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ORIGINAL PAPER

Blastochloris tepida, sp. nov., a thermophilic species of the bacteriochlorophyll *b*-containing genus *Blastochloris*

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Abstract

A new taxon is created for the thermophilic purple nonsulfur bacterium previously designated as *Rhodopseudomonas* strain GI. Strain GI was isolated from a New Mexico (USA) hot spring microbial mat and grows optimally above 40 °C and to a maximum of 47 °C. Strain GI is a bacteriochlorophyll *b*-containing species of purple nonsulfur bacteria and displays a budding morphology, typical of species of the genus *Blastochloris*. Although resembling the species *Blc. viridis* in many respects, the absorption spectrum, carotenoid content, and lipid fatty acid profile of strain GI is distinct from that of *Blc. viridis* strain DSM133^T and other recognized *Blastochloris* species. Strain GI forms its own subclade within the *Blastochloris* clade of purple nonsulfur bacteria based on comparative 16S rRNA gene sequences, and its genome is significantly larger than that of strain DSM133^T; average nucleotide identity between the genomes of *Blc. viridis* and strain GI was below 85%. Moreover, concatenated sequence analyses of PufLM and DnaK clearly showed strain GI to be distinct from both *Blc. viridis* and *Blc. sulfoviridis*. Because of its unique assortment of properties, it is proposed to classify strain GI as a new species of the genus *Blastochloris*, as *Blc. tepida*, sp.n., with strain GI^T designated as the type strain (= ATCC TSD-138 = DSM 106918).

Keywords *Blastochloris tepida* · Bacteriochlorophyll *b* · Purple nonsulfur bacteria · Hot spring phototrophic bacteria

Introduction

Several species of phototrophic purple bacteria are thermophilic, and the first of these to be isolated was the purple sulfur bacterium *Thermochromatium* (formerly *Chromatium*) *tepidum*. This phototroph grows to a maximum temperature of 57 °C and has a growth temperature optimum near 50 °C (Madigan 1984, 1986; Castenholz and Pierson 1995). No purple nonsulfur bacteria are known that grow at 50 °C but several species grow optimally at 40 °C, and these include species of *Rhodopseudomonas*, *Rhodoplanes* (*Rpl.*), and *Rubrivivax* (Namsaraev et al. 2003; Okamura et al. 2007; Stadtwald-Demchick et al. 1990; Favinger et al. 1989; Hiraishi 2017a, b; Hisada et al. 2007). *Rhodoplanes* (formerly *Rhodopseudomonas*) *cryptolactis* (now *Rpl. tepidamans*) and *Rhodocista centenaria* (formerly *Rhodospirillum centenum*) are the most thermotolerant of these phototrophs, growing up to 45 °C. All of these purple bacteria

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Genomic accession numbers *Blastochloris tepida* strain GI^T 16S rRNA gene sequence, MG725814; *Blastochloris tepida* strain GI^T complete genome sequence, AP018907. Culture accession numbers: *Blastochloris tepida* strain GI^T DSM 106918; ATCC TSD-138.

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contain bacteriochlorophyll (BChl) *a* as their chlorophyllous pigment.

Nearly 30 years ago, two of us isolated a budding purple bacterium containing BChl *b* from a hot spring microbial mat and reported on its basic physiology, including in particular the fact that the organism grew optimally at 42 °C and had a maximum growth temperature of 47 °C (Resnick and Madigan 1989). Since phylogenetic data were lacking at that time, this phototroph was not formally described as a new species and was simply referred to as *"Rhodopseudomonas* strain GI", although it was assumed that the isolate was related to the BChl *b*-containing budding species *Blastochloris* (then *Rhodopseudomonas*) *viridis* (Resnick and Madigan 1989; Drews and Giesbrecht 1966; Hiraishi 1997). In addition to its moderately thermophilic phenotype, strain GI displayed several other properties typical of purple nonsulfur bacteria containing BChl *b* (Madigan and Jung 2009; Hoogewerf et al. 2003; Keppen and Gorlenko 1975) but not previously reported in thermotolerant species, including the ability to fix N_2 at temperatures as high as 48 °C Resnick and Madigan 1989).

Here we complement our original study of strain GI with new data on the morphology, spectral properties, fatty acid and pigment compositions, and phylogeny and genomics of this organism, and assemble these data to support the establishment of a new species of the genus *Blastochloris* (*Blc.*), *Blc. tepida*, to accommodate strain GI and closely related isolates. The proposed species epithet reflects the thermophilic nature of strain GI, a phenotype unlike that of any of the currently recognized species of *Blastochloris*: *Blc. viridis*, *Blc. sulfoviridis*, and *Blc. gulmargensis* (Drews and Giesbrecht 1966; Keppen and Gorlenko 1975; Ramana et al. 2011).

Materials and methods

Isolation and microscopy

Strain GI was enriched and isolated at 45 °C from a sample of a cyanobacterial microbial mat that developed in the outflow of a small hot spring (47 °C) at Soda Dam, located within the Santa Fe National Forest (New Mexico, USA; GPS N35°793′, W106°685′); the microbial mat was dominated by species of the filamentous cyanobacteria *Oscillatoria* and *Phormidium* (Resnick and Madigan 1989). Cultures of strain GI were purified in repeated agar shake tubes and grown phototrophically (anoxic/light) in the mineral salts–malate medium described in Resnick and Madigan (1989) supplemented with 0.05% (final concentration) each of sodium ascorbate and yeast extract. Cultures of strain GI were stored at − 80 °C until accessioned into the DSMZ and ATCC in 2018.

Electron microscopy of cells of strain GI was performed as described in Cole et al. (2014) with the exception that an Orion helium ion microscope (Zeiss, Peabody, MA, USA) was used in place of a conventional scanning electron microscope. Fatty acid determinations were performed by MIDI (Newark, DE, USA) using their standard fatty acid methyl ester methods on lipids extracted from cells of strain GI grown at 43 °C. Absorption spectra were performed on membrane suspensions (chromatophores) prepared as described by Nagatsuma et al. (2019).

Phylogenetic and genomic analyses

Phylogenetic analysis of strain GI based on 16S rRNA gene sequence comparisons with other purple nonsulfur bacteria were performed as previously described in Kempher and Madigan (2012).

To obtain the genome sequence of strain GI, genomic DNA was extracted from a cell pellet using the Genome-Tip 500G DNA extraction kit (Qiagen, Venlo, The Netherlands). A sequencing library was prepared using the Accel-NGS XL Library Kit (Swift Biosciences, MI, USA) according to the manufacturer's protocol, and DNA fragments of less than 20 kb were removed using the BluePippin size-selection system (Sage Science, MA, USA). The final gene library was run on a PacBio RSII sequencer with SMRT cell v3 and P6-C4 v2 chemistry, yielding a total of 64,481 reads and 973,705,006 bp.

PacBio reads of more than 10 kb were assembled using the HGAP3 assembler program with default settings (Chin et al. 2013). In addition, a standard Illumina 600-bp paired-end library was constructed and sequenced on a HiSeq 2500 sequencer (Illumina, CA, USA). Sequences of 250 bases comprising 1,585,689 × 2 raw reads were used to improve the assembled contig with the Pilon (version 1.22) software tool (Walker et al. 2014). Gene prediction and functional annotation were carried out using the DDBJ Fast Annotation and Submission Tool (DFAST) (Tanizawa et al. 2017).

Amino acid sequences of PufL, PufM, and DnaK proteins from *Blc. viridis*, *Blc. sulfoviridis*, and *Rpl. elegans* in the public sequence database were aligned with those of strain GI using MAFFT version 7.407 with default parameters (Katoh and Castresana 2014). Poorly aligned regions were excluded using GBlocks version 0.91 b with default parameters (Talavera and Castresana 2007). After concatenating the three-protein sequence alignments, a maximum likelihood tree was inferred using RAxML version 8.2.10 with the WAG + Γ model and 1000 bootstrap replicates (Stamatakis 2014).

Results and discussion

Morphology, pigments, and fatty acids

Pure cultures of strain GI were grown routinely under phototrophic conditions at 42 °C. Cells were motile and stained Gram-negative. Individual cells measured approximately 1 × 1.5–2 µm and showed a budding morphology. Although the diameter of cells was fairly constant, cell length varied depending on what stage a given cell was in during the budding process, as shown in scanning electron micrographs (Fig. 1a). Thin sections of cells revealed a lamellar-type photosynthetic membrane system running parallel to the long axis of the cell (Fig. 1b), a feature characteristic of budding purple bacteria, including *Blc. viridis* (Drews and Giesbrecht 1966; Imhoff et al. 1984). Negatively stained transmission electron micrographs showed cells to contain one or more polar flagella (Fig. 1c).

Phototrophic cultures of strain GI were greenish yellow in color, and absorption spectra of chromatophore preparations were similar to yet distinct from that of the type strain of *Blc. viridis* (strain DSM 133^T), as shown in Fig. 2. The near infrared (Q_y) absorption maximum of strain GI lies at 1011 nm compared with that of 1014 nm in *Blc. viridis*; absorption maxima beyond 1000 nm is the major characteristic of BChl *b*-containing phototrophs (Scheer et al. 1974; Tsukatani et al. 2019). Other major maxima in the absorption spectra of *Blc. viridis* and strain GI were similar, although absorbance in the carotenoid region of the spectrum of *Blc. viridis* was noticeably greater than that of strain GI when both spectra were normalized to absorbance at their respective Q_y bands of BChl *b* in light-harvesting complex I around 1010 nm (Fig. 2); this suggests differences in the carotenoid composition between the two species. In this connection, a major carotenoid of strain GI was 1,2-dihdyrolycopene. Only trace amounts of this pigment are present in cells of *Blc. sulfoviridis*, and although detectable in *Blc. viridis*, 1,2-dihdyrolycopene content of this species is only one-fifth that of strain GI (Table 1). Strain GI also produced significant levels of lycopene and 1,2-dihydro-3,4-didehydrolycopene—carotenoids present in only trace amounts in *Blc. viridis* and *Blc. sulfoviridis*—and had the lowest levels of 1,2-dihydroneurosporene of all *Blastochloris* species (Table 1). Taken collectively, these results suggest that phytoene desaturase (CrtI), a major enzyme in the biosynthetic pathway of lycopene (which is a precursor of downstream lycopene derivatives), may be expressed at higher levels in strain GI than in other *Blastochloris* species.

Significant differences were also observed in the fatty acid composition of strain GI compared with other *Blastochloris* species. Strain GI showed over twice the $C_{16:0}$ content of *Blc. viridis* and *Blc. sulfoviridis* (Kompantseva et al. 2007) and nearly twice that of *Blc. gulmargensis* (Table 1). Moreover, although the $C_{18:0}$ fatty acid content of strain GI was notably higher than that of the other species, the unsaturated C_{18} content of strain GI was lower. Collectively, these differences likely reflect the thermophilic phenotype of strain GI, where saturated fatty acids would be predicted to lend more stability to membrane lipids.

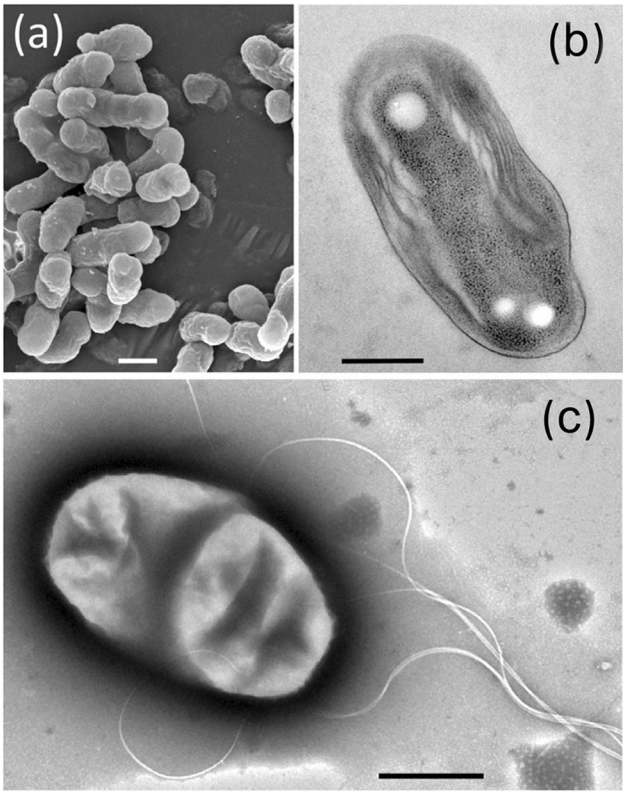

Fig. 1 Electron micrographs of cells of strain GI. **a** SEM of phototrophic cells, marker bar 1 µm. **b** Thin sectioned TEM, marker bar 0.5 µm. Note lamellar membranes. **c** Negatively stained TEM, marker bar 0.5 µm. Note polar flagella

Physiology

Several aspects of the physiology of strain GI were summarized in Resnick and Madigan (1989), in particular aspects of temperature requirements and tolerances, carbon and nitrogen nutrition, growth modes, and nitrogen fixation. Strain GI was isolated from an alkaline calcareous warm spring microbial mat composed primarily of cyanobacteria and *Chloroflexus* and in most respects displayed the physiology of a typical purple nonsulfur bacterium. That is, growth of strain GI was best under photoheterotrophic conditions at neutral pH with malate or pyruvate as carbon sources. In the presence of bicarbonate, butyrate was also a good carbon source for strain GI; this fatty acid is not used by

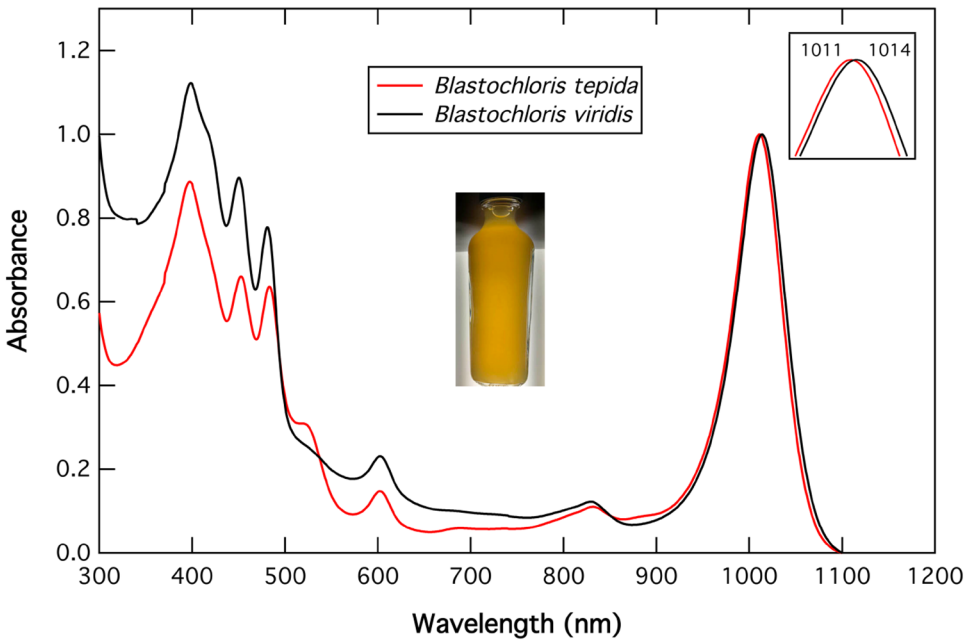

Fig. 2 Absorption spectra of chromatophores prepared from cells of *Blastochloris tepida* strain GI and *Blastochloris viridis* strain DSM 133^T. The bottle culture is a phototrophic culture of strain GI

Table 1 Major carotenoids and fatty acids of *Blastochloris tepida* strain GI compared with those from *Blastochloris viridis*, *Blastochloris sulfoviridis*, and *Blastochloris gulmargensis*

	Blc. tepida^T	*Blc. viridis*^{T}	*Blc. sulfoviridis*^{T}	*Blc. gulmargensis*^{T}
Carotenoid (mol %)				
Neurosporene	4	11	4[a]	10
1,2-Dihydroneurosporene	47	80	70	72
Lycopene	5	1	ND	2
1,2-Dihydrolycopene	34	7	1	10
1,2-Dihydro-3,4-didehydrolycopene	11	1	2	5
Fatty acid (% of total)				
$C_{16:0}$	22.3	9.7	9.6	13.8
$C_{16:1}\omega7c$	4.2	8.4	6.3	6
$C_{16:3}\omega7c$	1.7	ND	ND	ND
$C_{17:0}/_{17:0}$ 10-methyl	3.5	ND	ND	ND
$C_{17:1}\omega8c$	ND	1.9	0.6	ND
$C_{18:0}/_{18:0}$ 3OH	5.1	0.6	0.6	1.2
$C_{18:1}\omega7c$	57	76	77	68
$C_{20:1}\omega7c$	1.8	3.6	3.0	3
Sum C_{16}	29.6	18.7	17.3	23.8
Sum C_{18}	63.6	76.9	78.1	68.9

Carotenoid data for *Blc. sulfoviridis* and fatty acid data for *Blc. viridis*, *Blc. sulfoviridis,* and *Blc. gulmargensis* taken from Ramana et al. (2011) and Kompantseva et al. (2007)

ND not detected

Blc. viridis (Resnick and Madigan 1989; Trüper and Imhoff 1989). Although some aromatic compounds are used by select *Blastochloris* isolates (Zengler et al. 1999), benzoate was not used by strain GI or by *Blc. viridis* (Resnick and Madigan 1989; Trüper and Imhoff 1989). Chemotrophic dark growth on malate occurred in strain GI, but only at reduced oxygen tensions. In addition, strain GI required a reduced source of sulfur for biosynthetic purposes (thiosulfate or low levels of sulfide were preferred sources) and biotin as a growth factor (Resnick and Madigan 1989). In its natural habitat, this reduced biosynthetic sulfur source

requirement is likely met by the ~ 100 µM sulfide detected in the Soda Dam spring water.

In requiring a reduced sulfur source, strain GI resembles *Blc. sulfoviridis*, which also has such a requirement (Keppen and Gorlenko 1975); other *Blastochloris* species can use sulfate for biosynthetic sulfur needs (Ramana et al. 2011; Trüper and Imhoff 1989). In requiring biotin, strain GI resembles all other *Blastochloris* species. All *Blastochloris* species other than strain GI also require para-aminobenzoic acid (PABA) and some species require pyridoxal phosphate as well (Ramana et al. 2011; Trüper and Imhoff 1989).

A defining feature of the physiology of strain GI is its growth temperature profile compared with that of other *Blastochloris* species and supporting data in this regard were published in Resnick and Madigan (1989). Optimal growth of strain GI occurred at 42 °C, and no growth occurred at 28 °C or 48 °C; the growth temperature range was 30–47 °C. This stands in contrast to the temperature profiles of *Blc. viridis* and *Blc. sulfoviridis*, both of whose growth temperature optima lie under 30 °C (Drews and Giesbrecht 1966; Keppen and Gorlenko 1975; Trüper and Imhoff 1989).

At 47 °C, strain GI grew on either ammonia or N_2 as nitrogen sources, indicating that this *Blastochloris* species synthesizes a thermostable nitrogenase system (nitrogenase activity could still be detected at 48 °C, slightly above the maximum growth temperature, Resnick and Madigan 1989). Since the microbial mat from which strain GI was isolated had a temperature of 47 °C and such mats are typically N-deficient, it seems likely that strain GI fixes N_2 in situ. If so, this organism may supply fixed nitrogen not only to itself but also to the filamentous nonheterocystous cyanobacteria and *Chloroflexus* that formed the matrix of the Soda Dam microbial mat; all of these phototrophs are nondiazotrophic (Resnick and Madigan 1989; Heda and Madigan 1986). Indeed, because of their N_2-fixing abilities and their unique absorption properties, BChl *b*-containing purple bacteria may be widely distributed in hot spring microbial mats. The observation that strain GI-like purple bacteria exist in Japanese (Hisada et al. 2007) and Russian (Namsaraev et al. 2003) hot spring mats containing filamentous cyanobacteria and *Chloroflexus*, supports this hypothesis.

Phylogeny and correlation with temperature tolerances

The phylogeny of strain GI was determined by 16S rRNA gene sequencing, and the results are shown in Fig. 3. The genus *Blastochloris* forms a clade within the *Alphaproteobacteria* and its closest relatives are species of the genus *Rhodoplanes* (*Rpl.*), in particular, the thermotolerant BChl *a*-containing species *Rpl. tepidicaeni*, *Rpl. tepidamans*, and *Rpl. azumiensis* (Hiraishi 2017a, b). More distant relatives include the well-known purple nonsulfur bacteria *Rhodopseudomonas palustris*, and *Rhodoblastus acidophilus* (Fig. 3). All of these phototrophs are united by their characteristic budding growth mode, lamellar photosynthetic membrane systems, and production of BChl *a* (Hiraishi 2017a, b; Trüper and Imhoff 1989).

Within the *Blastochloris* clade, three subclades exist, one containing *Blc. viridis* and *Blc. gulmargensis*, one containing *Blc. sulfoviridis* strains, and a third containing strain GI (*Blc. tepida*) and strain TUT3225, a *Blastochloris*

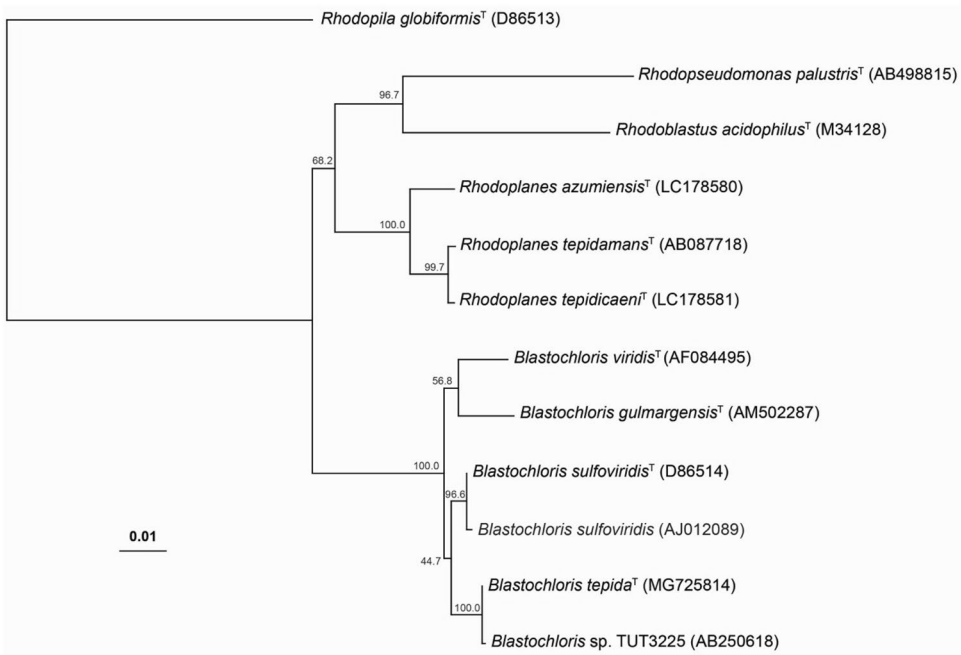

Fig. 3 Phylogenetic tree of species of *Blastochloris* and *Rhodoplanes* and nearby relatives of purple nonsulfur bacteria based on 16S rRNA gene sequence comparisons. Approximately 1400 nucleotides were used in the analysis and bootstrap values (1000 replications) are indicated at the nodes. Strain designations are as follows: *Rpi. globiformis* DSM 161^T; *Rps. palustris* ATTC 17001^T; *Rbl. acidophilus* DSM 137^T; *Rpl. azumiensis* NBRC 112816^T; *Rpl. tepidamans* DSM 9987^T; *Rpl. tepidicaeni* NBRC 112815^T; *Blc. viridis* DSM 133^T; *Blc. gulmargensis* DSM 19786^T; *Blc. sulfoviridis* DSM 729^T; *Blc. sulfoviridis* ToP1; *Blc. tepida* DSM 106918^T; *Blc.* sp. TUT3225

sp. isolated from a Japanese hot spring (Hisada et al. 2007) (Fig. 3). Strain TUT3225 grows up to 45 °C with an optimum at 42 °C (Hisada et al. 2007), thus it is slightly less thermotolerant than strain GI. The three *Rhodoplanes* species shown in Fig. 3 all grow optimally at 40 °C and grow up to a maximum of 45 °C. However, none are capable of growth at 47 °C, as is strain GI (Resnick and Madigan 1989). In contrast to these moderately thermophilic species, *Blc. viridis*, *Blc. sulfoviridis*, and *Blc. gulmargensis* are all mesophilic phototrophs, with temperature optima of 25–30 °C (Drews and Giesbrecht 1966; Keppen and Gorlenko 1975; Ramana et al. 2011; Zengler et al. 1999). Thus, strain GI is the most thermophilic of all known hot spring purple nonsulfur bacteria.

Table 2 Comparison of the genome features between *Blc. viridis* and *Blc. tepida*

Feature	*Blastochloris* species	
	Blastochloris tepida GI^T	*Blastochloris viridis* DSM 133Ta
Genome size (bp)	3,949,390	3,726,627
Plasmids	None	None
G + C content	68.3%	67.9%
Predicted open-reading frames	3606	3298
Average-coding sequence (bp)	935	963
Total coding ratio (%)	85.3	85.2
tRNA	56	53
rRNA	6	9

[a]Deposited data for the genome sequence of *B. viridis* DSM 133 (AP014854.2) was obtained from the GenBank database and then re-annotated by the DFAST pipeline (Tanizawa et al. 2017), which was also used for the annotation for the *Blc. tepida* genome sequence (AP018907) in this study. Note that the data shown for *Blc. viridis* are based on the re-annotation

Genome sequence and functional gene comparisons

The genome of strain GI was sequenced and assembled into a single contig, and a summary of the genome characteristics is presented in Table 2. Compared with the genome of *Blc. viridis* DSM 133^T (Tsukatani et al. 2015; Liu et al. 2016), the strain GI genome is significantly larger, containing 308 additional genes (Table 2). The GC content of the two genomes also differs slightly between the two organisms, but both genomes reside on single chromosomes with no plasmids detected (Table 2).

Virtually all of the genes that encode proteins of the reaction center and light-harvesting complexes and enzymes of BChl and carotenoid biosynthesis in purple nonsulfur bacteria are grouped together in the genome in a "photosynthetic gene cluster" (PGC) (Alberti et al. 1995; Nagashima and Nagashima 2013). PGCs also exist in *Blc. viridis* and strain GI; however, gene synteny in the PGCs of strain GI and *Blc. viridis* were not identical. In particular, the *bchIDO-crtIB* region of the two species PGCs were inverted (Fig. 4). Moreover, an unusual characteristic of the PGC of *Blc. viridis* compared with that of other phototrophic purple bacteria is that some genes encoding Calvin cycle proteins (*cbb* and related genes) are embedded within its PGC (Tsukatani et al. 2015; Liu et al. 2016); this was also true of the strain GI PGC (Fig. 4).

Computational analysis of the potential comprehensive functions (functionome) of the *Blc. viridis* and strain GI genomes with the MAPLE system (Takami et al. 2016) showed no significant difference in metabolic pathway content of the two species. Many genome rearrangements and frequent repetitive sequences were observed, and overall, the syntenic structure of the two species' genomes was only weakly conserved. This was supported by an average nucleotide identity between *Blc. viridis* and strain GI protein-encoding genes of 84.1%; values below 85% correlate strongly with genomic DNA:DNA hybridization values of

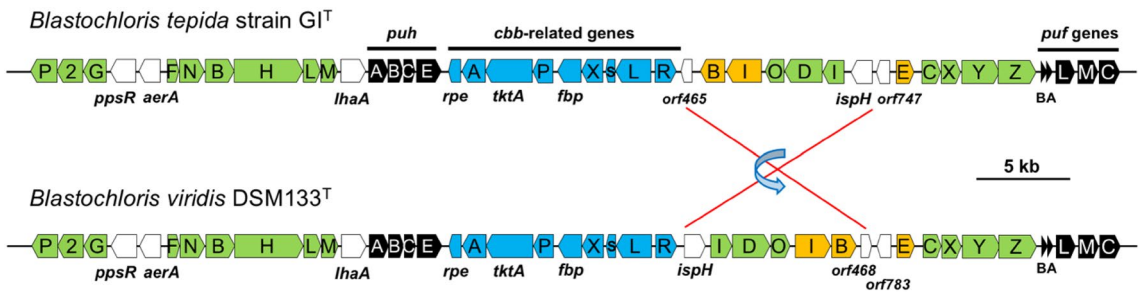

Fig. 4 Comparison of the photosynthetic gene clusters in the genomes of *Blc. viridis* strain DSM133^T and *Blc. tepida* strain GI. Genes are represented by rectangles pointing in the direction of transcription. Genes for BChl (*bch*) and carotenoid (*crt*) biosynthesis are shown in green and orange, respectively. The *puf* and *puh* genes encoding the reaction center and light-harvesting 1 complexes and related products are shown in black. Calvin cycle genes are shown in blue. Open-reading frames without an assigned gene name were annotated as hypothetical proteins

70% or lower, a standard used for years to delineate distinct species (Goris et al. 2007). We thus conclude that *Blc. viridis* and strain GI are distinct species.

The genome of *Blc. sulfoviridis* has not been sequenced and so whole genome comparisons of the type done between strain GI and *Blc. viridis* were not possible. However, because *Blc. sulfoviridis*, like *Blc. viridis*, is a close relative of strain GI (Fig. 3), additional genomic evidence was sought to differentiate strain GI from *Blc. sulfoviridis*. Three functional genes of phylogenetic importance have been sequenced in the type strain of *Blc. sulfoviridis* and are available in public databases; these include *dnaK*, *pufL*, and *pufM*. The two *puf* genes encode structural proteins in the photosynthetic reaction center of purple bacteria while *dnaK* encodes a key molecular chaperone in the bacterial heat shock response. Sequence comparisons of *pufLM* have been shown to be useful for resolving the phylogenies of purple bacteria (Swingley et al. 2009; Tank et al. 2009). The sequence of *dnaK* is highly conserved but also contains a variable region whose sequence has proven to be an alternative marker to 16S rRNA gene sequences for resolving the phylogenies of a broad range of *Alphaproteobacteria*, including phototrophic purple bacteria such as *Blastochloris* (Stepkowski et al. 2003).

The amino acid sequences of PufLM and DnaK deduced from the genome sequence of strain GI were, therefore, aligned with those of *Blc. viridis*, *Blc. sulfoviridis*, and *Rpl. elegans* in a concatenated analysis, and the results are shown in Fig. 5. The maximum likelihood tree constructed from this analysis clearly distinguishes strain GI from all other species of *Blastochloris*. In fact, the concatenated three-protein tree shows strain GI to be a closer relative to *Blc. viridis* than to *Blc. sulfoviridis* (Fig. 5). And, since *Blc. viridis* and strain GI are clearly distinct species on the basis of whole genome analyses, we conclude that strain GI and *Blc. sulfoviridis* are similarly distinct and separate species.

Final remarks and taxonomic conclusions

The earliest breakthroughs in studies of the structure of photosynthetic reaction center complexes occurred with the purple bacterium *Blc. viridis* (Michel 1982; Deisenhofer et al. 1985). This phototroph was ideal for several reasons, but in particular because it produced only a core and not a peripheral antenna complex. Now with the discovery of strain GI, a thermophilic species of *Blastochloris* is available that will likely produce more thermotolerant biomolecules than mesophilic species of *Blastochloris*. These, along with a complete genome sequence to support structural studies, suggests that BChl *b*-containing purple bacteria may continue to provide ideal model systems for unraveling the functional details of early events in photosynthesis.

Based on the assemblage of phenotypic properties of strain GI along with its unique phylogenetic position and genomic characteristics, this organism is proposed as a new species of the genus *Blastochloris*, as *Blastochloris tepida* sp.n. Strain GI^T has been accessioned into the American Type Culture Collection as ATCC TSD-138 and into the Deutsche Sammlung von Mikroorganismen und Zellkulturen as DSM 106918.

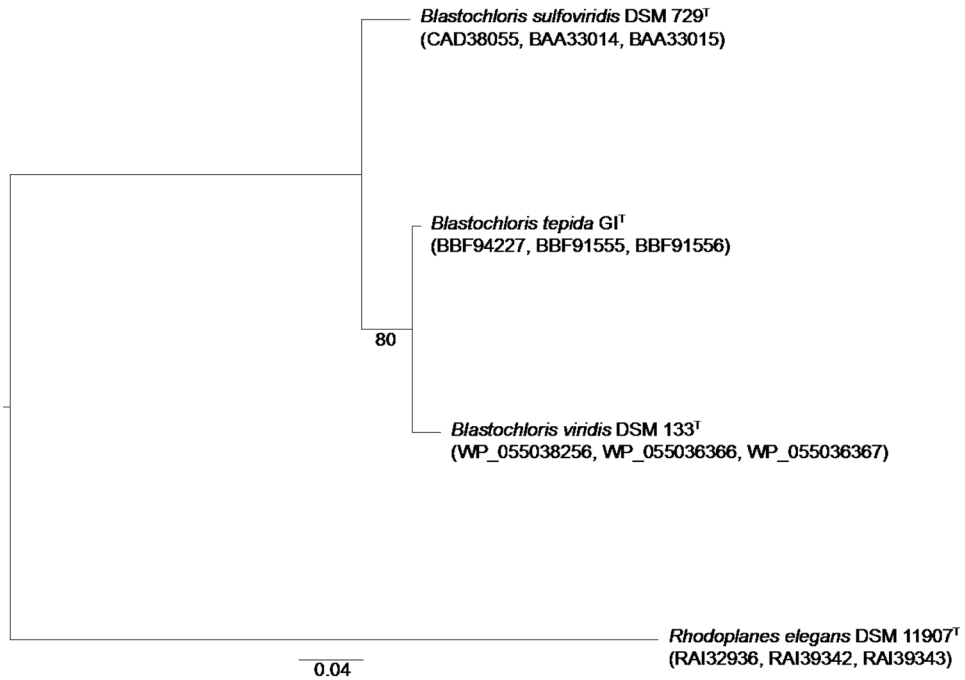

Fig. 5 Phylogenetic tree based on the concatenated protein sequences of PufLM and DnaK. Approximately 570 sites were used in the analysis. Bootstrap values (1000 replications) are indicated at the nodes

Description of *Blastochloris tepida* sp. nov

Blastochloris tepida (te'pi.da. L. fem. adj. *tepida* lukewarm).

Cells are Gram-negative phototrophic budding rods measuring 1 × 1.5–2 µm and motile by polar flagella. Phototrophic cultures are greenish yellow in color and intracytoplasmic photosynthetic membranes are present as lamellae running parallel to the long axis of the cell. Contains BChl *b* and 1,2-dihydroneurosporene, 1,2-dihydrolycopene, and 1,2-dihydro-3,4-didehydrolycopene as major carotenoids; lycopene and neurosporene are minor carotenoids. Membranes from phototrophic cells show absorption maxima at 1011, 602, 521, 483, 452, and 397 nm. Photoheterotrophic growth is best in mineral media containing 0.05% yeast extract and either malate or fumarate as primary carbon sources; glucose and fructose support lesser growth. Acetate, butyrate, and pyruvate support good growth but only in the presence of bicarbonate. Succinate is a poor growth substrate, and benzoate, pentoses, short-chain alcohols, and the amino acids aspartate and glutamate are not used. A reduced source of sulfur and the B-vitamin biotin are required for growth (0.01% thiosulfate and biotin fully replace yeast extract for optimal growth). Chemotrophic dark (respiratory) growth occurs only at reduced oxygen tensions. Ammonia, glutamine, and N_2 are the best nitrogen sources for growth. Growth is optimal at 42 °C and the growth temperature range is 30–47 °C. Best growth occurs at pH 6.8 and in media lacking NaCl. The type strain of *Blastochloris tepida* is strain GI^T. Cultures of *Blc. tepida* strain GI^T have been accessioned into the DSMZ (DSM 106918) and ATCC (TSD-138). The 16S gene sequence of *Blc. tepida* strain GI^T has been deposited into DDBJ/Genbank as MG725814 and its complete genome sequence as AP018907.

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Compliance with ethical standards

Conflict of interest The authors declare they have no financial or other conflicts of interest.

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