



Complete Genome Sequence of the Photoautotrophic and Bacteriochlorophyll *e*-Synthesizing Green Sulfur Bacterium *Chlorobaculum limnaeum* DSM 1677^T

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ABSTRACT *Chlorobaculum limnaeum* DSM 1677^T is a mesophilic, brown-colored, chlorophototrophic green sulfur bacterium that produces bacteriochlorophyll *e* and the carotenoid isorenieratene as major pigments. This bacterium serves as a model organism in molecular research on photosynthesis, sulfur metabolism, and bacteriochlorophyll biosynthesis. We report here the complete genome sequence.

Chlorobaculum limnaeum DSM 1677^T is a brown-colored, chlorophototrophic green sulfur bacterium (GSB) that belongs to the family *Chlorobiaceae* within the phylum *Chlorobi* (1, 2). It was originally isolated from Lake Kinneret, a monomictic, freshwater lake in Israel (<https://www.dsmz.de/catalogues/details/culture/DSM-1677.html>). Like all other members of the *Chlorobiaceae*, *C. limnaeum* is an anaerobic, anoxygenic chlorophototroph, and all known required genes for this capability were detected in the genome (3). Light energy is harvested by chlorosomes, which are also found in the phototrophic *Chloroflexaceae* (4), the recently discovered *Chloracidobacterium thermophilum* (5), and “*Candidatus Thermochlorobacter aerophilum*” (2). Sulfide is the preferred electron donor, and CO₂ fixation occurs through the reverse tricarboxylic acid cycle (3). GSB that are green in color synthesize chlorosomes containing bacteriochlorophyll (BChl) *c* or/and BChl *d* and the carotenoid chlorobactene, but brown-colored GSB produce chlorosomes containing BChl *e* and, usually, the carotenoid isorenieratene (3).

In contrast to the thermophilic, BChl *c*-containing, and naturally transformable *Chlorobaculum tepidum* TLS^T, *C. limnaeum* only recently became a model organism for studies on the evolution of photosynthesis, sulfur metabolism, and biosynthesis of BChls and carotenoids in brown-colored GSB. For example, the genomic data for *C. limnaeum* helped produce a mutant with chlorosomes containing only BChl *f* by inactivation of the *bchU* gene (6). BChl *f* is C-20 demethyl Bchl *e* and is analogous to the C-20 demethyl BChl *c*, namely, BChl *d*, which is found in green-colored GSB (6). Another recent study demonstrated that BciD of *C. limnaeum* is a radical *S*-adenosyl-L-methionine enzyme that converts the C-7 methyl group of bacteriochlorophyllide *c* or *d* into the formyl group of bacteriochlorophyllide *e* or *f*, respectively (7).

The closed genome of *C. limnaeum* DSM 1677^T consists of a single chromosome with 2,797,276 bp and a G+C content of 56.4 mol%; the genome is comparable in size to those of other GSB genomes (2.0 to 3.1 Mbp) (3). No plasmids were detected. Annotation using the NCBI Prokaryotic Genome Annotation Pipeline (https://www.ncbi.nlm.nih.gov/genome/annotation_prok/) predicted 2,452 protein-coding genes, 49 genes

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encoding tRNAs, and 2 rRNA operons with identical 5S, 16S, and 23S nucleotide sequences.

C. limnaeum DSM 1677^T was grown under anoxic conditions at ~25°C in CL⁻ medium (8) at ~50 μmol photons m⁻² s⁻¹. Purified genomic DNA was sequenced on a 454 pyrosequencer (GS FLX+; Roche) to an average depth of >60×. Reads (1,281,523 reads; average length, 381 ± 123 bp) were assembled using the Newbler assembler (Roche) into 89 contigs of at least 500 bp. The contigs were compared to the genome of *C. tepidum* (9) using PGA (10) to predict the arrangement of and the connections between the contigs. PCR amplification and Sanger sequencing were used for gap closure. The final assembly was carried out using the phred/phrap/consed software package.

Accession number(s). The genome sequence has been deposited at DDBJ/EMBL/GenBank under the nucleotide sequence with accession number [CP017305](https://doi.org/10.1093/nar/gkn168).

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REFERENCES

1. Imhoff JF. 2003. Phylogenetic taxonomy of the family *Chlorobiaceae* on the basis of 16S rRNA and *fmo* (Fenna-Matthews-Olson protein) gene sequences. *Int J Syst Evol Microbiol* 53:941–951. <https://doi.org/10.1099/ijs.0.02403-0>.
2. Liu Z, Klatt CG, Ludwig M, Rusch DB, Jensen SI, Kühl M, Ward DM, Bryant DA. 2012. ‘*Candidatus Thermochlorobacter aerophilum*’: an aerobic chlorophoto-heterotrophic member of the phylum *Chlorobi* defined by metagenomics and metatranscriptomics. *ISME J* 6:1869–1882. <https://doi.org/10.1038/ismej.2012.24>.
3. Bryant DA, Liu Z, Li T, Zhao F, Costas AMG, Klatt CG, Ward DM, Frigaard N-U, Overmann J. 2012. Comparative and functional genomics of anoxygenic green bacteria from the taxa *Chlorobi*, *Chloroflexi*, and *Acidobacteria*, p 47–102. In Burnap R, Vermaas W (ed), *Advances in photosynthesis and respiration: functional genomics and evolution of photosynthetic systems*, vol 33. Springer, Dordrecht, The Netherlands.
4. Hanada S, Pierson BK. 2006. The family *Chloroflexaceae*, p 815–842. In Dworkin M, Falkow S, Rosener E, Schleifer KH, Stackebrandt E (ed), *The prokaryotes: a handbook on the biology of bacteria*, vol 7. Springer, New York, NY.
5. Tank M, Bryant DA. 2015. *Chloracidobacterium thermophilum* gen. nov., sp. nov.: an anoxygenic, microaerophilic and chlorophotoheterotrophic acidobacterium. *Int J Syst Evol Microbiol* 65:1426–1430. <https://doi.org/10.1099/ijs.0.000113>.
6. Vogl K, Tank M, Orf GS, Blankenship RE, Bryant DA. 2012. Bacteriochlorophyll *f*: properties of chlorosomes containing the “forbidden chlorophyll”. *Front Microbiol* 3:298. <https://doi.org/10.3389/fmicb.2012.00298>.
7. Thweatt JL, Ferlez BH, Golbeck JH, Bryant DA. 2016. BciD is a radical S-adenosyl-L-methionine (SAM) enzyme that completes bacteriochlorophyllide *e* biosynthesis by oxidizing a methyl group into a formyl group at C-7. *J Biol Chem* 292:1361–1373. <https://doi.org/10.1074/jbc.M116.767665>.
8. Frigaard NU, Bryant DA. 2001. Chromosomal gene inactivation in the green sulfur bacterium *Chlorobium tepidum* by natural transformation. *Appl Environ Microbiol* 67:2538–2544. <https://doi.org/10.1128/AEM.67.6.2538-2544.2001>.
9. Eisen JA, Nelson KE, Paulsen IT, Heidelberg JF, Wu M, Dodson RJ, Deboy R, Gwinn ML, Nelson WC, Haft DH, Hickey EK, Peterson JD, Durkin AS, Kolonay JL, Yang F, Holt I, Umayam LA, Mason T, Brenner M, Shea TP, Parksey D, Nierman WC, Feldblyum TV, Hansen CL, Craven MB, Radune D, Vamathevan J, Khouri H, White O, Gruber TM, Ketchum KA, Venter JC, Tettelin H, Bryant DA, Fraser CM. 2002. The complete genome sequence of *Chlorobium tepidum* TLS, a photosynthetic, anaerobic, green-sulfur bacterium. *Proc Natl Acad Sci U S A* 99:9509–9514. <https://doi.org/10.1073/pnas.132181499>.
10. Zhao F, Zhao F, Li T, Bryant DA. 2008. A new pheromone trail-based genetic algorithm for comparative genome assembly. *Nucleic Acids Res* 36:3455–3462. <https://doi.org/10.1093/nar/gkn168>.