Chloracidobacterium thermophilum gen. nov.,
sp. nov.: an anoxygenic microaerophilic chlorophotoheterotrophic acidobacterium

Marcus Tank¹ and Donald A. Bryant¹,²

¹Department of Biochemistry and Molecular Biology, The Pennsylvania State University,
University Park, PA, 16802 USA
²Department of Chemistry and Biochemistry, Montana State University, Bozeman, MT, 59717 USA

A novel anoxygenic photoheterotrophic member of the phylum Acidobacteria,
Chloracidobacterium thermophilum strain B sp. nov., was isolated from a cyanobacterial
enrichment culture derived from microbial mats associated with Octopus Spring, Yellowstone
National Park, WY. C. thermophilum sp. nov. was a Gram-stain-negative rod (diameter,
approximately 0.8–1.0 μm; variable length, approximately 2.5 μm), which formed greenish-brown
liquid suspension cultures. It was a moderately thermophilic microaerophile and grew in a defined
medium at 51 °C (Topt; range 44 to 58 °C) and in the pH range 5.5 to 9.5 (pHopt = ~7.0). The
DNA G+C content was 61.3 mol%, and phylogenetic analysis, based on the 16S rRNA
sequence, showed that C. thermophilum sp. nov. belongs to subdivision 4 (Acidobacteriaceae) of
the Acidobacteria. C. thermophilum sp. nov. was unable to synthesize branched-chain amino
acids, L-lysine, and vitamin B₁₂, which were required for growth. Although the organism lacked
genes/enzymes for autotrophic carbon fixation, bicarbonate was required. Growth was stimulated
by other amino acids and 2-oxoglutarate. Cells produced chlorosomes containing a diverse
mixture of bacteriochlorophyll (BChl) c derivatives, and additionally, synthesized BChl aP, Chl aP,
and Zn-BChl aP, which occurred in type-1 homodimeric reaction centres. The carotenoids
included echinenone, canthaxanthin, lycopene, β-carotene and α-carotene. C. thermophilum sp.
no.v. produced iso-diabolic acid as its major fatty acid and synthesized three hopanoids
(diploptene, bacteriohopanetetrol and bacteriohopanetetrol cyclitol ether). Based upon its
phenotypic and genotypic properties, the name Chloracidobacterium thermophilum gen. nov., sp.
no.v. is proposed for this isolate; the type strain is C. thermophilum strain B¹ (ATCC BAA-
2647 = JCM 30199).

‘Candidatus Chloracidobacterium thermophilum’ was first
detected by bioinformatics analyses of metagenomic sequence
data derived from phototrophic microbial mats that occur
in alkaline (~pH 8.2) siliceous hot springs (50–65 °C) in
Yellowstone National Park, Wyoming, USA (Bryant et al.,
2007). C. thermophilum strain B is the first characterized
chlorophototrophic member of the phylum Acidobacteria
(Barns et al., 1999; Bryant et al., 2007), and together with

Abbreviations: BChl, bacteriochlorophyll; Chl, chlorophyll; MK, menaquinone;
P, phytol; PD, Δ2,6-phytadienol.

Footnote: The names Chloracidobacterium gen. nov. and Chloracidobacterium thermophilum sp. nov. are effectively published in this paper.
Due to restrictions placed on the distribution of the type strain of the
proposed species, valid publication of these names is currently not
possible.

Two supplementary figures and two supplementary tables, plus details of
the HPLC methods used, are available with the online Supplementary
Material.

Pyrinomonas methylaliphatogenes, Blastocatella fastidiosa,
Aridibacter famidurans and Aridibacter kawangonensis, is
one of the few cultivated strains from subdivision 4 (Crowe et al., 2014; Foesel et al., 2013; Huber et al., 2014). Strain B
was isolated from a cyanobacterial enrichment culture, B’-
NACy100, which was derived from a sample collected by
Allewalt et al. (2006) from Octopus Spring at a site tempera-
ture of 51–61 °C on July 10, 2002. The enrichment culture
was first simplified by elimination of the Synechococcus sp.,
which produced a stable co-culture of C. thermophilum sp.
nov., Anoxybacillus sp. and Meiothermus sp. as previously
described (Bryant et al., 2007; Garcia Costas et al., 2012a).
Physico-chemical studies that led to the establishment of an
axenic culture will be published elsewhere (Tank & Bryant,
2015).

Studies on the pure culture of strain B were carried out
using the ‘C. thermophilum Midnight Medium’ (CTM-
Medium) as the basal medium (Table S1, available in the

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online Supplementary Material). If not stated, the incubation temperature was 52.5 °C, the pH was 7 to 8.5, and the irradiance was 20 to 50 μmol photons m⁻² s⁻¹ provided from a 40 W tungsten bulb. Growth of *C. thermophilum* sp. nov. was routinely monitored by recording absorption changes in the Q₅ band of monomeric BChl c in methanol at 667 nm with a Genesys 10 scanning spectrophotometer (Thermo Spectronic). Phase-contrast and epifluorescence microscopy were performed with a Nikon Eclipse E400 microscope (Nikon). Transmission electron microscopy was performed using osmium tetroxide and potassium permanganate tandem fixation and the described protocol (Hohmann-Marriott et al., 2005). Images of ultrathin sections were taken with a JEOL-JEM 1200 EXII transmission electron microscope.

Embedded colonies of *C. thermophilum* sp. nov. in solidified agar plates or shakes were greenish-brown, displayed a lentiform shape and appeared after about 10 days under optimal microoxic growth conditions. Liquid cultures of *C. thermophilum* sp. nov. were also greenish-brown (Fig. S1a), and the absorbance spectrum of whole cells had maxima at 461 and 745 nm, which is consistent with the presence of chlorosomes (Fig. S1b). The cells did not float and mostly consisted of individual cells, pairs, short chains and clumps were infrequently observed (Fig. S2a). Consistent with the genomic data (Garcia Costas et al., 2012a), neither flagella nor gas vesicles were observed. Cell division typically occurred by binary fission, but unequal, budding-like division was rarely observed. Growth only occurred between 44 and 58 °C (Topt of ~51 °C) and between pH 5.5 and 9.5 with a broad optimum at circum-neutral pH. No growth occurred under anoxic conditions (in a chamber with an atmosphere of H₂, 2.68 Mb, while the smaller was 1.01 Mb, and both chromosomes encoded single-copy, essential genes (Garcia Costas et al., 2012a). 16S rRNA gene sequences were aligned in the ARB software program package and manually refined (Ludwig et al., 2004). Maximum-likelihood tree calculations were performed in MEGAl.0 using the general time reversal model, default parameters and 100 bootstrap resamplings (Tamura et al., 2013). *C. thermophilum* sp. nov. is presently the earliest diverging cultivated organism within subdivision 4 (Acidobacteiraceae) of the phylum Acidobacteria (Fig. 1). Table 1 summarizes and compares the properties of *C. thermophilum* sp. nov. with other members of subdivision 4.

The *C. thermophilum* sp. nov. genome predicted that the bacterium does not synthesize the branched-chain amino acids, l-isoleucine, l-leucine and l-valine, but that it should be capable of degrading these amino acids (Garcia Costas et al., 2012a). Growth tests confirmed that these three amino acids are essential, and further testing showed that l-lysine is also essential (Tank & Bryant, 2015). *C. thermophilum* sp. nov. could be grown on a mixture of 20 amino acids, which serve as the nitrogen, carbon and/or sulfur sources. As long as the branched-chain amino acids and l-lysine were included, *C. thermophilum* sp. nov. grew with all combinations of amino acids. Interestingly, although photoautotrophic growth with bicarbonate as the sole carbon source was not observed, bicarbonate was essential under all growth conditions, possibly because of the importance of anaplerotic reactions and the limited solubility of CO₂/bicarbonate at elevated temperatures.

*C. thermophilum* sp. nov. was unable to use sulfate as a sulfur source and instead relied on reduced sulfur sources. Thioglycollate, l-methionine and l-cysteine/cystine, sulfur and thiosulfate could all serve as the sole sulfur source for *C. thermophilum* sp. nov. The novel bacterium is predicted to require vitamin B₁₂ for l-methionine synthesis, but its genome lacks the genes for vitamin B₁₂ synthesis (Garcia Costas et al., 2012a). Testing showed that vitamin B₁₂ is essential, but *C. thermophilum* sp. nov. did not require any other vitamins (Tank & Bryant, 2015).
Table 1. Characteristics of *Chloracidobacterium thermophilum* strain B\(^T\) and other subdivision 4 Acidobacteria

| Taxa: 1, *C. thermophilum* strain B(T); 2, *Pyrrinomonas methylaliphagenes* K22\(^T\); 3, *Blastocella tarda* A2-16\(^T\); 4, *Aridibacter famidurans* A22_HD_4H\(^T\); 5, *Aridibacter kawangonensis* Ac_23_E3\(^T\). All taxa are Gram-stain-negative. +, Positive; −, negative; ND, not determined; NP, not present; D GTA, diacylglycerylhydroxymethyl-N, N, N-trimethyl-\(\beta\)-alanine; D PC, diphasatydylcholine; PC, phosphatydylcholine; PE, phosphatidylethanolamine; PME, phosphatidylmonomethylethanolamine; PG, phosphatidylglycerol; MK-8, menaquinone-8; MK-8(H\(_2\)), dihydromenaquinone-8; i, iso-; ai, anteiso-.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
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<tr>
<td>Cell shape</td>
<td>Mainly rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
<td>Rod</td>
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<tr>
<td>Cell size (d x l; (\mu)m)</td>
<td>0.8 to 1.0 x ~2.5</td>
<td>0.3–0.6 x 1–4</td>
<td>0.8–0.9 x 0.8–12.0</td>
<td>2.5–3.0 x 0.9</td>
<td>2.5–3.0 x 0.6–0.7</td>
</tr>
<tr>
<td>Cell division</td>
<td>Binary fission, budding-like</td>
<td>Binary fission</td>
<td>Binary fission/budding</td>
<td>Binary fission</td>
<td>Binary fission</td>
</tr>
<tr>
<td>Motility</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>Temperature range (°C) (optimum)</td>
<td>44–58 (50–51)</td>
<td>50–69 (65)</td>
<td>14–40 (29–35)</td>
<td>15–44 (24–36)</td>
<td>12–47 (36–44)</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>5.5–9.5 (7.0)</td>
<td>4.1–7.8 (6.5)</td>
<td>4.0–10.0 (5.0–7.5)</td>
<td>4.0–9.5 (5.5–9.0)</td>
<td>3.5–10.0 (5.5–8.0)</td>
</tr>
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<td>Metabolism</td>
<td>Anoxyganic</td>
<td>Photoheterotroph</td>
<td>Chemo-organotroph</td>
<td>Chemo-organotroph</td>
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<td>Colour</td>
<td>Greenish-brown</td>
<td>ND</td>
<td>Orange/pink</td>
<td>Yellow/pink</td>
<td>White/bright pinkish hue</td>
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<td>(Bacterio)-Chlorophylls</td>
<td>BChl (c) (major), BChl (a_\beta), Chl (a_\beta) (a_\gamma), Zn-BChl (a_\gamma)</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
<td>NP</td>
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<td>Major carotenoids</td>
<td>Echinone, canthaxanthin, lycopene, (\gamma)- and (\beta)-carotene</td>
<td>NP</td>
<td>ND</td>
<td>ND</td>
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<td>Polar lipids</td>
<td>D GTA, PE, PME, PC, PE, PC</td>
<td>PE, PC</td>
<td>i15:0, i17:0, i19:0, i21:0</td>
<td>PE, PC</td>
<td>i15:0, i17:0, i19:0, i21:0</td>
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<td>Major fatty acids</td>
<td>i14:0, i15:0, i15:0, i15:0, i16:0, i16:1, i19:0, i21:0</td>
<td>PE, PC</td>
<td>i15:0, i17:0, i19:0, i21:0</td>
<td>PE, PC</td>
<td>i15:0, i17:0, i19:0, i21:0</td>
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<td>Quinone</td>
<td>MK-8(H(_2))</td>
<td>MK-8</td>
<td>MK-8</td>
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<td>G + C content of DNA (mol%)</td>
<td>61.3</td>
<td>59.6</td>
<td>46.5</td>
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<td>B12 requirement</td>
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The pigment composition and lipids of strain B have previously been analysed. *C. thermophilum* sp. nov. synthesized four Chls: BChl \(c\), BChl \(a_\beta\), Chl \(a_\gamma\) and Zn-BChl \(a_\gamma\) (Garcia Costas et al., 2012b; Tsukatani et al., 2012). Detailed analyses of the Chls showed that BChl \(c\) can be esterified with six or more alcohols, of which octadecanol was the most abundant (Garcia Costas et al., 2012b). Additionally, the BChl \(c\) produced by *C. thermophilum* sp. nov. can be methylated at both the C-8\(^2\) and C-12\(^1\) positions, typical of members of the family *Chlorobiaceae* (Garcia Costas et al., 2012b). The homodimeric, type-1 photochemical reaction centres of *C. thermophilum* sp. nov. are the first that are known to contain three different Chls: BChl \(a_\beta\), Chl \(a_\gamma\) and Zn-BChl \(a_\gamma\) (Tsukatani et al., 2012). The BChl \(a\) derivatives are esterified with phytol, and Chl \(a\) is esterified with \(\Delta-2,6\)-phytadienol, as in *Chlorobaculum tepidum* (Chew & Bryant, 2007). The major carotenoids in the chlorosomes were the keto-carotenoids, canthaxanthin and echinenone, but additionally *C. thermophilum* sp. nov. synthesized lycopene, \(\gamma\)-carotene, \(\beta\)-carotene and derivatives of deoxyflexixanthin (Garcia Costas et al., 2012b; Tsukatani et al., 2012). *C. thermophilum* sp. nov. contained n-C14:0, iso-C15:0, anteiso-C15:0, iso-C16:0, n-C16:1, n-C16:1, iso-C17:0, anteiso-C17:0, 5-methyl iso-diabolic acid and iso-diabolic acid (13, 16-dimethyl octacosanediocic acid) as the major fatty acids, as well as substantial amounts of a C-18 \(n\)-alkane (Garcia Costas et al., 2012b; Sinninghe Damsté...
Microaerophilic, moderately thermophilic, anoxygenic, chlorophotoheterotrophic eubacterium. Cells are Gram-stain-negative, non-motile rods that divide by binary fission. Cells have chlorosomes as light-harvesting organelles, and the BCHl a-binding Fenna-Matthews-Olson protein for light energy transfer to homodimeric type-1 reaction centres. Cells synthesize bacteriochlorophylls ε and ap, chlorophyll apD, Zn-bacteriochlorophyll a′p, echinenone and canthaxanthin as the major pigments. Based upon 16S rRNA sequence analysis, the new genus is assigned to subdivision 4 (Acidobacteriaceae) of the phylum Acidobacteria. The type species is *Chloracidobacterium thermophilum*.

**Description of Chloracidobacterium thermophilum, sp. nov.**

*Chloracidobacterium* (Chlor.a.ci.do.bac.te’ri.um. Gr. adj. chloros, greenish-yellow, pale green; Acidobacterium, a bacterial genus; *Chlorobacterium*, a green Acidobacterium).

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**Description of Chloracidobacterium thermophilum, sp. nov.**

*Chloracidobacterium thermophilum* (ther.mo’phi.lum. Gr. n. thermé, heat; N.L. adj. philus -a -um (from Gr. adj. philos -é -on), friend, loving; N.L. neut. adj. thermophilus, heat-loving).

Additional characteristics to those given in the genus description. Colonies on solid medium are greenish/brownish and lenticular in shape. Cells do not float in liquid medium and predominantly occur as solitary cells. The type species is *Chloracidobacterium thermophilum* (Crowe *et al.*, 2014; Foesel *et al.*, 2014; Garcia Costas *et al.*, 2012b) identified dihydrogenated menaquinone-8 as the predominant quinone in *C. thermophilum* sp. nov. Menaquinone 8 is also the predominant quinone found in the other members of subdivision 4 (Table 1) (Crowe *et al.*, 2014; Foesel *et al.*, 2014; Huber *et al.*, 2014).
sulfur source are included. Bicarbonate and vitamin B_{12} are also essential medium components. Weak growth in the dark occurs with mannose, 2-oxoglutarate and amino acids. Unable to grow on nitrate (2.5 mM), ammonia (1 mM) and 80% (v/v) dinitrogen gas. Thioglycolate, L-methionine, i-cysteine/cystine, sulfur and thiosulfate can serve as sulfur sources. Fatty acids include n-C_{14} : 0, iso-C_{15} : 0, anteiso-C_{15} : 0, iso-C_{16} : 0, n-C_{16} : 1n9, n-C_{16} : 0, iso-C_{17} : 0, anteiso-C_{17} : 0, and 5-methyl iso-diabolic acid, but iso-diabolic acid is the most abundant. Shows substantial amounts of a C-18 n-alkane. Polar lipids are diacylglycerolhydroxymethyl-N, N, N-trimethyl-β-alanine, phosphatidylethanolamine, phosphatidylmonomethylethanolamine and phosphatidylcholine. Synthesizes three hphanoids: diploptene, bacteriohophanetetrolcylitol ether.

The type strain, strain B^T (ATCC BAA-2647, JCM 30199), was isolated from the phototrophic microbial mat community located in the effluent channel of Octopus Spring in the Lower Geyser Basin of Yellowstone National Park, Wyoming, USA. The genomic DNA G+C content of the type strain was 61.3 mol% (by sequence).

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References


