

**Imhoffiella gen. nov.. a marine phototrophic member of family Chromatiaceae including the description of Imhoffiella purpurea sp. nov. and the reclassification of Thiorhodococcus bheemlicus Anil Kumar et al. 2007 as Imhoffiella bheemlica comb. nov.**

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The GenBank/EMBL/DDJB accession number for the 16S rRNA gene sequence of strain AK35<sup>T</sup> is HF562219.

A coccoid-shaped phototrophic purple sulfur bacterium was isolated from a coastal surface water sample collected from Visakhapatnam, India. Strain AK35<sup>T</sup> was Gram-negative, motile, purple colored, containing bacteriochlorophyll *a* and the carotenoid rhodopinal as major photosynthetic pigments. Strain AK35<sup>T</sup> was able to grow photoheterotrophically and could utilize a number of organic substrates. It was unable to grow photoautotrophically. Strain AK35<sup>T</sup> was able to utilize sulfide and thiosulfate as electron donors. The main fatty acids present were identified as C<sub>16:0</sub>, C<sub>18:1 ω7c</sub> and C<sub>16:1 ω7c</sub> and/or iso-C<sub>15:0</sub> 2OH (Summed feature 3) were identified. Strain AK35<sup>T</sup> contained diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and six unidentified lipids as polar lipids. The G+C content of the DNA of strain AK35<sup>T</sup> was 63.1 mol%. The 16S rRNA gene sequence comparisons indicated that the isolate represented a member of the family *Chromatiaceae*. The 16S rRNA gene sequence analysis indicated that strain AK35<sup>T</sup> is phylogenetically distinctly positioned outside the groups of most members of the genus *Thiorhodococcus*, clustered with members of the genera *Marichromatium* and *Phaeochromatium*, but was most closely related to *Thiorhodococcus bheemlicus* with a pair-wise sequence similarity of 98.75%. Based on DNA–DNA hybridization between strains AK35<sup>T</sup> and *Thiorhodococcus bheemlicus* MTCC 8120<sup>T</sup> a relatedness of 39.46% was established. Distinct morphological, physiological and genotypic differences from these previously described taxa supported the classification of this isolate as a representative of a novel species in a new genus, for which the name *Imhoffiella purpurea* gen. nov., sp. nov. is

proposed. The type strain of *Imhoffiella purpurea* is AK35<sup>T</sup> (= JCM 18851<sup>T</sup> = KCTC 15575<sup>T</sup> = MTCC 12304<sup>T</sup>). In addition, *Thiorhodococcus bheemlicus* is recognized as another species of this genus and transferred to *Imhoffiella bheemlica* comb. nov.

**Keywords:** *Imhoffiella purpurea*, 16S rRNA gene-based phylogeny, chemotaxonomy, *Gammaproteobacteria*

The purple sulfur bacteria (PSB) are one of the groups of anoxygenic phototrophic bacteria. They belong to the class *Gammaproteobacteria* capable of photosynthesis without using water as reducing agent and without liberating oxygen. As an alternative, PSB uses hydrogen sulfide, which is oxidized to sulfur granules inside or outside the cells and further oxidized to sulfate. PSB grow anaerobically or microaerobically and are mainly found in illuminated anoxic stagnant aquatic habitats where hydrogen sulfide accumulates. PSB belongs to two families, *Chromatiaceae* and *Ectothiorhodospiraceae*, which are differentiated by the position of sulfur granules, internal in *Chromatiaceae* and external in *Ectothiorhodospiraceae* and also from the structure of their internal membranes. Marine habitats are a tremendous recess for purple sulfur bacterial diversity. Several novel species of PSB belonging to the genera *Allochromatium*, *Halochromatium*, *Isochromatium*, *Lamprobacter*, *Marichromatium*, *Phaeobacterium*, *Rhabdochromatium*, *Thioalkalicoccus*, *Thiococcus*, *Thioflavicoccus*, *Thiohalocapsa*, *Thiophageococcus*, *Thiorhodococcus*, *Thiorhodospira* and *Thiorhodovibrio* were isolated from diverse marine habitats like aquaculture ponds, estuarine waters, man-made and natural lagoons, marine sulfur springs, mangroves, marine tidal waters, salt marshes and solar salterns (Anil Kumar *et al.*, 2008a, b; Anil Kumar *et al.*, 2009; Imhoff, 2001, 2005a; Nupur *et al.*, 2015; Srinivas *et al.*, 2009). The genus *Thiorhodococcus* comprises five valid species names, *Thiorhodococcus minor* (Imhoff *et al.*, 1998) (originally described as *Thiorhodococcus minus*; Guyoneaud *et al.*, 1997), *Thiorhodococcus mannitoliphagus* (Rabold *et al.*, 2006), *Thiorhodococcus bheemlicus*, *Thiorhodococcus kakinadensis* (Anil Kumar *et al.*, 2007) and *Thiorhodococcus modestalkaliphilus* (Sucharita *et al.*, 2010) which were isolated from an anoxic sediment of a fishpond (manmade coastal lagoon), a microbial mat of an estuary, a marine aquaculture pond, marine tidal waters from a fishing harbor and Chilika Lagoon (Odisha, India), respectively. The type species of the genus is *Thiorhodococcus minor*. In the course of bacterial diversity studies of marine samples, a bacterial strain, AK35<sup>T</sup>, was isolated from a tidal water sample collected near Visakhapatnam, Andhra Pradesh, India. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain AK35<sup>T</sup> was closely related to *Thiorhodococcus bheemlicus* and distantly related to the other members of the genus *Thiorhodococcus* 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 AK35<sup>T</sup>.

The strain AK35<sup>T</sup> was isolated from a tidal water sample collected from Rama Krishna beach (GPS Positioning: 17°42' 37.09"N 83°19' 04.56"E), Visakhapatnam, Andhra Pradesh, India. The temperature and pH of the water sample were 30 °C and 8.3, respectively. The anoxygenic phototrophic bacterium AK35<sup>T</sup> was isolated using photolithoheterotrophic enrichments of the water sample, and subsequent purification was performed as previously described by 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 h 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 1000 x magnification and also by transmission electron microscope (Jeol JEM 2100) at an operating voltage of 200 kV. The Gram reaction was determined by using the Gram staining kit from HIMEDIA (Mumbai, India) as described by manufacturer's protocol. Motility was assessed under light microscopy using the hanging drop method.

Physiological and biochemical characteristics were determined as previously described (Anil Kumar *et al.*, 2008a, b; Srinivas *et al.*, 2006). *In vivo* absorption spectra were measured with a Spectronic Genesys 2 spectrophotometer in sucrose solution (Trüper & Pfennig, 1981). Absorption spectra of pigments extracted with acetone were also recorded. The carotenoid composition was analyzed by using C<sub>18</sub>-HPLC (Takaichi & Shimada, 1992).

Strain AK35<sup>T</sup> was 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 method) and were analyzed using Sherlock Microbial Identification System (MIDI-6890 with database TSBA50) by the protocol described by the manufacturer. At the time of harvesting, the cells were at the logarithmic phase of growth. Freeze-dried 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 molybdato-phosphoric acid, molybdenum blue, ninhydrin and Molisch reagents) (Komagata & Suzuki, 1987).

Genomic DNA was isolated by using the procedure of Marmur (1961) and the mol% 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 AK35<sup>T</sup> 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 strains of the genera *Marichromatium* and *Thiorhodococcus* as well as type species of other phototrophic 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 was drawn to scale, with branch lengths measured in the number of substitutions per site. All positions containing gaps and missing data were eliminated. There was a total of 1247 positions in the final dataset. Evolutionary analyzes 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). DNA–DNA hybridization was performed by the membrane filter method (Tourova & Antonov, 1987) as described previously (Rakshak *et al.*, 2013).

Cells of strain AK35<sup>T</sup> were Gram-negative, coccoid-shaped, non-sporulating, motile with single flagellum and were 1.7-2.0  $\mu\text{m}$  in diameter (Fig. 1). Colonies were 1.5-2.2 mm in diameter, circular to oval, smooth, purple, opaque and raised with entire margin on modified Pfennig medium. Strain AK35<sup>T</sup> grows photolithoheterotrophically [anaerobic, light (2000 lux),  $\text{Na}_2\text{S}/\text{Na}_2\text{S}_2\text{O}_3$  (0.5 mM), pyruvate (0.3% w/v)] and photoorganoheterotrophically [anaerobic, light (2000 lux), pyruvate (0.3%, w/v)]. Photolithoautotrophically [anaerobic, light (2000 lux),  $\text{Na}_2\text{S}/\text{Na}_2\text{S}_2\text{O}_3$  (0.5 mM),

NaHCO<sub>3</sub> (0.1%, w/v)], chemolithoautotrophic growth [aerobic, dark, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (0.5 mM), NaHCO<sub>3</sub> (0.1%, w/v)], chemolithoheterotrophic growth [aerobic, dark, Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (0.5 mM), pyruvate (0.3%, w/v)], chemoorganoheterotrophic growth [aerobic, dark with 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 4% (w/v, NaCl), the optimum NaCl concentration being 1–2% (w/v). The pH range for growth of strain AK35<sup>T</sup> was 7.0–8.5 with the optimum at 7.5–8.0. The temperature range for growth was from 20–37 °C and the optimum temperature was at 30 °C. Growth factors were not required. The color of photosynthetically grown cell suspensions was purple. The whole-cell absorption spectrum of strain AK35<sup>T</sup> in sucrose solution showed maxima at 400, 500, 536, 594, 800, 864 nm, confirming the presence of bacteriochlorophyll *a* and carotenoids (Supplementary Fig. S1a). The absorption spectrum of acetone-extracted pigments showed maxima at 446, 474, 502 nm, indicating the presence of carotenoids (Supplementary Fig. S1b). The carotenoid composition of strain AK35<sup>T</sup>, as determined by using C<sub>18</sub>-HPLC analysis, was rhodopinal (75%), rhodopin (15%), lycopene (8%), dihydroxylycopene (1%) and dihydroxylycopene-glucoside (1%). They were identified based on absorption spectra in the C<sub>18</sub>-HPLC eluent of methanol, and the retention times on the C<sub>18</sub>-HPLC (Takaichi & Shimada, 1992). Major carotenoids were purified and their relative molecular masses were analyzed. Other physiological characteristics are given in the species description and in Tables 1 and 2.

The cellular fatty acid composition of strain AK35<sup>T</sup> showed a profile of eight fatty acids including C<sub>12:0</sub> (2.0%), C<sub>14:0</sub> (2.3%), C<sub>16:0</sub> (18.8%), iso-C<sub>16:0</sub> 2OH (1.4%), C<sub>16:1</sub> *ω*7*c* alcohol (0.8%), C<sub>18:1</sub> *ω*5*c* (1.0%), C<sub>18:1</sub> *ω*7*c* (38.2%) and C<sub>16:1</sub> *ω*7*c*/iso-C<sub>15:0</sub> 2OH (Summed feature 3) (35.5%). Unsaturated fatty acids constituted 75.5%. The major polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG) and six unidentified lipids (L1–L6) (Fig. 3a). The DNA base composition of strain AK35<sup>T</sup> was 63.1 mol% G+C (*Tm*).

The composition of polar lipids and carotenoids of *Thiorhodococcus bheemicus* MTCC 8120<sup>T</sup> was very similar to those of strain AK35<sup>T</sup>. Polar lipids were diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), one unidentified aminophospholipid (APL1) and three unidentified lipids (L1, L4, L7) (Fig. 3b) and carotenoids were rhodopinal (74%), rhodopin (18%), lycopene (6%), dihydroxylycopene-glucoside (2%) and dihydroxylycopene (trace).

For phylogenetic assessment of strain AK35<sup>T</sup>, the 16S rRNA gene sequence (1465 bp) 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 *Thiorhodococcus bheemicus* with a pair-wise sequence similarity of

strain AK35<sup>T</sup> to *Thiorhodococcus bheemicus* of 98.75%. Sequence similarities to other members of the genus *Thiorhodococcus*, including the type species *Thiorhodococcus minor*, were between 95.77 and 96.56%. In a phylogenetic trees based on the neighbour joining method (Fig. 2) and on the maximum likelihood method (Supplementary Fig. S2) strain AK35<sup>T</sup> was associated with members of the genera *Marichromatium* and *Phaeochromatium* (Fig. 2). In all phylogenetic trees of the 16S rRNA gene sequences strain AK35<sup>T</sup> clustered closely together with *Thiorhodococcus bheemicus* (Supplementary Fig. S2 and 3).

As the type strain of *Thiorhodococcus bheemicus* MTCC 8120<sup>T</sup> is phylogenetically close to strain AK35<sup>T</sup>, their relationship by DNA-DNA hybridization was determined and phenotypic differences between the two strains were evaluated and a comparison of different phenotypic characteristics are shown in Table 1. Properties of strain AK35<sup>T</sup> differed from the *Thiorhodococcus bheemicus* MTCC 8120<sup>T</sup> for instance with respect to cell size, the colour of cell suspension, NaCl growth range and optimum, temperature growth optimum, pH growth range and optimum, sulfate assimilation, carbon substrate utilization, polar lipid content and DNA G + C content (mol%) (Table 1). The average relatedness of the strain AK35<sup>T</sup> with *Thiorhodococcus bheemicus* MTCC 8120<sup>T</sup> by DNA-DNA hybridization was 39.46%, which were determined from three experimental values with a standard deviation of 3.81%.

Based on the phylogenetic analysis a comparison was made between the characteristics of strains AK35<sup>T</sup>, MTCC 8120<sup>T</sup> and all the genera of the family *Chromatiaceae*, *Allochromatium*, *Halochromatium*, *Isochromatium*, *Lamprocystis*, *Marichromatium*, *Nitrosococcus*, *Phaeobacterium*, *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. Strains AK35<sup>T</sup> and MTCC 8120<sup>T</sup> also is in particular different from most members of the genus *Thiorhodococcus*, including the type species *Thiorhodococcus minor*. Thus, the cumulative differences that strains AK35<sup>T</sup> and MTCC 8120<sup>T</sup> exhibited with the above type strains unambiguously supported the creation of a new genus *Imhoffiella*, and a new type species of this genus, for which the name *Imhoffiella purpurea* sp. nov., and the reclassification of *Thiorhodococcus bheemicus* Anil Kuma *et al.* 2007 as *Imhoffiella bheemlica* comb. nov. were proposed.

#### **Description of *Imhoffiella* gen. nov.**

*Imhoffiella* (Im.hoff.i.e'l'a. N.L. fem. dim. n. *Imhoffiella* genus in honor of Johannes F. Imhoff, a German microbiologist who has made a significant contribution to our knowledge of anoxygenic phototrophic bacteria).

Cells are spherical, single or diplococci, multiply by binary fission and are motile by means of a single 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 globules within the cell. In the presence of sulfide and bicarbonate, organic substrates can be photoassimilated. Salt and growth factors are not required for growth. The DNA G+C content is approximately 63–65.5 mol%. The type species is *Imhoffia purpurea*.

#### **Description of *Imhoffiella purpurea* sp. nov.**

*Imhoffiella purpurea* (pur.pu're.a. L. fem. adj. *purpurea* purple).

Cells are Gram-staining-negative, coccoid-shaped, 1.7-2.0 µm in diameter, motile, divide by binary fission and purple pigmented. Colonies grown for 3 days at 30 °C on modified Pfennig medium are circular, 1.5-2.0 mm in diameter, circular-oval, smooth, purple, opaque and raise with an entire margin. The *in vivo* absorption spectrum of intact cells in sucrose exhibits maxima at 400, 500, 536, 594, 800, and 864 nm. The absorption spectrum in acetone extracted pigments gives absorption maxima at 446, 474, 502 nm. Major photosynthetic pigments are bacteriochlorophyll *a* and rhodospinal. The type strain is mesophilic (range 20–37 °C, optimum 30 °C), can grow both in neutral and alkaline conditions (pH range 7.0–8.5, optimum 7.5-8.0) and does not require NaCl for growth but can tolerate concentrations of NaCl up to 4% w/v with optimum growth between 1-2% NaCl. Positive for oxidase and catalase activities but negative for β-galactosidase activity. Strain can able to grow photolithoheterotrophic and photoorganoheterotrophic growth modes. Photolithoautotrophic, chemolithoautotrophic, chemolithoheterotrophic, chemorganoheterotrophic and fermentative growth modes are not possible. Photoorganoheterotrophy with various organic compounds is the preferred growth mode. Substrates that are utilized as carbon source/electron donors under photoheterotrophic condition include fumarate, casamino acids, glucose, glycerol, succinate and valerate but not acetate, butyrate, crotonate, ethanol, formate, fructose, citrate, methanol, malate, propanol, propionate,

pyruvate, and yeast extract. Ammonium chloride, nitrate, glutamine, and yeast extract are utilized as nitrogen sources but not nitrite. Thiosulfate (2 mM), sulfur (0.1% w/v) and sulfide (1 mM) are utilized as sulfur sources under photoheterotrophic condition. There is no growth factor requirement. The major fatty acids are C<sub>16:0</sub>, C<sub>18:1</sub> ω7c and C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2OH (Summed feature 3). The predominant polar lipids are diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol and six unidentified lipids. The G+C content of the genomic DNA of the type strain of type genus is 63.1 mol%.

The type strain AK35<sup>T</sup> (= JCM 18851<sup>T</sup> = KCTC 15575<sup>T</sup> = MTCC 12304<sup>T</sup>) was isolated from a tidal water sample collected from Rama Krishna beach, Visakhapatnam, India.

#### **Transfer of *Thiorhodococcus bheemicus* to *Imhoffiella bheemlica* comb. nov,**

*Imhoffiella bheemlica* (*Thiorhodococcus bheemicus* Anil Kumar *et al.*, 2007) (bheem'li.ca. N.L. fem. adj. bheemlica named after Bheemli, the place from which the type strain was isolated). The description is the same as that for *Thiorhodococcus bheemicus* (Anil Kumar *et al.*, 2007) with the following modifications. The major fatty acids are C<sub>16:0</sub>, C<sub>18:1</sub> ω7c, C<sub>18:1</sub> ω9c and summed feature 3. The polar lipids consist of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylglycerol, one unidentified aminolipid and three unidentified lipids. Major carotenoid is rhodopinal.

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**Figure Legends:**

**Fig. 1.** Electron micrograph of negatively stained cells of strain AK35<sup>T</sup>. Bar, 1.0 µm.

**Fig. 2.** Neighbor-joining tree based on 16S rRNA gene sequences representing the phylogenetic relationships between the strain AK35<sup>T</sup> and the species of the other genera belongs to phototrophic

members of the family *Chromatiaceae*. Numbers at the nodes are bootstrap values >50%. *Escherichia coli* KCTC 2441<sup>T</sup> (EU014689) was taken as the outgroup organism. Bar, 0.02 substitutions per nucleotide position.

**Fig. 3.** Two-dimensional thin-layer chromatogram of the total polar lipids of the strain AK35<sup>T</sup> (a) and *Imhoffiella bheemica* MTCC 8120<sup>T</sup> (b) after spraying the plates with molybdato-phosphoric acid. Abbreviations: DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL, unidentified aminophospholipid; L, unidentified lipid. The spots identified as phospho-, amino- or glycolipids by spraying molybdenum blue, ninhydrin and  $\alpha$ -naphthol reagents, respectively.

**Table 1.** Phenotypic features that distinguish strain AK35<sup>T</sup> with the closely related type strain of the genus *Thiorhodococcus*

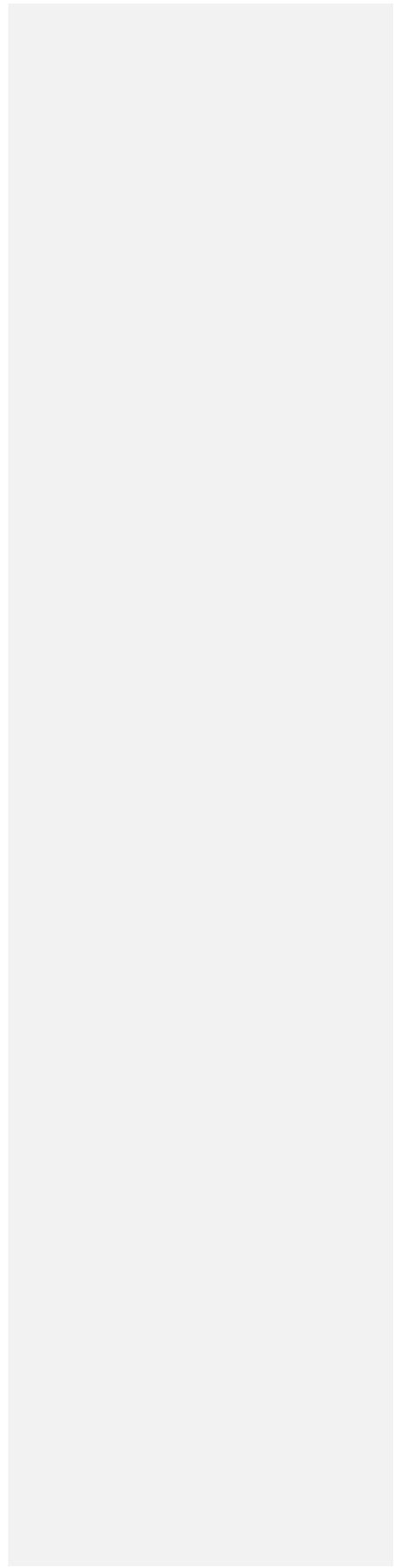
Data is from the present study. Both strains are coccoid in shape and motile; contain rhodopinal as major carotenoid; organic substrate utilization was tested during photoheterotrophic growth; fumarate was utilized; grow photolithoheterotrophically and photoorganoheterotrophically. Both strains cannot able to grow by chemolithoautotrophic, chemolithoheterotrophic, chemoorganoheterotrophic and fermentative growth modes; cannot utilize hydrogen, formate, propionate, malate, fructose, ethanol, propanol and crotonate; donot require vitamin for growth.

Characteristic	<i>Imhoffiella purpurea</i> AK35 <sup>T</sup>	<i>Imhoffiella bheemica</i> MTCC 8120 <sup>T</sup>
Cell size (µm)	1.7-2.0	4.0-6.0
Aggregate formation	Single cocci	Mostly diplococci
Color of cell suspensions	Purple	Purple-violet
<i>In vivo</i> bchl <i>a</i> absorption peak (nm)	400, 500, 536, 594, 800, 864	371, 460, 491, 530, 590, 803, 857
Photolithoautotrophy	-	+
Sulfate assimilation	+	-
pH range	7.0-8.5	6.5-8.0
pH optimum	7.5-8.0	7.0-7.5
Temperature optimum (°C)	30	25-30
Temperature range (°C)	20-37	20-30
Salinity range (%)	0-4	0.5-6
NaCl optimum (%)	1-2	1-3
<b>Carbon/ e- donor</b>		
Sulfide	w	+
Thiosulfate	w	+
Sulfur	+	-
Sulfite	+	-
Acetate	-	+
Butyrate	-	w
Lactate	-	+
Succinate	w	-
Glucose	w	+
Glycerol	w	+
Pyruvate	-	+
Glycolate	-	+
Valerate	w	+
Casamino acids	w	+
Polar lipids	DPG, PE, PG, L1-L6	DPG, PE, PG, APL1, L1, L4, L7
DNA G+C content (mol%)	63.1	65.5

+, substrate utilized or present; -, substrate not utilized or absent; w, weak growth; DPG, diphosphatidylglycerol; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; APL, aminophospholipid; L, lipid. Summed feature 3 is C<sub>16:1</sub> ω7c and/or iso-C<sub>15:0</sub> 2OH.

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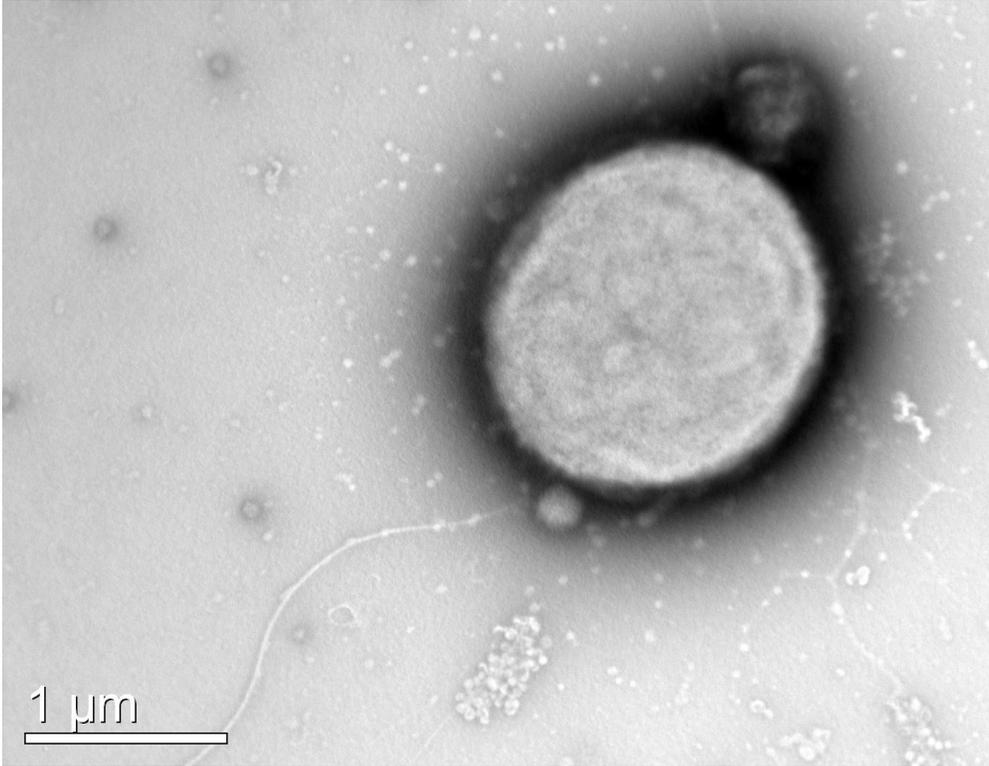
**Table 2.** Phenotypic features that distinguish strains AK35<sup>T</sup> and MTCC 8120<sup>T</sup> with the type species of the genera, *Chromatiaceae*

1. Strain AK35<sup>T</sup>; 2. Strain MTCC 8120<sup>T</sup>; 3. *Allochroamatium* (Imhoff, 2005b); 4. *Halochroamatium* (Imhoff & Caumette, 2005a); 5. *Isochromatium* (Imhoff, 2005c); 6. *Lamprocystis* (Imhoff, 2001); 7. *Marichroamatium* (Imhoff, 2005d); 8. *Nitrosococcus* (Koops & Roser, 2005); 9. *Phaeobacterium* (Nupur *et al.*, 2015); 10. *Phaeochroamatium* (Sucharita *et al.*, 2010 & Shivali *et al.*, 2012); 11. *Rhabdochroamatium* (Imhoff, 2005e); 12. *Rheinheimeria* (Brettar *et al.*, 2002); 13. *Thermochroamatium* (Imhoff & Madigan, 2005); 14. *Thioalkalicoccus* (Imhoff, 2005f); 15. *Thiobaca* (Rees *et al.*, 2002); 16. *Thiocapsa* (Imhoff & Caumette, 2005b); 17. *Thiococcus* (Imhoff, 2005g); 18. *Thiocystis* (Imhoff, 2005h); 19. *Thiodictyon* (Imhoff, 2005i); 20. *Thioflavicoccus* (Imhoff & Pfennig, 2001); 21. *Thiohalocapsa* (Imhoff & Caumette, 2005c); 22. *Thiolamprovum* (Guyoneaud *et al.*, 1998); 23. *Thiopedia* (Imhoff, 2005j); 24. *Thiophageococcus* (Anil Kumar *et al.*, 2008b); 25. *Thiorhodococcus* (Guyoneaud *et al.*, 1997); 26. *Thiorhodovibrio* (Overmann *et al.*, 1992); 27. *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	26	27
Cell shape	Co	Co	R	RO	R	SO	R	SE	R	R	R	RC	R	SO	R	S	S	R, SO	R	Co	S	SO	SO	S	SO	VS	RS
Cell size (µm)	1.7-2	4-6	1-4	2-4	3.5-4.5	2-3.5	1-1.7	1.5-2.5	2-4	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	1.2-1.6	2.5-4
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	-	-	+	+	+	+
Aggregate formation	-	Di co	-	-	-	B	Spe	Si pi	-	-	-	-	-	-	-	Si/Pi	tet	-	Irr	-	-	-	Plt	Rec	-	Si, Pi, irr	-
Colour of cell suspension	Pu	Puv	Ob	Pr	Pv-Puv	Pv	Br Pur	NA	Rb	Db	BO B	Dbl-Lbl	NA	Ybr, Ob	Br	P, Rr	Ob	Puv	Puv	Ybg-Ob	Pur	P, Rr	Pur	S-B	Bo	P	Y-Ob
Gas vesicles	-	-	-	-	-	+	-	NA	-	NA	NA	NA	-	-	-	±	-	-	+	-	-	+	+	-	-	NA	NA
Internal photosynthetic Membranes	V	V	V	V	V	V	V	EI	V	V	V	-	V	T	V	V	T	V	V	T	V	V	V	V	V	V	V
Slime capsule	-	-	-	-	-	+	-	NA	-	NA	NA	NA	NA	-	NA	-	-	+	NA	-	+	-	-	-	-	NA	NA
Bacteriochlorophyll	a	a	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	RA	RA	RA	SP	RA	RA	SP Ok	NA	RN	RN	LY	NA	SP	THS	LY	SP	THS	RA	RA	THS	Ok	SP	OK	LY	SP	LY, RN	
pH optimum	7.5-8.0	7.0-7.5	6.5-7.6	7.2-7.6	6.5-7.6	7.0-7.3	6.5-7.6	NA	7.5	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)	20-37	20-30	25-35	20-35	25-30	20-30	25-37	NA	22-37	NA	20-35	4-30	48-50	25	25-30	20-35	20-35	20-35	20-30	20-30	20-30	37	20	20-35	30-35	14-20	20-25
Salt requirement	-	+	-	+	+	-	+	+	-	+	+	-	-	+	-	±	±	-	-	+	+	-	-	-	+	-	-
Growth mode(s)	PLH, POH	PLA, PLH	PLA	PLA, CLA, COH	PLA	PLA, CLA	PLA, CLA, COH	CL H	PO H	PL H	PL A	COH	PL A	PLA	PLH, POH	PLA, POH, CLA, COH	PLA	PLA, CLA, COH	PLA	PLA	PLA, POH, CLA, COH	PLA, CLA	PLA	PL A	PLA, CLA	PL A	PLA
Sulfate assimilation	+	-	+	+	+	NA	+	NA	-	NA	+	-	+	NA	-	+	-	±	NA	+	-	-	-	-	-	+	+
Vitamin B <sub>12</sub> requirement	-	-	±	±	+	NA	-	NA	-	-	NA	NA	-	-	NA	-	-	-	NA	-	+	-	-	-	-	-	+
DNA G+C content (mol%)	63.1	65.5	55.1-66.3	64.6-66.3	62.2-62.8	63.4-64.1	68.9-70.4	50.5	65.5	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; Di co, diplococci; B, branching; Irr, Irregular; Plt, platelet; Rec, rectangular; Spe, 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; Pu, purple; 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; THS, tetrahydrospirilloxanthin; Ok, okenone; LY, lycopenal; §, marine strains may tolerate low concentrations of NaCl; PLA, PL

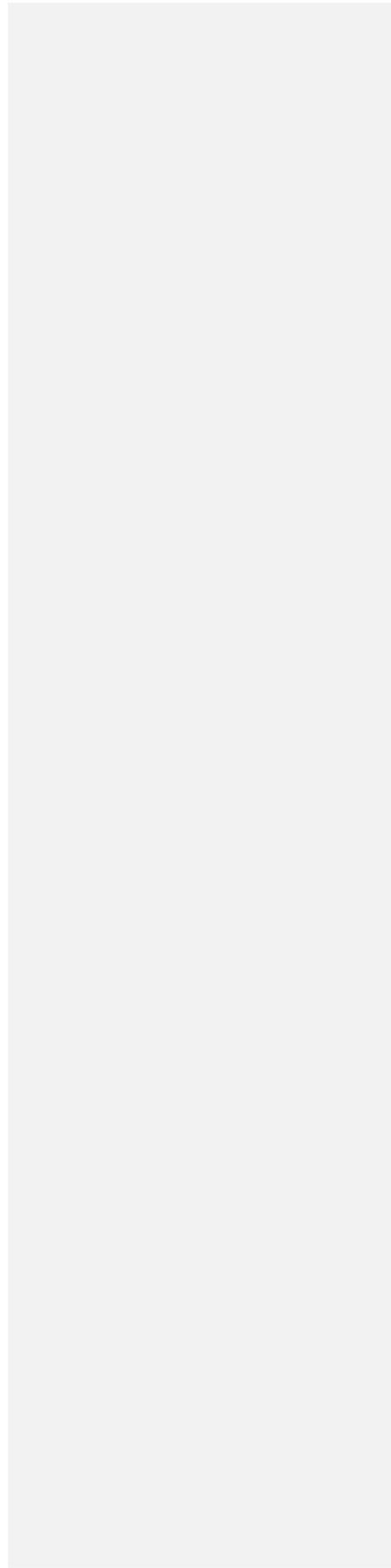
| photolithoautotrophic; PLH, photolithoheterotrophic; POH, photoorganoheterotrophic; CLA, chemolithoautotrophic; CLH, chemolithoheterotrophic; COH, chemoorganoheterotrophic; +, present; -, absent; ±, variable; NA, no data available.



**Fig. 1**

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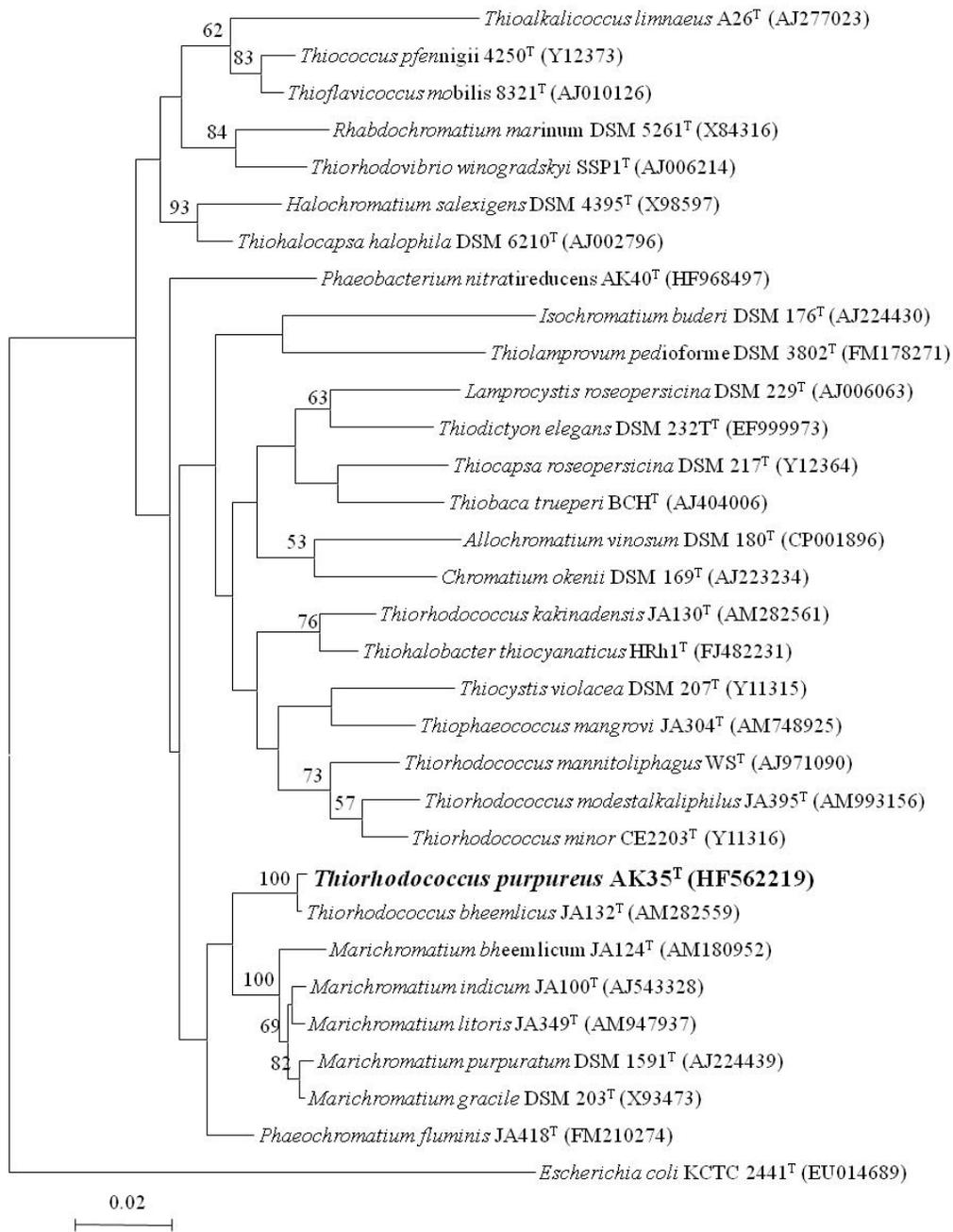


Fig. 2

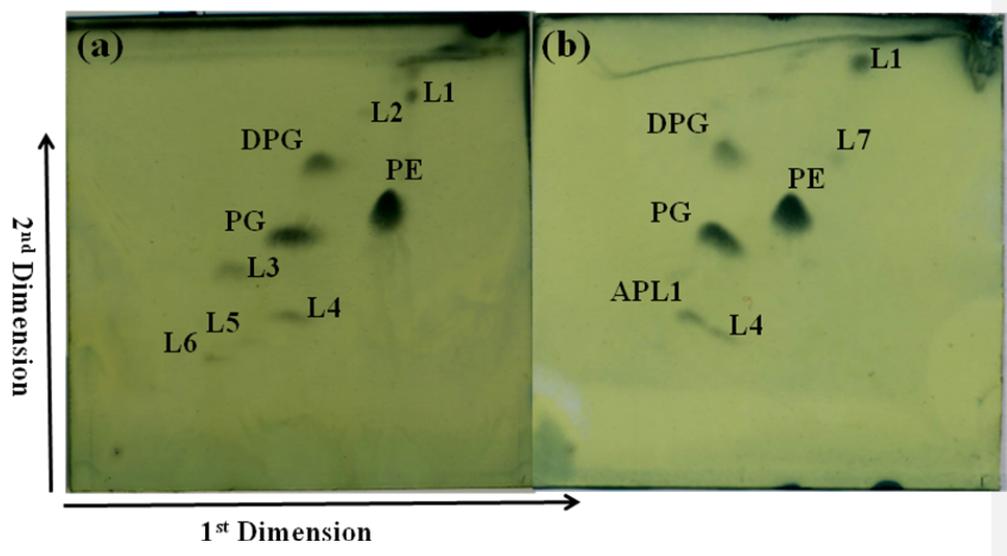


Fig. 3