Thiobaca trueperi gen. nov., sp. nov., a phototrophic purple sulfur bacterium isolated from freshwater lake sediment

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Two strains of a novel species of phototrophic micro-organism were isolated from the sediments of a shallow, freshwater, eutrophic lake. Both strains grew photolithoheterotrophically with sulfide as an electron donor, transiently accumulating intracellular sulfur globules. Photolithoautotrophic growth was not observed. One strain was designated BCH[T] (the type strain) and was studied in most detail. Cells contained bacteriochlorophyll *a*, and the dominant carotenoid was lycopene. Cell suspensions were brown. The photosynthetic membranes had a vesicular arrangement. Acetate, propionate, pyruvate, succinate and fumarate were each used as electron donors and carbon sources in the presence of sulfide and bicarbonate. In the presence of light, growth did not occur with hydrogen, thiosulfate or iron(II). The optimum temperature for growth was between 25 and 30 °C, the maximum being 36 °C. The G+C content of the genomic DNA of strain BCH[T] was 63 mol%. Analysis of the 16S RNA genes showed that both strains belonged to the γ-subclass of the *Proteobacteria* but were phylogenetically distinct from any described phototrophic organisms within the *Chromatiaceae*. On the basis of phylogenetic and physiological differences from other phototrophic micro-organisms, strain BCH[T] is described as a novel species of a new genus, *Thiobaca trueperi* gen. nov., sp. nov.

Keywords: *Thiobaca trueperi*, phototrophic bacteria, freshwater lakes

INTRODUCTION

Many investigations into the presence of phototrophic bacteria in aquatic ecosystems have been stimulated by conspicuous coloured accumulations occurring in anoxic waters or surface mud (Pfennig & Trüper, 1992; Pfennig, 1987). If sulfide is produced at depth in aquatic environments, populations of purple and green phototrophic bacteria can grow by using the sulfide as an electron donor for photosynthesis; this often leads to prolific communities of phototrophic organisms (Sorokin, 1970; Takahashi & Ichimura, 1970; Overmann *et al.*, 1996). Other phototrophic bacteria that do not use sulfide, but instead use a range of reduced organic compounds as electron donors, also occur in

freshwater systems (Imhoff & Trüper, 1992). These phototrophs tend not to form dense, coloured blooms, but numerous studies have demonstrated their presence in a wide variety of freshwater ecosystems. In this study, we examined sediments of a freshwater lake that undergoes summer stratification. The lake has extensive growth of macrophytes, leading to a high carbon loading in the lake, resulting in apparently high rates of methane formation in the sediments. Since previous observations indicated the methanogenic nature of the study site, we examined the presence of phototrophic bacteria growing with relatively low levels of sulfide. Enrichment cultures led to the purification of brown-coloured organisms that were shown subsequently to be related to purple sulfur bacteria. Phylogenetic and physiological studies showed that the organisms differed significantly from any described in the literature, and we conclude that the strains represent a novel species of a new genus. We

propose the name *Thiobaca trueperi* gen. nov., sp. nov., to encompass these novel strains.

METHODS

Source of organisms. Normans Lagoon is a eutrophic oxbow lake that is between 2 and 4 m deep and is situated adjacent to the River Murray, Albury, Australia. The lake has areas of open water surrounding beds of the emergent macrophyte *Eleocharis sphacelata*. Beds of *Brasenia schreberi* are associated with the edges of the lake, and portions of the surface often show growth of the aquatic fern *Azolla*. The lake is fringed, in part, by river red gums (*Eucalyptus camaldulensis*). Sediment was collected from the open-water regions, and enrichment cultures were made by adding 0·5 ml wet sediment to 10 ml basal medium (see below) that contained acetate (5 mM), malate (5 mM) and sulfide (0·5 mM). Enrichment cultures were subcultured several times and pure cultures were obtained by application of the agar-shake dilution method (Pfennig, 1978).

Growth conditions. Basal medium (Biebl & Pfennig, 1981) was used throughout this study and contained (g l^{-1} distilled water): KH_2PO_4 (0·5), $MgSO_4 . 7H_2O$ (0·2), NaCl (0·4), NH_4Cl (0·4), $CaCl_2 . 2H_2O$ (0·05) and yeast extract (0·2, Oxoid). Basal medium was prepared and dispensed into Hungate tubes or 30 ml serum bottles, with N_2 as the headspace gas, as described by Rees *et al.* (1995). Bicarbonate was added to culture vessels after autoclaving to give a final concentration of 30 mM. Sodium sulfide was added to give a final concentration of either 0·5 or 1·5 mM, depending on the desired growth conditions. The final pH of the medium was between 7·2 and 7·4. Additional carbon sources were added to the medium from sterile anoxic stock solutions. Organic compounds were prepared as anoxic solutions, which were generally sterilized by autoclaving. Sodium pyruvate, carbohydrates, sodium thiosulfate and sodium sulfite were filter-sterilized through 0·2 μm pore-size membrane filters. A vitamin solution (Widdel & Bak, 1992) containing vitamin B_{12} was added to sterile medium to give a final concentration of 50 μg vitamin B_{12} l^{-1}. Cultures were routinely incubated at 25 °C, with illumination provided by tungsten lamps giving a light intensity of 120–140 lux.

Cellular and physiological characterization. All physiological tests were carried out in basal medium. When growth with organic compounds was not unequivocal, strains were subcultured into medium containing the same substrate. Photoautotrophy and vitamin requirements were examined by subculturing strains through five successive passages in the appropriate media.

When testing the use of ferrous iron as an electron donor for phototrophic growth, a solution of $FeSO_4$ was prepared under anoxic conditions and added to the medium to give a final concentration of 5 mM. Sufficient sodium bicarbonate was added to the medium to ensure that the pH was maintained following addition of the iron(II) solution.

To test the ability to fix atmospheric nitrogen, a 1% (v/v) inoculum was used to transfer cells through three successive subcultures in basal medium without yeast extract or ammonium chloride, but with nitrogen in the headspaces of the culture bottles. After the third subculture, in addition to continued subculturing of the cells in the yeast extract-/ammonium chloride-free medium, the latter cells were used to inoculate two further experimental systems, which subsequently were serially passaged. The additional growth conditions were yeast extract-/ammonium chloride-free medium with argon in the headspace and ammonium-containing medium with argon in the headspace.

Whole-cell spectra were obtained as described by Pfennig & Trüper (1992). For acetone extracts, cells were collected on glass-fibre filters and extracted overnight in the dark at 4 °C. Scans were obtained using a Cary 3 (Varian) spectrophotometer. Photosynthetic pigments were analysed by HPLC as described previously (Dilling *et al.*, 1995).

Phase-contrast micrographs were prepared by immobilizing cells on agar-coated slides and examining the slides with a Zeiss Axioskop microscope. Nile blue A (Ostle & Holt, 1982) was used to determine whether cells contained lipid inclusions. For electron microscopy studies, exponential-phase cells of strain BCH^T were chemically fixed with 2·5% (v/v) glutaraldehyde in the carbonate-buffered growth medium (pH 7·0) at ambient temperature for 5 days. Fixed cells were immobilized in 2% (w/v) Noble agar (Difco). Cubes (1 mm^3) of immobilized cells were cut and then washed three times for 10 min each at room temperature with 100 mM cacodylate buffer (pH 7·3). Post-fixation with 1% (w/v) OsO_4/100 mM cacodylate buffer (pH 7·3), further preparation steps and electron microscope analysis were done according to Yakimov *et al.* (1998).

DNA base composition and phylogenetic analyses. The G+C content (mol%) of the genomic DNA was determined by HPLC as described by Janssen *et al.* (1996). The 16S rRNA genes of the novel strains were sequenced and the sequence data used to infer evolutionary relationships between these strains and other bacteria, as described by Janssen & O'Farrell (1999). Evolutionary analyses were carried out using software implemented in the Australian Genomic Information Service (ANGIS) system (Littlejohn *et al.*, 1996). The 16S rRNA gene sequences were compared with known 16S rRNA gene sequences by carrying out a BLAST analysis (Altschul *et al.*, 1990) in the GenBank database (Benson *et al.*, 2000), allowing the identification of close relatives. The gene sequences from the two novel strains and selected reference sequences (identified by the BLAST analyses and by comparisons of the inferred phylogenies determined in other studies; Guyoneaud *et al.*, 1998; Imhoff *et al.*, 1998) were aligned using the PILEUP program. The alignment was then checked manually and edited, using the secondary structure of the *Escherichia coli* 16S rRNA (Neefs *et al.*, 1993) as a template. Regions for which homology could not be determined were eliminated from the entire dataset; these were helices 10 and 11, the terminal loops of helices 16, 29 and 43 and part of the basal region of helix P23-1 (helix numbering is that of Neefs *et al.*, 1993). In addition, 5' and 3' termini for which sequence information was lacking for one or more of the sequences were not used in the phylogenetic analyses. Evolutionary distances between pairs of edited sequences were determined according to the nucleotide substitution model of Jukes & Cantor (1969), implemented in the EDNADIST program in the ANGIS system. Phylogenetic trees were reconstructed on the basis of these evolutionary distances by using the EFITCH program, which uses a least-squares method devised by Fitch & Margoliash (1967), based on 1203 unambiguously aligned nucleotide positions, with a random order input and the global rearrangement option, as implemented in the ANGIS system. The 16S rRNA gene of *Escherichia coli* was used as an outgroup. The significance of each node was tested by bootstrap analysis (1000 data resamplings) as described elsewhere (Janssen & O'Farrell, 1999). Maximum-parsi-

mony and maximum-likelihood analyses were performed using the EDNAPARS and EDNAML programs in the ANGIS system.

RESULTS

Isolation of strains

Enrichment cultures transferred to agar-shake dilution tubes yielded a variety of colony types. Two strains were subsequently purified from brown-coloured colonies and were designated strain BCH[T] and strain OCH-PHB. Early growth experiments showed that both strains grew with sulfide and acetate but not malate, and the latter was subsequently eliminated from the routine subculturing medium. Liquid-medium cultures stored in the dark at 4 °C required subculture within 2 months, as longer incubation led to lysis and loss of culture viability. Cultures formed capsule material during stationary phase, which held the cells in a loose aggregation. Invariably, experiments showed that the physiological properties of strains BCH[T] and OCH-PHB were almost identical and that these strains possess only minor differences in their 16S rRNA gene sequence. For this reason, strain BCH[T] was studied in most detail and is described here as the type strain of a novel species in a new genus.

Morphological characteristics

Cells of strains BCH[T] and OCH-PHB were 1·5 μm in diameter and 3–4·5 μm in length (Fig. 1). Cells of both strains often occurred in pairs, division occurring by binary fission. Rapid, single-directional motility consistent with flagella-driven motility was observed. No evidence for gliding motility was seen. The cells stained
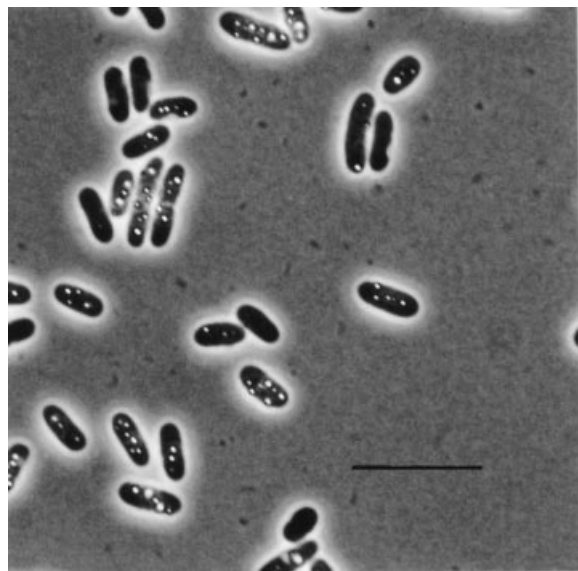

Fig. 1. Phase-contrast micrograph of cells of *Thiobaca trueperi* strain BCH[T] containing refractile sulfur globules. Bar, 10 μm.

Gram-negative. The cells produced opaque intracellular inclusions that had intense fluorescence after staining with Nile blue A, indicating that the inclusions were poly-3-hydroxybutyrate. Gas vesicles were never observed. The distinct morphology and cellular ultrastructure were apparent in cells growing exponentially under anaerobic and photoheterotrophic conditions (Fig. 2a, b). Besides the prominent, electron-translucent, spherical deposits of poly-3-hydroxybutyrate, dark-stained inclusions of varying dimensions were also recognized (Fig. 2, rhombus). These electron-dense inclusions obviously represent paracrystalline bodies since, to a certain extent, they showed a regular periodic substructure (Fig. 2c; white arrowheads) with edge-angles of approximately 120°. Often, these inclusions were connected or surrounded by an amorphous, particulate, dark cytoplasmic substance, which may represent precursor material used for the synthesis of the inclusion (Fig. 2c; open arrowhead). The cytoplasm was characterized by the presence of large numbers of vesicles (Fig. 2c, d; v) of medium electron transparency, with a mean diameter of $40·1 \pm 3·9$ nm ($n = 44$). Thin sections confirmed a rather thick, Gram-negative cell wall that had an overall thickness of $61·1 \pm 5·6$ nm ($n = 27$), supplemented by an S layer (Fig. 2d, e; sl). This S layer was generally present on most of the cells (Fig. 2a; single arrowhead), but was occasionally absent from others (Fig. 2a; double arrowheads). Characteristically, the S layer showed a triple-layered organization, the individual layers being of similar thickness (approx. 13 nm) (Fig. 2e; S1, S2, S3).

Photosynthetic pigments

Cultures of strain BCH[T] were brown. Absorption maxima of *in vivo* preparations occurred at 380, 462, 489, 524, 598, 805 and 875 nm. The peaks at 380, 598, 805 and 875 nm demonstrate the presence of bacteriochlorophyll *a* (Fig. 3). The *in vivo* bacteriochlorophyll absorption maxima that occurred at 805 and 875 nm shifted to 773 nm when extraction was in acetone:water (90%:10%, v/v). The absorption maxima at 462, 489 and 524 nm indicate that lycopene and rhodopin are present. Analysis of cell extracts by HPLC confirmed that the dominant carotenoid was lycopene. Other carotenoids were present in small quantities and were not analysed further.

Physiological characteristics

Strain BCH[T] grew photoheterotrophically under anoxic conditions. When agar-shake tubes were incubated with the caps open (atmospheric conditions), strain BCH[T] grew only in the very deepest parts of the tubes. Heterotrophic growth did not occur in the absence of light. In the presence of sulfide and bicarbonate, the following compounds were used as growth substrates (tested at 5 mM final concentration): acetate, propionate, fumarate, succinate, lactate and pyruvate. Substrates that were not used included formate,
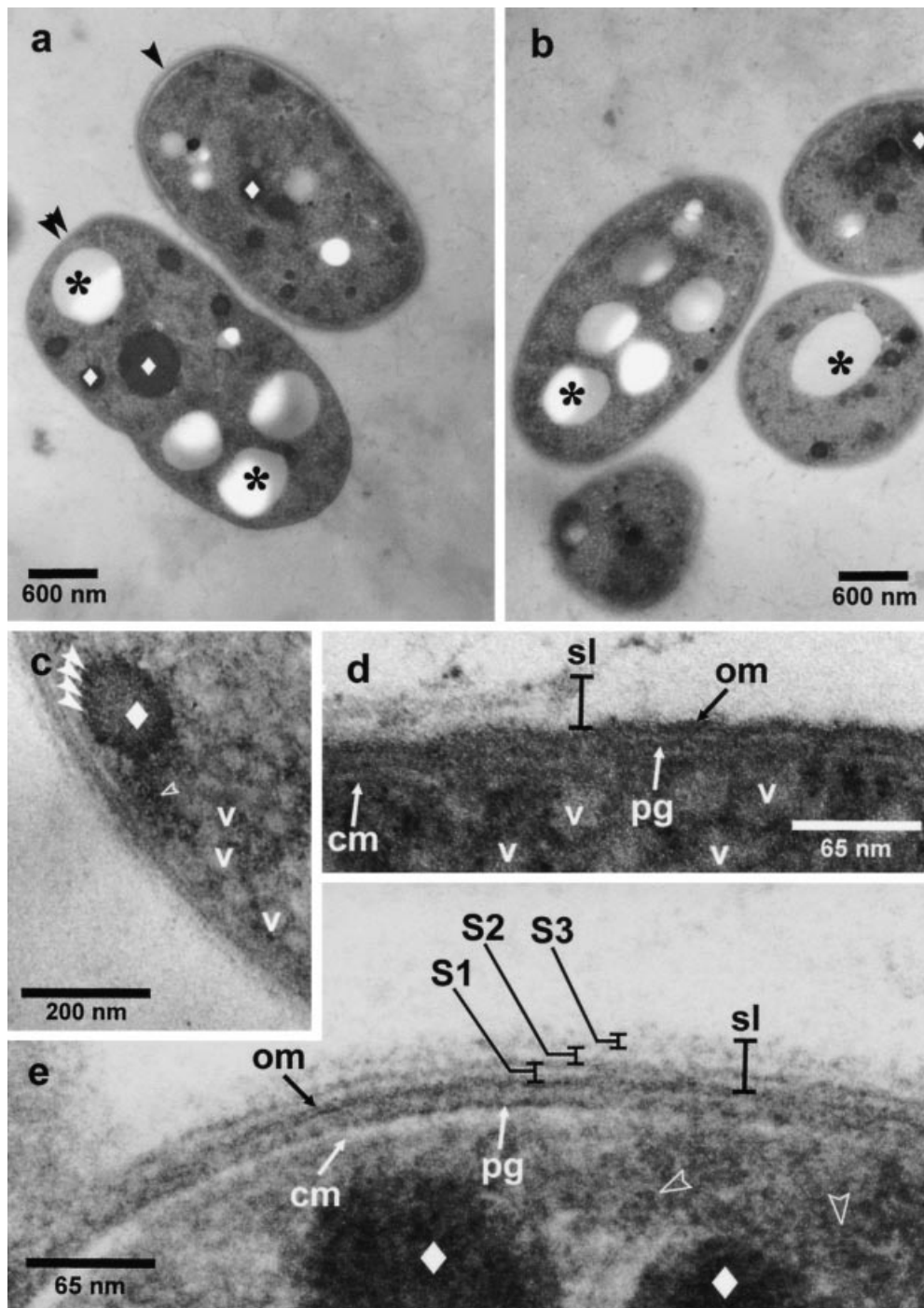
Fig. 2. Ultrastructure of *Thiobaca trueperi* strain BCH[T]. (a, b) Transversely and longitudinally sectioned cells, which contain electron-translucent poly-3-hydroxybutyrate inclusions (asterisks) and dark inclusions of various sizes (rhombuses). Double arrowheads indicate a cell that has lost its S layer, whereas a single arrowhead points to the S layer of a second cell. (c) The cytoplasm characteristically contains densely packed, electron-translucent vesicles (v). Dark inclusions, which often appear dark-rimmed, are characterized by periodic elements (white arrowheads) and are surrounded by an amorphous, dark, cytoplasmic substance (open arrowhead). (d) The S layer (sl) is partially present on the left. (e) Details of the cell wall. The overall thickness of the S layer is shown (bar labelled 'sl') and the triple-layered substructure is indicated (S1, S2, S3). The cytoplasmic membrane (cm) surrounds the cytoplasm, the murein layer (pg) is densely stained and the outer membrane (om) shows the typical 'double-track' features.
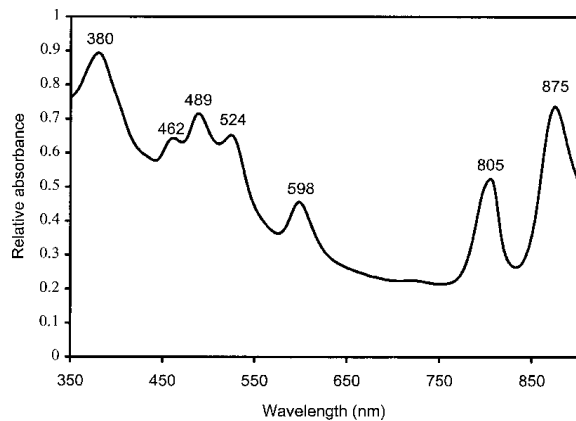
Fig. 3. Whole-cell spectral scan of strain BCH^T.

butyrate, valerate, palmitate, benzoate, ethanol, malate, citrate and glucose.

Strain BCH^T stored intracellular sulfur globules when incubated in the presence of hydrogen sulfide, and the sulfur globules were utilized upon extended incubation. No growth occurred with thiosulfate or molecular hydrogen. Both strains grew in the presence of iron(II) plus acetate; however, growth occurred at the expense of acetate, and iron(II) was not oxidized. No growth occurred in medium containing iron(II) without acetate. Growth occurred in medium containing up to 2·5 % (w/v) NaCl, optimum growth occurring between 0 and 0·5 % (w/v) NaCl. The optimum temperature for growth was between 25 and 30 °C, and 36 °C was the maximum temperature at which growth occurred. Although nitrogen fixation was not demonstrated by acetylene-reduction or $^{15}N_2$ studies, strain BCH^T continued to grow after five subcultures in medium with atmospheric nitrogen as the only nitrogen source. Similarly, growth occurred in ammonium-containing medium with argon in the headspace, but no growth occurred in ammonium-free medium under an argon atmosphere.

DNA base composition

The DNA base compositions for strains BCH^T and OCH-PHB were respectively 62·9 (SD = 0·67, $n = 5$) and 63·9 (SD = 0·04, $n = 5$) mol% G+C.

Phylogeny

Fig. 4 shows a dendrogram depicting the phylogenetic relationships of strains BCH^T and OCH-PHB within the family *Chromatiaceae*. Including other reference sequences or eliminating some resulted in some changes to the branching order, but these changes were only ever at nodes where bootstrap values were less than 70 %. Maximum-parsimony and maximum-likelihood analyses maintained those clusters that were

found using distance methods and supported by high bootstrap values. These clusters were (i) strains BCH^T and OCH-PHB, (ii) the three *Thiocapsa* species, (iii) strain Thd2, *Lamprocystis roseopersicina* and *Lamprocystis purpurea*, (iv) the four *Thiocystis* species, the two *Allochromatium* species, *Thermochromatium tepidum* and *Chromatium okenii*, (v) the two *Marichromatium* species and (vi) *Rhabdochromatium marinum* and *Thiorhodovibrio winogradskyi*. The order of branching was different, however, from that obtained using distance methods (data not shown). The low bootstrap confidences ascribed to the nodes between the clusters in the distance analyses, and the intracluster coherence but lack of stability in the intercluster relationships obtained in maximum-parsimony and maximum-likelihood analyses, suggest that the six clusters are phylogenetically coherent groupings and do not point to a closer relationship for strains BCH^T and OCH-PHB with respect to any one of the other five clusters.

The pairing of strains BCH^T and OCH-PHB separately from all other groupings was maintained and supported by all analyses (100 % bootstrap). The 16S rRNA genes of strains BCH^T and OCH-PHB had only 14 differences over the 1367 common nucleotide positions analysed. The Jukes–Cantor-corrected evolutionary distances to the closest relatives were as follows: 4·2, 7·8 and 6·0 % for *Thiocapsa roseopersicina*, *Thiocapsa rosea* and *Thiocapsa pendens*, respectively; and 5·0, 5·6 and 5·3 % for *L. roseopersicina*, *L. purpurea* and strain Thd2, respectively.

DISCUSSION

Normans Lagoon is an oxbow lake that contains extensive macrophyte growth and riparian vegetation. The consequence is that the lake sediments receive high organic carbon loads, leading to significant methanogenic activity in the sediments. The apparent domination of terminal oxidation by methanogenic organisms, as compared to sulfate-reducing species, led us to include only low levels of sulfide in the enrichment medium in the presence of malate and acetate (substrates commonly used by non-sulfur phototrophic micro-organisms; Imhoff & Trüper, 1992). The resulting brown-coloured pure cultures isolated after enrichment with this medium, namely strains BCH^T and OCH-PHB, were subsequently shown to be able to use sulfide as an electron donor for phototrophic growth. These strains were characterized and found to display characteristics of members of the family *Chromatiaceae*.

Phylogenetic analyses of the 16S rRNA gene sequences of strains BCH^T and OCH-PHB indicated that the two strains were closely related (98·7 % similarity) and that both strains were members of the family *Chromatiaceae*. However, the calculated evolutionary distances do not support a closer relationship with any particular genus included in our analyses. Strains BCH^T and OCH-PHB shared very similar morpho-
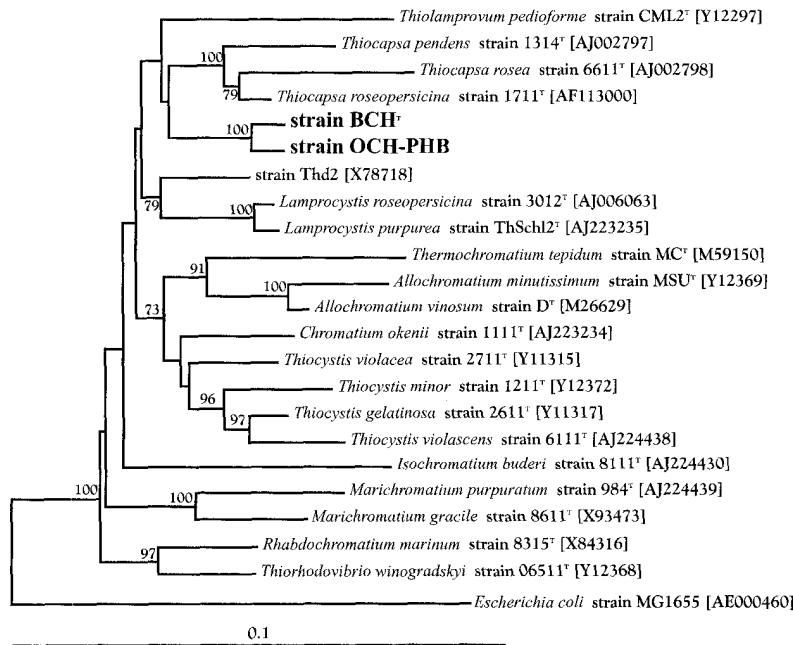
Fig. 4. Dendrogram depicting the phylogenetic relationships of strains BCH^T and OCH-PHB within the family *Chromatiaceae*, determined from 16S rRNA gene sequence analysis from pairwise evolutionary distances constructed using a least-squares method. Bar, 0·1 inferred nucleotide substitutions per nucleotide position. Numbers given at the nodes represent bootstrap percentage values (1000 analyses, values of less than 70 % not shown). GenBank/EMBL/DDBJ accession numbers are given in brackets.

Table 1. Summary of characteristics of *Thiobaca trueperi* and related phototrophic bacteria

Taxa are listed as: 1, *Thiobaca trueperi* BCH^T; 2, *Thiocapsa roseopersicina* 1711^T; 3, *Thiocapsa pendens* 1314^T; 4, *Thiocapsa rosea* 6611^T; 5, *Thiolamprovum pedioforme* CML2^T; 6, *Lamprocystis roseopersicina* 3012^T; 7, *Lamprocystis purpurea* ThSch12^T; 8, strain Thd2. Data were taken from this study and from Guyoneaud *et al.* (1998) (*Thiocapsa* species), Eichler & Pfennig (1988) (*Thiolamprovum pedioforme*), Pfennig *et al.* (1968) (*Lamprocystis roseopersicina*), Eichler & Pfennig (1986) and Tindall (1999) (*Lamprocystis purpurea*) and Ehrenreich & Widdel (1994) (strain Thd2). All taxa listed contain bacteriochlorophyll *a* and utilize acetate and pyruvate.

Characteristic	1	2	3	4	5	6	7	8
Cell morphology	Rod	Spherical	Spherical	Spherical	Spherical to oval	Spherical	Spherical to oval	Rod
Aggregate pattern	Single or pairs	Tetrads, small irregular aggregates	Irregular aggregates	Irregular aggregates	Platelets	Diplococci or long chains of aggregates	Single or forming irregular clumps	Single or forming irregular aggregates
Cell size (μm):								
Diameter	1·6	1·0–3·0	1·5–2·0	2·0–3·0	2·0	3–3·5	1·9–2·3	1·3
Length	3–4·5	–	–	–	2·0–3·0	–	2·0–3·2	2·0–3·0
Gas vesicles	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Carotenoid	Lycopene	Spirilloxanthin	Spirilloxanthin	Spirilloxanthin	Spirilloxanthin	Lycopenal	Okenone	NR
Colour of cell suspension	Brown	Pink-red	Pink-red	Pink-red	Pink to rose-red	Purple	Purple-red	Purple to brownish red
G+C content (mol%)	62·9	65·3	65·3	64·3	65·5	63·8	63·5	NR
Substrates used:								
Hydrogen	–	+	–	–	–	NR	–	+
Hydrogen sulfide	+	+	+	+	+	+	+	–
Thiosulfate	–	+	+	+	+	+*	+	–
Formate	–	–	–	–	–	–*	+	–
Propionate	+	+	+	+	–	+*	+	–
Malate	–	+	+	–	–	–*	–	–
Succinate	+	+	–	–	–	–*	–	–
Fumarate	+	+	–	–	–	–*	–	–
Glucose	–	–	+	–	–	+*	+	+
Glycerol	–	+	–	–	–	–*	–	NR

* B. Eichler and N. Pfennig (personal communication).

NR, Not reported.

logical properties but differed from their closest relatives (Table 1). The predominant morphology of *Thiocapsa* species, *L. purpurea* and *Thiolamprovum pedioforme* is spherical or spherical to oval-shaped cells, whereas the novel strains occur predominantly as rod-shaped cells. *Thiocapsa* species, *L. purpurea* and *Thiolamprovum pedioforme* also produce varied cell aggregates (Eichler & Pfennig, 1986; Pfennig & Trü-

per, 1992; Imhoff, 2001). A loose form of aggregation occurred in strain BCH[T] but the aggregation was only associated with stationary-phase cultures.

Like other members of the *Chromatiaceae*, strains BCH[T] and OCH-PHB possess bacteriochlorophyll *a*. The carotenoids present in members of the *Chromatiaceae* are diverse. The dominant carotenoid in *Thiocapsa roseopersicina*, *Thiocapsa pendens*, *Thiocapsa rosea* and *Thiolamprovum pedioforme* is spirilloxanthin, whereas *L. purpurea* possesses okenone and *L. roseopersicina* possesses lycopenal. Strains BCH[T] and OCH-PHB, in contrast, possess lycopene.

Strain BCH[T] possesses the vesicular arrangement of internal photosynthetic membranes characteristic of members of the family *Chromatiaceae*. We also observed dark-stained inclusions that morphologically resemble the 'polyhedral bodies' or 'carboxysomes' that have been described for *Thiobacillus neapolitanus* (Shively *et al.*, 1973) and the cyanobacterium *Gloeothece* (Golecki & Heinrich, 1991). However, the 'membrane' that surrounds the carboxysomes in *Thiobacillus neapolitanus* was not observed in strain BCH[T]. Although a high constitutive level of autotrophic enzymes was found in *Thiobacillus neapolitanus* (Kuenen & Beudeker, 1982), it is questionable whether the inclusions we observed would represent 'carboxysomes' *sensu stricto*, given the heterotrophic growth conditions used. Furthermore, because these inclusions did not show electron-dense contrast in unstained samples, and since no iron could be detected by electron energy-loss spectroscopy, they are not similar to the magnet-sensitive inclusions found in *Ectothiorhodospira shaposhnikovii* and *Rhodopseudomononas palustris* (Vainshtein *et al.*, 1997). The exact nature of the dark-stained inclusions remains unknown at this stage.

Strain BCH[T] differs in a number of physiological aspects from its nearest relatives (Table 1). Both *Thiocapsa roseopersicina* and strain BCH[T] are able to use a relatively diverse range of organic compounds, but strain BCH[T] is unable to use hydrogen. Strain BCH[T] also differs from *Thiocapsa roseopersicina* in its inability to use thiosulfate. Strain BCH[T] shares some physiological characteristics with other representatives of the genus *Thiocapsa*, such as the ability to use acetate and pyruvate; however, these characteristics appear to be spread widely across this group of phototrophic bacteria and so cannot be used as discriminatory characteristics. Strain BCH[T] is able to use succinate and fumarate but not thiosulfate, whereas the opposite applies to *Thiocystis* species. Strain Thd2 is closely related to strain BCH[T] but possesses several physiological characteristics that distinguish it from strain BCH[T]. Strain Thd2 is able to oxidize iron(II), hydrogen and sulfide when the latter is supplied as iron sulfide, but it cannot use free sulfide or thiosulfate. Strain BCH[T] is unable to use iron(II).

Evolutionary analyses do not support a closer relationship between our novel strains and members of any genus of the currently described phototrophic sulfur bacteria. Similarly, morphological and physiological characteristics do not lend support to the inclusion of strains BCH[T] and OCH-PHB in any of the currently described genera. We therefore propose that the latter strains represent a novel species of a new genus, *Thiobaca trueperi* gen. nov., sp. nov.

Description of *Thiobaca* gen. nov.

Thiobaca (Thi.o.ba′ca. Gr. n. *thios* sulfur; L. fem. n. *baca* berry, especially an olive; N.L. fem. n. *Thiobaca*, a berry with sulfur).

Cells are rod-shaped, occurring as single cells or in pairs. Motility is consistent with the presence of flagella. Cells stain Gram-negative. Division is by binary fission. Bacteriochlorophyll *a* and carotenoids are present. The internal photosynthetic membrane is of the vesicular type. Grows photolithoheterotrophically or photoorganoheterotrophically under anoxic conditions with sulfide as electron donor. Sulfur globules are accumulated transiently inside cells. The type species is *Thiobaca trueperi*.

Description of *Thiobaca trueperi* sp. nov.

Thiobaca trueperi (true′pe.ri. N.L. gen. n. *trueperi* of Trüper, named after Hans G. Trüper, a German microbiologist who has made a significant contribution to our knowledge of anoxygenic phototrophic bacteria).

Cells are rod-shaped, 1·5 μm in diameter, 3–4·5 μm long and motile. Gas vesicles are not formed. Cell suspension is brown in colour. Photosynthetic membranes are of the vesicular type. Contains bacteriochlorophyll *a*. Lycopene is the dominant carotenoid. Sulfate is not assimilated. Uses hydrogen sulfide as an electron donor for photolithoheterotrophic growth. Sulfur globules are stored inside the cells as intermediate products. No growth occurs with hydrogen, thiosulfate or iron(II). Acetate, propionate, pyruvate, succinate and fumarate are used in the presence of sulfide and bicarbonate. The optimum temperature for growth is between 25 and 30 °C, the maximum being 36 °C. The G + C content of the DNA is 63 mol % The habitat is freshwater sediment. The type strain is strain BCH[T]. Strains BCH[T] and OCH-PHB have respectively been deposited at the DSMZ as DSM 13587[T] and DSM 13588 and at the ATCC as ATCC BAA-132[T] and ATCC BAA-133.

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