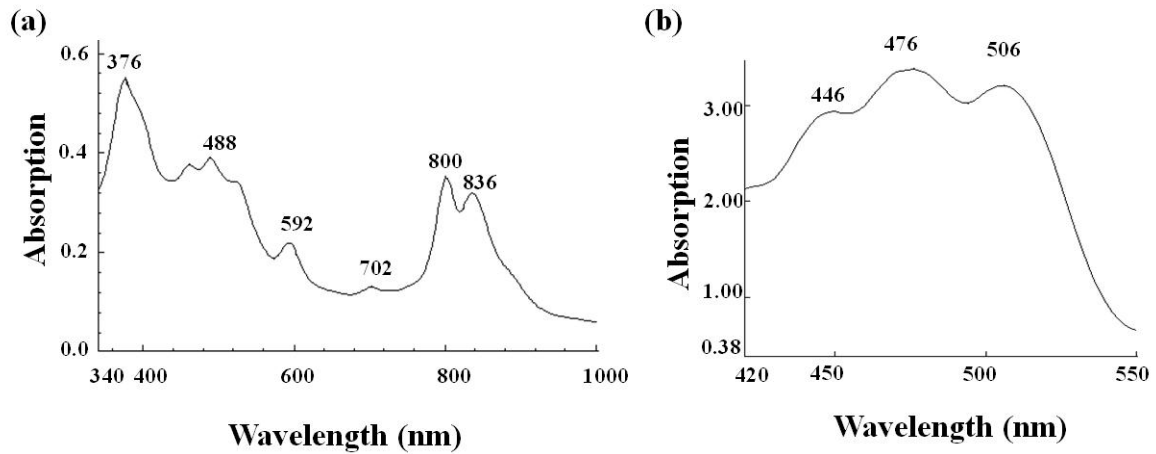
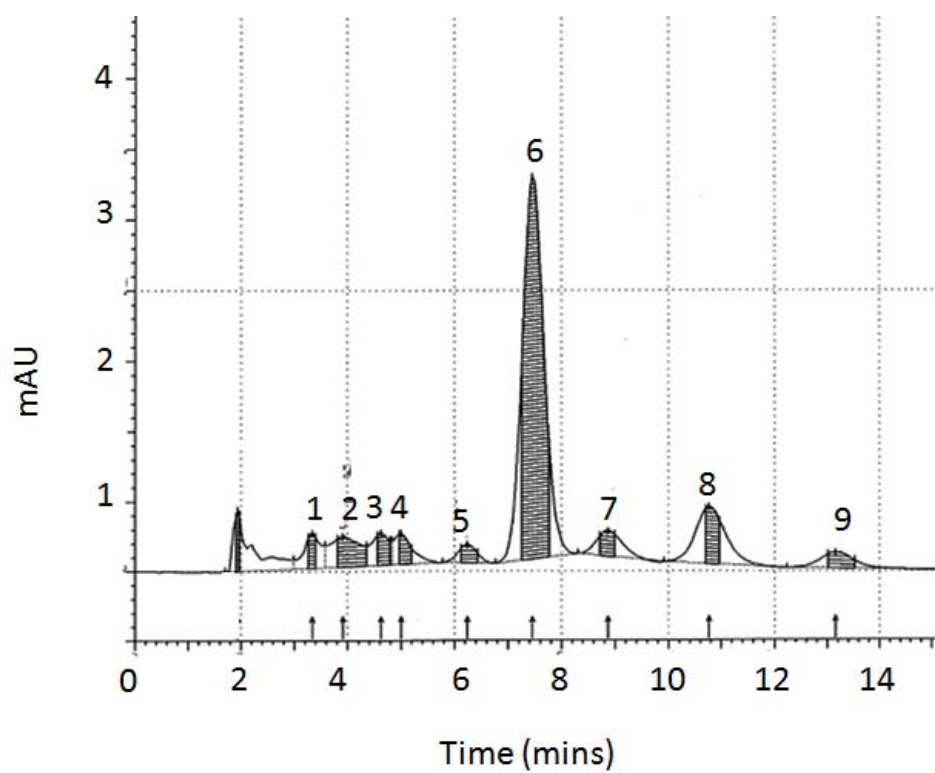


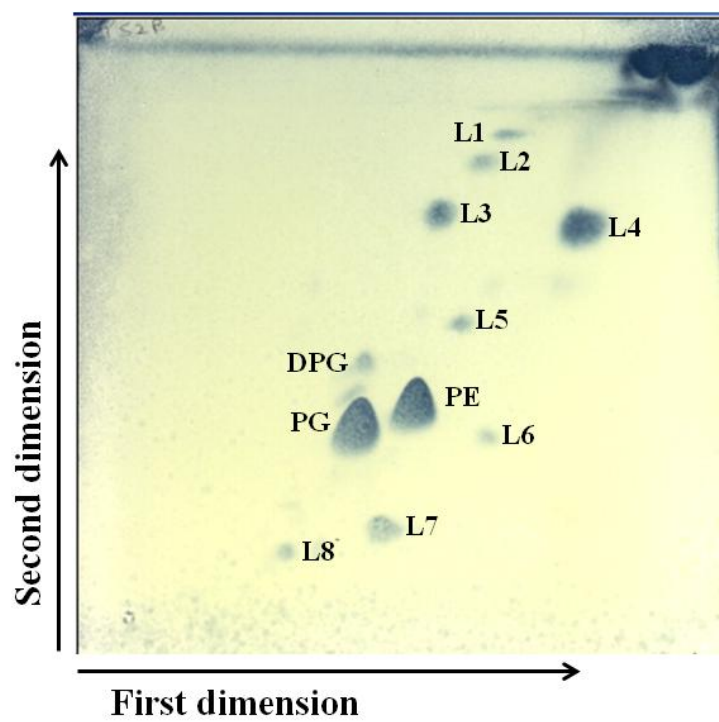
Fig. 2



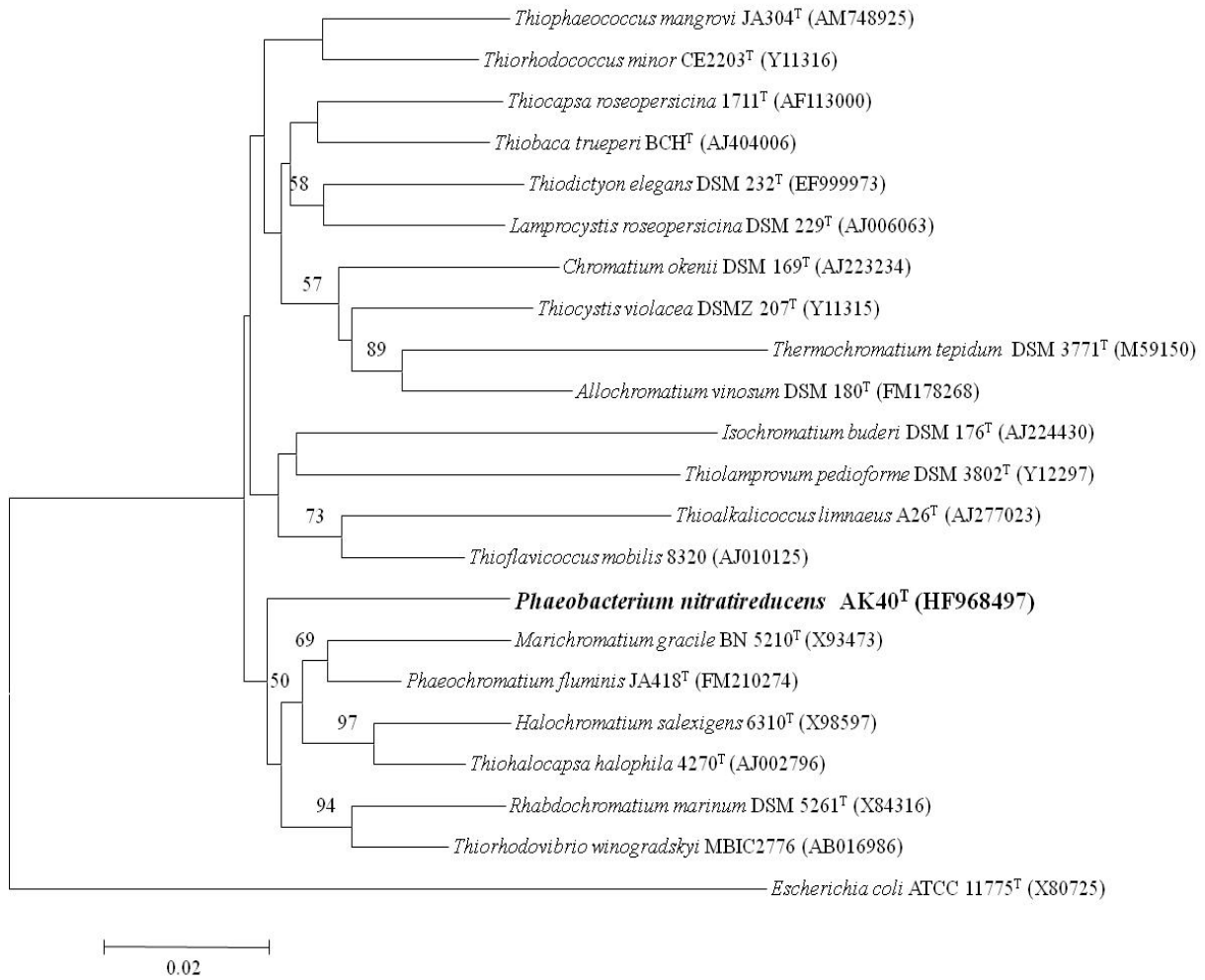
Supplementary Fig. S1



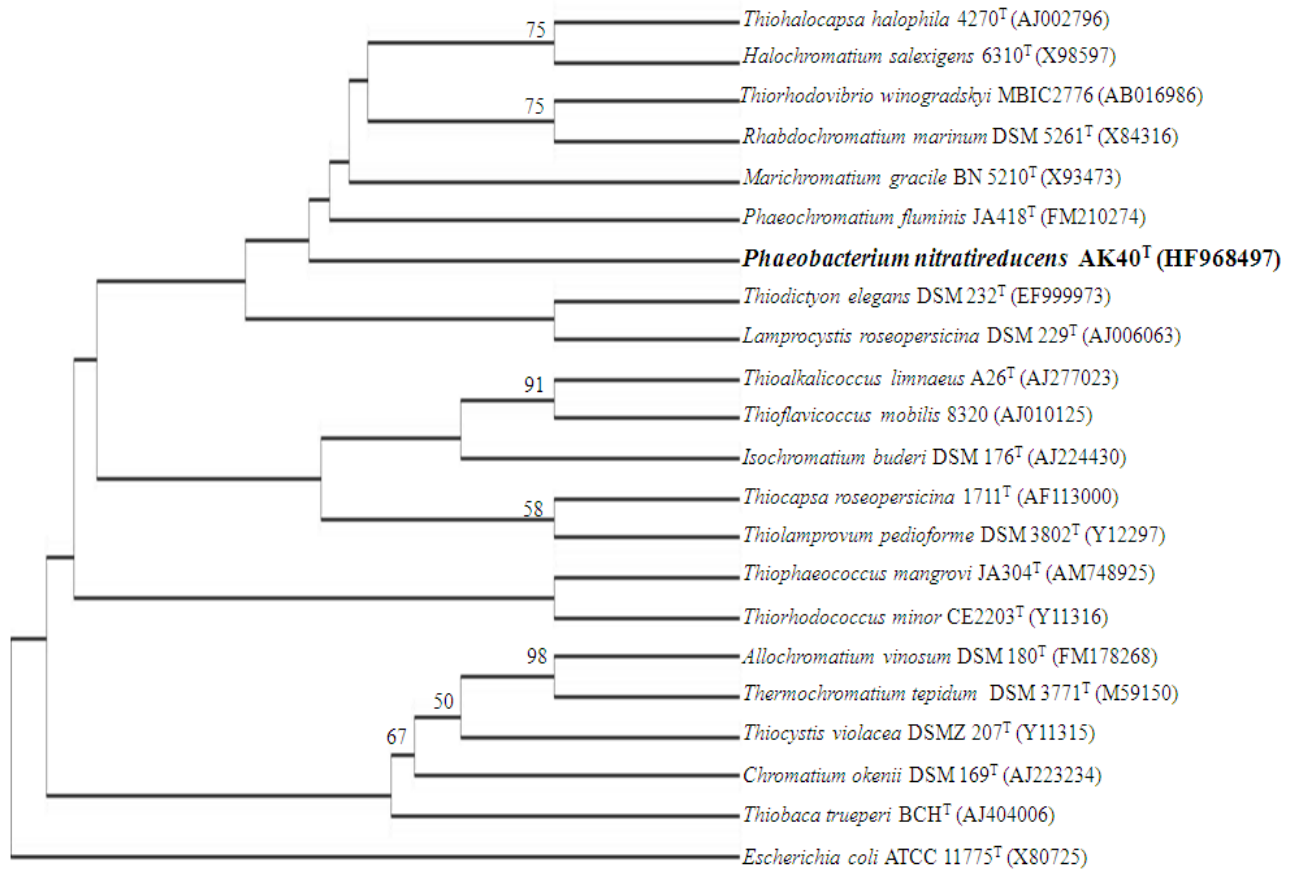
Supplementary Fig. S2



Supplementary Fig. S3



Supplementary Fig. S4



1.0

Supplementary Fig. S5