The genome sequence of the giant phototrophic gammaproteobacterium *Thiospirillum jenense* gives insight into its physiological properties and phylogenetic relationships

Johannes F. Imhoff¹ · Terrance E. Meyer² · John A. Kyndt³

Received: 23 June 2020 / Revised: 21 July 2020 / Accepted: 24 July 2020 / Published online: 5 August 2020
© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

In a conserved culture of the purple sulfur bacterium *Thiospirillum jenense* DSM216^T, cells of this species were easily recognized by cell morphology, large-size spirilla and visible flagellar tuft. The *Tsp. jenense* genome is 3.22 Mb in size and has a GC content of 48.7 mol%. It was readily identified as a member of the *Chromatiaceae* by the complement of proteins in its genome. A whole genome comparison clearly placed *Tsp. jenense* near *Thiorhodovibrio* and *Rhabdochromatium* species and somewhat more distant from *Thiohalocapsa* and *Halochromatium* species. This relationship was also found with the sequences of the photosynthetic reaction center protein PufM. The genome sequence supported important properties of this bacterium: the presence of ribulose-bisphosphate carboxylase and enzymes of the Calvin cycle of autotrophic carbon dioxide fixation but the absence of carboxysomes, an incomplete tricarboxylic acid cycle and the lack of malate dehydrogenase, the presence of a sulfur oxidation pathway including adenylylsulfate reductase (*aprAB*) but absence of assimilatory sulfate reduction, the presence of hydrogenase (*hoxHMFYUFE*), nitrogenase and a photosynthetic gene cluster (*pufBALMC*). The FixNOP type of cytochrome oxidase was notably lacking, which may be the reason that renders the cells highly sensitive to oxygen. Two minor phototrophic contaminants were found using metagenomic binning: one was identified as a strain of *Rhodopseudomonas palustris* and the second one has an average nucleotide identity of 82% to the nearest neighbor *Rhodoferrax antarcticus*. It should be considered as a new species of this genus and *Rhodoferrax jenense* is proposed as the name.

Keywords *Thiospirillum jenense* · *Thiospirillum* genome · Purple sulfur bacteria · Chromatiaceae · New *Rhodoferrax* species

Introduction

It was the immense size of the bacterium, its colored cultures, the content of highly refractive sulfur globules combined with the active motility and phototactic behavior that made *Thiospirillum jenense* an interesting study object

for almost two centuries, especially during the early years of microbiology. The bacterium was found by Ehrenberg (1838) in calm water of a cove in a small creek near Ziegenhain (Jena, Germany), where it developed together with *Chromatium okenii*. He described this bacterium as *Ophidomonas jenensis* with a cell width of 3.5–4.5 µm and a length of 30–40 up to 100 µm, a giant among bacteria. Cultures of *Tsp. jenense* are pastel-colored orange-brown and due to the size, even single cells can be recognized as colored in the light microscope. In contrast to many other bacteria, the large polar flagellar tuft of *Tsp. jenense* may be visible in the light microscope (Fig. 1) and already Ehrenberg (1838) observed these flagella, for the first time with a living bacterium. The flagellar tuft pushes the cell forward, but at reversal of direction it turns down over the cell and then draws it through the water (Buder 1915). It was also Ehrenberg (1838) who reported on refractive inclusion bodies that were later recognized to be elemental sulfur globules

Communicated by Erko Stackebrandt.

✉ Johannes F. Imhoff
jimhoff@geomar.de

¹ GEOMAR Helmholtz Centre for Ocean Research RU Marine Symbioses, Düsternbrooker Weg 20, 24105 Kiel, Germany

² Department of Biochemistry, University of Arizona, Tucson, AZ, USA

³ College of Science and Technology, Bellevue University, Bellevue, NE 68005, USA

Fig. 1 Brightfield photomicrograph of *Tsp. jenense* DSM216 showing intracellular elemental sulfur globules and a polar tuft of flagella. Bar indicates 10 μ m (courtesy of N. Pfennig)

(van Niel 1932). The requirement of hydrogen sulfide as well as light for the development of *Tsp. jenense* and other purple sulfur bacteria was concluded by Buder (1919) from his ecological observations on the development of these bacteria.

All of the early studies on metabolism, motility and phototactic responses of *Tsp. jenense* were made with material taken from the natural environment (Winogradsky 1888; Buder 1915, 1919; Schlegel 1956; van Niel 1955). Although many attempts had been made in the early years, *Tsp. jenense* could not be grown in the laboratory, not even in crude cultures. It was Buder who was able to keep transient cultures of *Tsp. jenense* according to the Winogradsky method (Winogradsky 1888; Buder 1915). The source for many years was a pond in Ostrau near Halle, where these bacteria formed regular mass developments from April to July (Schlegel and Pfennig 1961). This material formed the basis for studies on the photobiology of *Tsp. jenense* and *Chromatium okenii* by H.G. Schlegel during his PhD studies in the lab of Johannes Buder (Schlegel 1956). The mechanism of photophobic tactic movements of *Tsp. jenense*, responsible for the reversal of flagellar rotation upon entering dark zones or dim light, was shown by studies of Buder and Schlegel (Buder, 1915, 1919; Schlegel 1956) and demonstrated to be responsible for accumulation of the cells in the light.

Years later Schlegel and Pfennig (1961) in great detail described enrichment conditions and culture media for the growth of *Tsp. jenense*. Norbert Pfennig was successful in the cultivation of *Tsp. jenense* and other large-cell Chromatiaceae including *Chromatium okenii* and *Allochro-matium warmingii*. As he describes in his memoirs (Pfennig 1993), he used the pond near Ostrau (in particular, well known by H.-G. Schlegel who used material from the same pond during his PhD thesis, and at that time was head of the Microbiology Institute in Göttingen) as a source

and a small bottle was smuggled from the eastern to the western part of Germany across the inner border which was at this time strictly closed. The *Thiospirillum* thrived for a while in a Winogradsky column set up with sewage sludge and eventually was brought into highly enriched and pure culture (Schlegel and Pfennig 1961). Pfennig discovered that it required the addition of vitamin B₁₂ and that it also was sensitive to the continuous bright lights in the laboratory and enjoyed a diurnal cycle of 16 h light and 8 h darkness. It would tolerate only 2–3 mM sulfide and had to be fed regularly to achieve dense populations in the cultures. Cultures of *Tsp. jenense* appear orange in color and the major carotenoids were identified as lycopene and rhodopin (Schmidt 1963; Schmidt et al. 1965). *Thiospirillum sanguineum* on the other hand is a marine bacterium and is rose-red in color (Paterek and Paynter 1988), which suggests that the carotenoids may be dominated by spirilloxanthin.

Pfennig also continued studies on the phototactic behavior and an impressive movie was produced that is still available online (Pfennig 1963). In this video, the phototactic movement and the reversal of the flagella rotation upon light/dark stimuli were demonstrated. Also the collection of cells in a small light frame due to their phototactic response and the accumulation of the cells at light of specific wavelength, in particular at the absorption maxima of bacteriochlorophyll *a*, were observed (Pfennig 1963). However, after this short period of active research on this bacterium, for more than five decades the problems in cultivation of *Tsp. jenense* and later also the missing availability of cultures have disabled further detailed studies. The original locations, where the *Tsp. jenense* was collected from no longer exist and therefore, frozen cultures currently represent the only source to obtain data on this interesting bacterium. No sequence information of this bacterium, even no 16S rRNA gene sequences, have been available so far. The data presented here for the first time allow the phylogenetic alignment of *Tsp. jenense* with other purple sulfur bacteria and give insight into its genomic capability and physiological properties. Despite the lack of pure cultures, bacteria resembling *Thiospirillum* appear to be more widespread in nature and developments in anaerobic habitats including both marine and freshwater environments of spiral-shaped bacteria containing different carotenoids have been observed (Paterek and Paynter 1988; Brie et al. 2007; Caldwell and Tiedje 1975). This includes a brackish pond in San Elijo Lagoon near La Jolla (California) where such a development of a pink-colored *Thiospirillum* was observed and maintained as enrichment for a while in a Winogradsky-type column by one of the authors (JFI). The genome sequence of *Tsp. jenense* should stimulate the search for and the identification of other *Thiospirillum* and related bacteria in nature, eventually leading to further isolation and characterization of other *Thiospirillum* species.

Materials and methods

Cultures

Cultures of *Thiospirillum jenense* DSM 216^T (Pfennig 1112, strain Ostrau) were received by JFI from N. Pfennig, who at this time (1991) worked at the University of Konstanz. Living cultures were maintained for some years in the Institute for Microbiology at the University of Bonn and in parallel the cultures were preserved immediately after transfer to Bonn and kept in liquid nitrogen. In 1993, the liquid nitrogen preserves were taken to Kiel as JFI took a position in Marine Microbiology at the GEOMAR Ocean Research Center Kiel (at that time named Institut für Meereskunde in Kiel). A 2-ml ampule of this preserved material was the source for the genomic analysis presented here.

gDNA sequencing assembly and annotation

Genomic DNA was extracted using the GeneJET DNA purification kit (Thermo Scientific). The quantity and purity of DNA were determined using Qubit and Nanodrop instruments and showed A260/280 ratio of 1.75. The DNA library was prepared with the Nextera DNA flex library prep kit (Illumina, Inc.). Genomes were sequenced using 500 µl of a 1.8-pM library with an Illumina MiniSeq instrument. Paired-end (2 × 150 bp) sequencing generated 2,195,084 reads and 178 Mbps. Quality control of the reads was performed using FASTQC within BaseSpace (Illumina, version 1.0.0), using a k-mer size of 5 and contamination filtering. The data were assembled de novo using Unicycler within PATRIC (Wattam et al. 2017). This assembly yielded 128 contigs (> 300 bp), the largest being 178,986 bp, and an N₅₀ of 86,232 bp. The genome sequence was annotated using RAST (Rapid Annotations using Subsystem Technology; version 2.0; Aziz et al. 2008). Taxonomic classification of the assembled contigs was performed with Kraken2 within PATRIC (Wood and Salzberg 2014). All database genomes were used for this taxonomic classification. A Kraken2 analysis of the raw, unassembled sequencing data was also performed and showed a representation of the *Rhodopseudomonas* and *Rhodoferrax* contaminants at 13 and 4% in the dataset, which were removed during the Unicycler assembly process. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under BioProject PRJNA638421 with the following genome accessions: *Thiospirillum jenense* DSM216: JABVCQ000000000, *Rhodoferrax jenense* 4810: JABVZS000000000, *Rhodopseudomonas palustris* 4810: JABVZT000000000.

Metagenomic binning

The sequencing reads were used to perform a metagenomic binning using the Metagenomic Binning service within PATRIC (Wattam et al. 2017). Paired-end reads were used as input and default parameters were used. Sets of contig bins were constructed (hits against contigs that have less than fourfold coverage or are less than 400 bp in length are removed). The contig pool was split into bins using reference genomes. Quality control of each bin was performed using checkM (Parks et al. 2014). Each bin was automatically annotated using PATRIC RAST and consistency checks of the annotation were performed, producing a coarse score (percentage of roles that are correctly present or absent) and a fine score (percentage of roles that are correctly absent or present in the correct number). Identified genomes were ranked based on their coarse score, fine score and completeness.

16S rRNA amplification

Degenerate primers were used for the 16S rRNA PCR reaction, described by Weisburg et al. (1991). Both the FD1 forward and RD1 reverse, and the FD1 forward and the RP1 reverse primer sets were used. Using each primer set on two different template concentrations (5–12 ng/µl), a total of four PCR reactions were performed. Amplified fragments were gel purified using the PureLink Quick Gel Extraction and PCR purification kit (Invitrogen), and sequenced by Sanger sequencing using the respective FD1, RD1 and RP1 sequencing primers (at the Iowa State University DNA core facility). The degenerate primers were designed to amplify most bacterial 16S rRNA and given the known impurities in the culture this could result in the amplification of 16S rRNA from different species, however all of the sequencing reactions gave the same result (based on CLUSTALW alignment of the sequenced fragments). Forward and reverse sequences were assembled into one 16S rRNA sequence of 1494 bp. This was used to BLAST against the assembled *Tsp. jenense* DSM 216^T genome, which identified the complete 16S rRNA sequence (located near the end of contig 73).

ANI and whole genome comparison

Average percentage nucleotide identity (ANIb) between the whole genomes was calculated using JSpecies (Richter et al. 2015). A whole genome-based phylogenetic tree was generated using the CodonTree method within PATRIC (Wattam et al. 2017), which used PGFams as homology groups. The aligned proteins and coding DNA from 349 single-copy genes were used for RAXML analysis (Stamatakis et al. 2008; Stamatakis 2014) for the *Thiospirillum jenense* DSM

216^T tree, and 389 single copy genes were used for the *Rhodoferrax jenense* tree. The support values for the phylogenetic tree were generated using 100 rounds of the ‘Rapid bootstrapping’ option of RaxML. iTOL was used for tree visualization (Letunik and Bork 2019).

Phylogenetic analysis of PufM

For phylogenetic analysis of PufM, selected sequences were aligned using ClustalX version 2.1 (Larkin et al. 2007) and trees were calculated by the neighbor-joining (NJ) method (Saitou and Nei 1987). NJplot was used to draw the phylogenetic trees expressed in the Newick phylogenetic tree format (Perrière and Gouy 1996). The tree topologies were evaluated with bootstrap analyses based on 1000 replicates and values > 70% indicated in the tree.

Synteny analysis

Comparative genome regions were generated using global PATRIC PGFam families to determine a set of genes that match a focus gene. Only reference and representative genomes were used in the search. The gene set is compared to the focus gene using BLAST and sorted by BLAST scores within PATRIC (Wattam et al. 2017). The photosynthetic reaction center M subunit (*pufM*) and nitrogenase *nifH* were used as focus genes to analyze synteny of the photosynthetic gene cluster and nitrogen fixing gene cluster regions in the *Tsp. jenense* genome.

Results and discussion

As known from microscopic examinations of the *Tsp. jenense* culture and the results from attempts to purify the cultures using the deep agar dilution method, the culture was contaminated with a strain resembling *Rhodopseudomonas palustris* and also another contaminant of thin cells similar to *Rubrivivax* or *Rhodoferrax* species (unpublished). A metagenomic binning on the entire genomic dataset obtained from the culture preserve of *Tsp. jenense* indeed led to the identification of the two contaminants. One of the binned genomes was identified as *Rps. palustris* and resulted in an assembled genome of 5.3 Mbp in 316 contigs (97% coarse consistency and 90% fine consistency with 100%

completeness) while the other gave reference to *Rhodoferrax* species and assembled into a 5.9-Mbp genome with 324 contigs (92% coarse consistency and 72% fine consistency with 100% completeness) (Table 1). Since the metagenomic binning is based on mapping of the binned contigs to reference genomes in the database, it is not surprising that the *Tsp. jenense* genome was not identified in this first process. However, a genome assembly of the sequencing reads using Unicycler provided a separate genome assembly, in addition to the two contaminants obtained by the metagenomic binning process. This third assembly yielded a genome size of 3.22 Mb in 128 contigs and a GC content of 48.7% (Table 1) which is close to the GC content of 45.5% for *Tsp. jenense* as determined by buoyant density (Mandel et al. 1971). An initial taxonomic classification of these assembled contigs using Kraken2 identified this genome as belonging to a Gammaproteobacterium (> 75% of the Proteobacteria identified). However, with almost 40% of the sequence fraction of *Thiospirillum jenense* unidentified using Kraken2, this genome quite likely belongs to a species that is not represented in the database.

It was beyond the capability of average nucleotide identity (ANI) to determine the position of *Tsp. jenense* in relation to other purple sulfur bacteria, because it showed less than 70% identity even to the closest relatives. However, we independently cloned and sequenced the 16S rRNA which allowed us to identify the RNA in the genome sequence. This showed a clear relationship to the other Chromatiaceae and for the first time allows identification of *Thiospirillum* using molecular methods. A whole-genome phylogenetic tree is shown in Fig. 2, which indicates that *Tsp. jenense* is not closely related to any other species of the Chromatiaceae and has *Thiorhodovibrio* species as the nearest neighbors. The *Rhodopseudomonas* was identified as a strain of *Rps. palustris* having ANI of 98% to the neotype strain DSM 123 (Imhoff et al. 2020). The other contaminant in the culture was found to have ANI of 82% to *Rfx. antarcticus* and 79% to *Rfx. fermentans* and therefore is considered to represent a new species of *Rhodoferrax*. We propose to call it *Rhodoferrax jenense* according to its origin from a coculture with *Thiospirillum jenense*. A whole-genome phylogenetic tree comparing this new *Rhodoferrax* species to its closest relatives is given in Fig. 3. Consistent with the ANI comparison, *Rfx. jenense* is positioned within the *Rhodoferrax* genus, but clearly different from the other species in that genus.

Table 1 Overview of the genome features of all of the genome sequences identified in the *Thiospirillum jenense* culture

Species	Genome size	GC content	Contigs	Coverage	N50	CDS	tRNAs	Genbank accession
<i>Thiospirillum jenense</i> DSM 216 ^T	3.22 Mb	48.7	128	55X	86,232	3260	46	JABVCQ000000000
<i>Rhodoferrax jenense</i> 4810	5.97 Mb	56.5	324	30X	47,617	5964	69	JABVZS000000000
<i>Rhodopseudomonas palustris</i> 4810	5.34 Mb	63.4	316	33X	34,048	5462	56	JABVZT000000000

Fig. 2 Phylogenetic tree of *Thiospirillum jenense* DSM 216 whole genome comparison to its closest relatives. The phylogenetic tree was generated using the codon tree method within PATRIC (Wattam

et al. 2017). *Ectothiorhodospira haloalkaliphila* was included as an outgroup. iTOL was used for the tree visualization (Letunic and Bork 2019)

Fig. 3 Phylogenetic tree of *Rhodoferax jenense* whole genome comparison to its closest relatives. The phylogenetic tree was generated using the codon tree method within PATRIC (Wattam et al. 2017).

Ectothiorhodospira haloalkaliphila was included as an outgroup. iTOL was used for the tree visualization (Letunic and Bork 2019)

The *Tsp. jenense* genome reflects its important physiological properties. According to its photoautotrophic growth with reduced sulfur compounds, it is not surprising that ribulose-bisphosphate carboxylase (Rubisco) and enzymes of the Calvin cycle of autotrophic carbon dioxide fixation are encoded. Carboxysomes and the genes encoding carboxysome peptides are absent. *Tsp. jenense* encodes two forms of Rubisco. The large subunit of the form-I enzyme is most similar to one copy of *Ach. vinosum* (*rbcAB* 96.8%), *Ach. warmingii* (96.4%) and *Thiorhodococcus mannitoliphagus* (96.2%), but only 82.4% to a second copy of *Ach. vinosum* (*rbcSL*), which is always associated with the carboxysome genes (Badger and Bek 2008; Weissgerber et al. 2011). The second gene of *Tsp. jenense* is most similar to

the form II genes of *Ach. warmingii* (91.6%), *Imhoffiella purpurea* (90.9%) and *Thiorhodococcus drewsii* (89.9%), but only 82.4% to the enzyme of *Rhodospirillum rubrum*, which only has the form II enzyme and was the first well-studied Rubisco enzyme (Tabita and McFadden 1974; Tabita et al. 2008). The tricarboxylic acid cycle is incomplete and malate dehydrogenase (MDH) is absent. This is in accord with the restricted ability of *Tsp. jenense* to photoassimilate organic compounds. This impairment is shared by its close phylogenetic neighbors *Thiorhodovibrio winogradskyi* (DSM 6702^T), *Thiorhodovibrio* 970 and *Rhabdochromatium marinum* (DSM 5261^T), *Lamprobacter modestohalophilus* (DSM 25653^T) and the *Halochromatium* species, *Halochromatium salexigens* (DSM 4395^T), *Hch. glycolicum* (DSM

11080^T) and *Hch. roseum*, (DSM 18859^T), but not with the *Thiohalocapsa* species.

Systems active in the oxidation of reduced sulfur compounds include a sulfide quinone reductase, which is considered active in the oxidation of sulfide to elemental sulfur. A dissimilatory sulfite reductase and associated genes (*dsrA-BEFHCMKLJ OPNRS*), which are essential for the oxidation of stored elemental sulfur (Weissberger et al. 2011), are encoded as a gene cluster similar to *Ach. vinosum*. Also polysulfide reductase (*psrABC*), heterodisulfide reductase (*hdrABCD*) and adenylylsulfate reductase (*aprAB*) are encoded. The absence of the Sox-system may explain the failure to grow on thiosulfate as electron donor for photosynthesis. Sulfate assimilation is lacking in *Tsp. jenense*. A gene cluster of sulfate assimilation encoding enzymes for sulfate uptake, activation to adenylylsulfate, its reduction to sulfite and further to sulfide similar to the *cysAWTPBI-HDN* cluster of *Ach. vinosum* (Neumann et al. 2000) is not present. A BLAST search using The SEED Viewer of the RAST platform (Altschul et al. 1997) supported the absence of the sulfate uptake system (*cysAWTP*), the two subunits of sulfate-adenylyltransferase, as well as assimilatory forms of adenylylsulfate reductase (*cysH*) and sulfite reductase (*cysI*) in *Tsp. jenense*.

The hydrogenase system enabling growth with hydrogen as electron donor is also found (*hoxHMFYUFE*). Nitrogen fixation is enabled by the molybdenum–iron nitrogenase (*nif*), alternative systems are not present. Denitrification enzymes are not encoded and ammonia is assimilated via the GS/GOGAT system. A comparison of the *nifHDKT-nafY* genomic region of *Tsp. jenense* to the genomes of its relatives shows a clear conservation of this gene cluster

(Fig. 4). The gene organization and relative gene spacing is well conserved, indicating that there is little evolutionary drift in this region.

A photosynthetic gene cluster with reaction center and light-harvesting complexes (*pufBALMC*) and the cytochrome BC1 complex are present. The phylogeny of PufM is in accord with the whole genome relations of *Tsp. jenense* to other Chromatiaceae and supports the close relation to *Thiorhodovibrio* and *Rhabdochromatium* species (Fig. 5). The knowledge of the *pufLM* sequences now opens up the possibility to use these genes for identification of *Tsp. jenense* and related *Thiospirillum* species in environmental samples as suggested and demonstrated earlier for other phototrophic purple bacteria (Tank et al. 2009, 2011; Thiel et al. 2010; Imhoff 2016). The photosynthesis genes are less organized as in many other phototrophic purple bacteria and in addition to three short clusters occur single or in pairs throughout the genome. A comparison of the synteny of the conserved *bchCXYZ-LHβα-pufLMC-LHβα* gene cluster of *Tsp. jenense* is shown in Fig. 6. The gene organization of reaction center (*puf*) and light-harvesting (*LH*) genes is well conserved in all of the close relatives, but there is somewhat more divergence in the *bch* synteny. *Marichromatium purpuratum*, *Thiocapsa marina*, *Imhoffiella purpurea* and *Thiorhodococcus drewsii* have a very similar *bch* gene organization and intergenic spacing to *Tsp. jenense*, while the spacing of the *Ach. vinosum bch* genes is significantly larger. This is consistent with the phylogenetic comparisons which placed *Ach. vinosum* evolutionary more distant from *Tsp. jenense* than the other species. In the *Thiorhodovibrio* sp. 970 genome the *puf* and light-harvesting genes are clustered, and conserved compared to *Tsp. jenense*, however

Fig. 4 Comparison of the *nifHDK-nafY* genomic region between *Tsp. jenense* and other purple sulfur bacteria. Genes are colored based on their family membership

Fig. 5 Phylogenetic tree of PufM sequences of *Tsp. jenense* DSM 216 and related Chromatiaceae. The phylogenetic tree was calculated by the neighbor-joining (NJ) method (Saitou and Nei 1987). NJplot was used to draw the phylogenetic trees expressed in the Newick phyloge-

netic tree format (Perrière and Gouy 1996). The tree topologies were evaluated with bootstrap analyses based on 1000 replicates and values > 70% indicated in the tree

instead of the *bchCXYZ* genes there are a series of hypothetical proteins (pink in Fig. 6) located upstream from the *puf* region. This was surprising since *Thiorhodovibrio* sp. 970 is the nearest relative to *Thiospirillum jenense* based on our phylogenetic and ANI comparisons. The *Thiorhodovibrio bchCXYZ* genes are located elsewhere on the genome and are actually clustered near the end of a different contig, which indicates that an error might have occurred in that genome assembly. Although located on a different contig, the *bch* genome region in *Thiorhodovibrio* is conserved compared to the other Chromatiaceae genomes. Moreover, when we reassembled the original SRA file for the *Thiorhodovibrio* sp. 970 genome (from Genbank accession SRR3927343) using Unicycler, the *bchCXYZ* gene cluster was positioned in exactly the same position relative to the *puf* and LH genes as in *Thiospirillum jenense* and the other closely related Chromatiaceae genomes (data not shown).

We found HiPIP (the soluble mediator between PufC and the BC1 complex), cytochromes C4, and C', glutathione amide reductase (unique to purple sulfur bacteria and close relatives), a diheme bacterial cytochrome c peroxidase (BCCP), an Fe-superoxide dismutase (sodB), and the CydAB quinol oxidase. Catalase and peroxidase are absent.

FixNOP, one of the oxidase systems with the highest affinities for oxygen ever measured (in the region of

7 nM, which is sixfold lower than for the Cox type oxidase), is commonly found in phototrophic and aerobic bacteria (Preisig et al. 1996), but is absent from *Tsp. jenense*. FixNOQP (ccoNOQP) encodes a membrane-bound heme/copper cytochrome oxidase specifically induced under oxygen limitation in aerobic bacteria and was shown to have a role in nitrogen fixation through protection of nitrogenase against oxygen damage (Preisig et al. 1996). The FixNOP oxidase is present in most purple sulfur bacteria, except *Chromatium okenii*, *Chromatium weissei*, and *Allochromatium warmingii*, all three of which were reported to be difficult to isolate and keep in culture similar to *Tsp. jenense* (Pfennig 1993). The lack of FixNOP may indeed be the reason that these four species including *Tsp. jenense*, are more sensitive to oxygen than others and difficult to isolate. Furthermore, the presence of the contaminants may have helped to remove traces of oxygen in the cultures, thus improving chances for survival during enrichment and growth. Methods for cultivation of strict anaerobes, particularly the Hungate technique (Hungate, 1969), might be used to advantage in this instance. It has been applied to isolate another species of *Thiospirillum* (Paterek and Paynter 1988).

Fig. 6 Comparison of the photosynthetic genomic region between *Tsp. jenense* and other purple sulfur bacteria. Genes are colored based on their family membership

Conclusions

Nothing was known prior to this study about the molecular genetics of *Thiospirillum jenense* and its genome sequence has widened the view on the phylogeny of the Chromatiaceae family. This unusually large bacterium is phylogenetically distant to all other purple sulfur bacteria. As already suspected from the low G + C content, the whole genome sequence and ANI comparisons confirmed the exceptional properties of *Tsp. jenense*. Although the analyses of the photosynthetic gene clusters and essential genes of sulfur and nitrogen metabolism in many points were in accord with other Chromatiaceae, the low degree of forming functional genomic clusters clearly distinguished *Tsp. jenense* from most other purple sulfur bacteria and supported its unique taxonomic placement. We pointed out some explanation behind the challenges of cultivating *Tsp. jenense* that have been limiting detailed studies in the past. One key component appears to be the lack of a high-affinity oxidase FixNOP, which presumably renders the cells highly sensitive to oxygen damage. In this context, the two identified sequenced contaminant species, *Rps. palustris* and *Rfx. jenense*, might help with the removal of traces of oxygen in the cultures. Thereby they support the growth, but also explain the difficulties with obtaining pure cultures of *Tsp. jenense*.

Having genomic and genetic data available for *Tsp. jenense* will undoubtedly aid the identification and study of related *Thiospirillum* species, which have been repeatedly observed in nature.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389–3402
- Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Fromsma K, Gerdes S, Glass EM, Kabul M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parello B, Push GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O (2008) The RAST server: rapid annotations using subsystems technology. *BMC Genomics* 9(75):1
- Badger MR, Bek EJ (2008) Multiple Rubisco forms in *Proteobacteria*: their functional significance in relation to CO₂ acquisition by the CBB cycle. *J Exp Bot* 59:1525–1541

- Briée C, Moreira D, López-García P (2007) Archaeal and bacterial community composition of sediment and plankton from a suboxic freshwater pond. *Res Microbiol* 158:213–227
- Buder J (1915) Zur Kenntnis des *Thiospirillum jenense* und seiner Reaktion auf Lichtreize. *JB Bot* 56:29–584
- Buder J (1919) Zur Biologie des Bacteriopurpurins und der Purpurbakterien. *JB Bot* 58:525–628
- Caldwell DE, Tiedje JM (1975) The structure of anaerobic bacterial communities in the hypolimnia of several Michigan lakes. *Can J Microbiol* 21:377–385
- Ehrenberg CG (1838) Die Infusionstierchen als vollkommene Organismen. L. Voss-Verlag, Leipzig
- Hungate RE (1969) A roll tube method for cultivation of strict anaerobes. In: Norris JR, Ribbons DW (eds) *Methods in microbiology*, vol 3B. Academic Press., New York, pp 117–132
- Imhoff JF (2016) New dimensions in microbial ecology – Functional genes in studies to unravel the biodiversity and role of functional microbial groups in the environment. *Microorganisms* 4(2):19. <https://doi.org/10.3390/microorganisms4020019>
- Imhoff JF, Meyer TE, Kyndt JA (2020) Genomic and genetic sequence information of strains assigned to the genus *Rhodopseudomonas* reveal the great heterogeneity of the group and identify strain *Rhodopseudomonas palustris* DSM 123T as the true type strain of this species. *Int J Syst Evol Microbiol*. <https://doi.org/10.1099/ijsem.0.004077>
- Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. *Bioinformatics* 23:2947–2948
- Letunic I, Bork P (2019) Interactive tree of life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 47:256–259. <https://doi.org/10.1093/nar/gkz239>
- Mandel M, Leadbetter ER, Pfennig N, Trüper HG (1971) Deoxyribonucleic acid base compositions of phototrophic bacteria. *Int J Syst Bacteriol* 21:222–230
- Neumann S, Wynen A, Trüper HG, Dahl C (2000) Characterization of the *cys* gene locus from *Allochromatium vinosum* indicates an unusual sulfate assimilation pathway. *Mol Biol Rep* 27:27–33
- Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW (2014) Assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 25:1043–1055
- Paterek JR, Paynter MJ (1988) Populations of anaerobic phototrophic bacteria in a *Spartina alterniflora* salt marsh. *Appl Environ Microbiol* 54:1360–1364
- Pfennig N (1963) *Thiospirillum jenense* (Thiorhodaceae)—Lokomotion und phototaktisches Verhalten. IWF. <https://doi.org/10.3203/IWF/E-678>
- Pfennig N (1993) Reflections of a microbiologist, or how to learn from the microbes. *Annu Rev Microbiol* 47:1–29
- Perrière G, Gouy M (1996) WWW-Query: an on-line retrieval system for biological sequence banks. *Biochimie* 78:364–369
- Preisig O, Zufferey R, Thöny-Meyer L, Appleby CA, Hennecke H (1996) A high-affinity *cbb3*-type cytochrome oxidase terminates the symbiosis-specific respiratory chain of *Bradyrhizobium japonicum*. *J Bacteriol* 178:1532–1538
- Richter M, Rosselló-Móra R, Glöckner FO, Peplies J (2015) JSpeciesWS: a web server for prokaryotic species circumscription based on pairwise genome comparison. *Bioinformatics* 32:929–931. <https://doi.org/10.1093/bioinformatics/btv681>
- Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 4:406–425
- Schlegel HG (1956) Vergleichende untersuchungen über die lichtempfindlichkeit einiger purpurbakterien. *Arch Protistenkunde* 101:69–97
- Schlegel HG, Pfennig N (1961) Die anreicherungskultur einiger schwefelpurpurbakterien. *Arch Mikrobiol* 38:1–39
- Schmidt K (1963) Carotenoids in Thiorhodaceae. II. Carotenoid composition of *Thiospirillum jenense* Winogradsky and *Chromatium vinosum* Winogradsky. *Arch Mikrobiol* 46:127–137
- Schmidt K, Pfennig N, Liaaen Jensen S (1965) Carotenoids of Thiorhodaceae. IV. The carotenoid composition of 25 pure isolates. *Arch Mikrobiol* 52:132–146
- Stamatakis A, Hoover P, Rougemont JJSB (2008) A rapid bootstrap algorithm for the RAxML web servers. *Syst Biol* 57:758–771
- Stamatakis AJB (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* 30:1312–1313
- Tabita FR, McFadden BA (1974) D-ribulose 1, 5-diphosphate carboxylase from *Rhodospirillum rubrum*. II. Quaternary structure, composition, catalytic and immunological properties. *J Biol Chem* 249:3459–3464
- Tabita FR, Satagopan S, Hanson TE, Kreeb NE, Scott SS (2008) Distinct form I, II, III, and IV rubisco proteins from the three kingdoms of life provide clues about rubisco evolution and structure/function relationships. *J Experim Bot* 59:1515–1524
- Tank M, Thiel V, Imhoff JF (2009) Phylogenetic relationship of phototrophic purple sulfur bacteria according to *pufL* and *pufM* genes. *Intern Microbiol* 12:175–185
- Tank M, Blümel M, Imhoff JF (2011) Communities of purple sulfur bacteria in a Baltic Sea coastal lagoon analyzed by *pufLM* gene libraries and the impact of temperature and NaCl concentration in experimental enrichment cultures. *FEMS Microbiol Ecol* 78:428–438
- Thiel V, Tank M, Neulinger SC, Gehrmann L, Dorador C, Imhoff JF (2010) Unique communities of anoxygenic phototrophic bacteria in saline lakes of Salar de Atacama (Chile). Evidence for a new phylogenetic lineage of phototrophic Gammaproteobacteria from *pufLM* gene analyses. *FEMS Microbiol Ecol* 74:510–522
- Van Niel CB (1932) On the morphology and physiology of the purple and green sulfur bacteria. *Arch Mikrobiol* 3:1–112
- Van Niel CB (1955) The microbe as a whole. In: Waksman SA (ed) *Perspectives and horizons in microbiology*. Rutgers University Press, New Brunswick, NJ, pp 3–12
- Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL (2017) Improvements to patric, the all-bacterial bioinformatics database and analysis resource center. *Nucleic Acids Res* 45:D535–D542
- Weissgerber T, Zigann R, Bruce D, Chang Y, Detter JC, Han C, Hauser L, Jeffries CD, Land M, Munk AC, Tapia R, Dahl C (2011) Complete genome sequence of *Allochromatium vinosum* DSM 180^T. *Stand Genomic Sci* 5:311–330
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703. <https://doi.org/10.1128/jb.173.2.697-703.1991>
- Winogradsky SN (1888) Beiträge zur Morphologie und Physiologie der Schwefelbakterien, Leipzig, Arthur Felix, pp 1–120

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.