

Kallotenue papyrolyticum gen. nov., sp. nov., a cellulolytic and filamentous thermophile that represents a novel lineage (*Kallotenuales* ord. nov., *Kallotenuaceae* fam. nov.) within the class *Chloroflexia*

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Several closely related, thermophilic and cellulolytic bacterial strains, designated JKG1^T, JKG2, JKG3, JKG4 and JKG5, were isolated from a cellulolytic enrichment (corn stover) incubated in the water column of Great Boiling Spring, NV. Strain JKG1^T had cells of diameter 0.7–0.9 µm and length ~2.0 µm that formed non-branched, multicellular filaments reaching >300 µm. Spores were not formed and dense liquid cultures were red. The temperature range for growth was 45–65 °C, with an optimum of 55 °C. The pH range for growth was pH 5.6–9.0, with an optimum of pH 7.5. JKG1^T grew as an aerobic heterotroph, utilizing glucose, sucrose, xylose, arabinose, cellobiose, CM-cellulose, filter paper, microcrystalline cellulose, xylan, starch, Casamino acids, tryptone, peptone, yeast extract, acetate, citrate, lactate, pyruvate and glycerol as sole carbon sources, and was not observed to photosynthesize. The cells stained Gram-negative. Phylogenetic analysis using 16S rRNA gene sequences placed the new isolates in the class *Chloroflexia*, but distant from other cultivated members, with the highest sequence identity of 82.5% to *Roseiflexus castenholzii*. The major quinone was menaquinone-9; no ubiquinones were detected. The major cellular fatty acids (>5%) were C_{18:0}, anteiso-C_{17:0}, iso-C_{18:0}, iso-C_{17:0}, C_{16:0}, iso-C_{16:0} and C_{17:0}. The peptidoglycan amino acids were alanine, ornithine, glutamic acid, serine and asparagine. Whole-cell sugars included mannose, rhamnose, glucose, galactose, ribose, arabinose and xylose. Morphological, phylogenetic and chemotaxonomic results suggest that JKG1^T is representative of a new lineage within the class *Chloroflexia*, which we propose to designate *Kallotenue papyrolyticum* gen. nov., sp. nov., *Kallotenuaceae* fam. nov., *Kallotenuales* ord. nov. The type strain of *Kallotenue papyrolyticum* gen. nov., sp. nov. is JKG1^T (=DSM 26889^T=JCM 19132^T).

The phylum *Chloroflexi* is a deeply branching lineage of bacteria composed of a limited number of cultivated representatives that display diverse metabolic strategies and

phenotypes. The class *Chloroflexia* contains gliding filamentous bacteria divided into two orders, *Chloroflexales* and *Herpetosiphonales*. The order *Chloroflexales* comprises anoxygenic phototrophs that possess bacteriochlorophyll and may contain chlorosomes (Dubinina & Gorlenko, 1975; Hanada *et al.*, 2002; Keppen *et al.*, 1994; Pierson & Castenholz, 1974; Pierson *et al.*, 1985). The order *Herpetosiphonales* includes only one genus, *Herpetosiphon*, the two described species of which, *Herpetosiphon geysericola*

Abbreviations: GBS, Great Boiling Spring; MK, menaquinone; SEM, scanning electron microscopy; TEM, transmission electron microscope.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of strains JKG1^T, JKG2, JKG3, JKG4 and JKG5 are JX848544, JX848545, JX848546, JX848547 and JX848548, respectively.

and *Herpetosiphon aurantiacus*, are mesophilic and aerobic heterotrophs. *Herpetosiphon aurantiacus* utilizes carbohydrates as carbon sources, with 6.5% of its genome devoted to carbohydrate transport and metabolism (Kiss *et al.*, 2011). However, *Herpetosiphon geysericola*, reported to degrade cigarette paper, is the only member of the class *Chloroflexia* known to hydrolyse either soluble or insoluble cellulose (Lewin 1970).

Five bacterial strains (JKG1^T, JKG2, JKG3, JKG4 and JKG5) were isolated from a lignocellulosic enrichment incubated in Great Boiling Spring (GBS). GBS is a circumneutral geothermal spring, located in north-western Nevada, USA, at 40° 39' 41" N 119° 21' 58" W (Costa *et al.*, 2009). Twenty grams of ammonia fibre explosion (AFEX)-treated corn stover were enclosed within a nylon filter bag of 100 µm pore size (Pentair Industrial) sewn shut with nylon thread. The bag was suspended in the spring water column of GBS ~10 cm below the air–water interface at Site 85 (85 °C) for 9 weeks (Peacock *et al.*, 2013). A modified version of Castenholz Medium D (Castenholz, 1969), designated Castenholz medium D VN (VN medium), was prepared with the addition of 1 × Wolfe's vitamins (Balch *et al.*, 1979) and 0.027 g NH₄Cl l⁻¹. Ten millilitres of VN medium was prepared in 30 ml glass screw-top culture tubes with 1% (w/v) filter paper (Whatman filter paper grade 1; GE Healthcare) as the sole carbon and energy source (VNF medium) and air as headspace. Material from the enrichment was obtained using sterile forceps and used to inoculate three screw-top tubes, each of which was incubated in the laboratory at 60, 70 or 80 °C. After 8 weeks of incubation a biofilm was visible in the 60 °C tube. The biofilm culture was maintained on VNF medium until pure cultures could be obtained. Traditional approaches of plating or dilution to extinction yielded weakly or non-cellulolytic isolates of the genera *Thermus*, *Geobacillus* and *Rhodothermus*; however, pure cultures were not obtained of a filamentous morphotype commonly observed at the medium/filter paper interface. Therefore, short filaments, representing one or a few cells, were separated from the mixed culture by using optical tweezers and a sterile microfluidic device (Dodsworth *et al.*, 2013; Youssef *et al.*, 2011) and recovered from the chip by flushing with VN medium. Five strains (JKG1, JKG2, JKG3, JKG4 and JKG5) of similar morphology were cultivated from single filaments. All five strains were checked for purity based on the recovery of a single colony type following plating on solid VN medium with 0.2% (w/v) glucose and buffered at pH 7.5 with 15 mM HEPES (VNG medium). VNG medium was solidified by the addition of 1.5% (w/v) agar (granulated; BD Difco). In addition, direct sequencing of 16S rRNA genes PCR-amplified from DNA prepared from each strain, described below, yielded a single, high quality sequence with no ambiguous nucleotides diagnostic of a mixed culture. Strain JKG1^T was characterized in detail.

The colony morphology of strain JKG1^T was observed after 7 days of incubation at 55 °C on VNG. Filament

morphology was observed using phase-contrast microscopy with an Olympus BX51 phase-contrast microscope and Olympus V-TV1 × –2 camera. Detailed morphology was visualized using a Tecnai T-12 TEM (FEI) transmission electron microscope (TEM) with LaB6 filament operating at 120 kV. Images were collected digitally using a 2 × 2K Ultrascan 1000 charge-coupled device with a 'U' scintillator (Gatan) calibrated to the TEM camera length to enable direct measurements correlated with the magnification of the acquired images. DigitalMicrograph (Gatan) software was used for imaging and analyses of cellular features. Scanning electron microscopy (SEM) samples were examined using a Helios 600 Nanolab Dual Beam microscope (FEI).

For TEM, cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C overnight and washed three times in 0.1 M cacodylate buffer. Cells were then osmicated with 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at room temperature and washed three times in 0.1 M cacodylate buffer. Cells were dehydrated in 30, 50 and 75% ethanol for 30 min each, and three times in 100% ethanol for 60 min. Cells were washed with a 1:1 mixture of LR white acrylic resin (Electron Microscopy Sciences) and ethanol for 30 min and then infiltrated in 100% resin, three washes, 4 h each. Samples were cured at 60 °C for 24 h. Polymerized blocks were sectioned to 70 nm thin sections with a Leica Ultracut UCT ultramicrotome, sections were mounted on Formvar-coated 100 mesh Cu TEM grids, sputtered with carbon and post-stained for 7 min with aqueous 2% uranyl acetate prior to TEM imaging.

For the whole mount preparation, 5 µl of a planktonic cell suspension was applied on a 100 mesh Cu grid with Formvar and carbon (Electron Microscopy Sciences). The cells were allowed to adhere to the grids for 30 s before blotting the liquid with a filter paper and then staining with 5 µl negative stain Nano-W (Nanoprobes) for 45 s, followed by air drying.

For SEM, cells were fixed in 2.5% glutaraldehyde in 0.1 M cacodylate buffer at 4 °C overnight, washed three times in buffer and dehydrated in an ethanol series (33%, 50%, 75%), followed by three washes in 100% ethanol. The samples were then critically point dried in a critical point dryer (CPD) instrument (Samdri-795; Tousimis) and processed according to an automated CPD scheme, with CO₂ as a transitional fluid. The CPD holder was lined with nanopore membrane to prevent cell loss during the processing. The cells were mounted on standard aluminium SEM stubs covered with double-sided carbon adhesive tape and sputter-coated with carbon.

Physiological tests carried out in VNG broth were done at 55 °C in the dark in glass 25 ml Balch tubes stoppered with butyl rubber septa (Wheaton) with an air headspace and orbital shaking at an empirically determined optimum (100 r.p.m., 20° angle), except where noted. Growth at the following pH was tested with 15 mM of the indicated

buffer: pH 5.5, 6.0 and 6.5, MES; pH 7.0, 7.5 and 8.0, HEPES; pH 7.5, 8.0, 8.5 and 9.0, Tris; and pH 8.5, 9.5 and 10.0 CHES. Growth temperature was tested in five-degree increments, from 40 to 75 °C. NaCl tolerance was tested at 0, 0.2, 0.4, 1.0, 1.5, 2.0, 2.5 and 3.0 % (w/v) NaCl.

VN medium buffered to pH 7.5 (at 55 °C) with 15 mM HEPES was used to test the utilization of a variety of potential growth substrates, tested at 0.2 % (w/v): D-glucose, sucrose, (+)-cellobiose, xylose, L-arabinose, pyruvate, Casamino acids (EMD), tryptone (BD Bacto), peptone (BD Bacto), yeast extract (EM Science, EMD), acetate, citrate, glycerol, lactate, CM-cellulose (Spectrum), filter paper (Whatman filter paper grade 1; GE Healthcare), microcrystalline cellulose (extra pure 90 µm; Acros Organics, Thermo Fisher Scientific), xylan (birch wood; Sigma-Aldrich) and starch (soluble potato starch powder; JT Baker, Avator Performance Materials). Three consecutive transfers were conducted for each substrate, with 72 h of growth allowed before each transfer, and the third transfer was performed in triplicate. Positive substrate utilization was determined by a final mean OD exceeding that of a no-carbon-source control by 0.050 OD units (Spectronic 20D spectrophotometer; Milton Roy). Growth under anaerobic conditions was tested using Balch tubes with N₂-gassed VN medium buffered to pH 7.5 with 15 mM HEPES and a headspace of N₂ using glucose, Casamino acids and yeast extract (0.2 %, w/v) as potential fermentation substrates. Anaerobic respiration was tested in VNG with the following possible terminal electron acceptors at 2 mM except where noted: nitrate, DMSO, fumarate, ferric iron [ferric-nitritotriacetic acid or 18 mM hydrous ferric oxide (Schwertmann & Cornell, 2000)], thiosulfate and sulfite.

Phototrophic growth was tested using N₂-gassed VN medium, N₂ as headspace and 0.3 mM Na₂S or 3 mM thiosulfate as possible electron donors. Tubes were incubated under the illumination of a full-spectrum LED bulb (Grow Lite; Stimulus Brands) or incandescent bulb. Photoheterotrophic growth was tested using glucose, citrate and yeast extract (0.2 %, w/v) as potential substrates. Photoautotrophic growth was tested by the addition of 50 mM NaHCO₃.

Chemotaxonomic analyses were conducted on lyophilized cells that were grown aerobically in R2A medium (Reasoner & Geldreich, 1985). Glucose was prepared separately as a sterile solution and added after autoclaving. Fatty acid methyl ester analysis was performed by MIDI Laboratories (Newark, DE, USA) (Sasser, 2006). Preparation of purified cell wall and peptidoglycan analysis were performed by TLC and HPLC as described by Schleifer (1985) and Tang *et al.* (2009). The whole-cell sugars were prepared and analysed according to the method described by Tang *et al.* (2009). Menaquinones were extracted as described by Collins *et al.* (1977) and Minnikin *et al.* (1984) and then analysed by HPLC (Tamaoka *et al.*, 1983).

DNA was extracted using the FastDNA Spin kit for Soil (MP Biomedicals) using modifications specified previously (Dodsworth *et al.*, 2011). 16S rRNA genes were amplified

by PCR using primers 9bF (Eder *et al.*, 1999) and 1512uR (Eder *et al.*, 2001) as described previously (Costa *et al.*, 2009) and sequenced at Functional Biosciences (Madison, WI, USA) using the PCR primers 704bR (TCTACG YATTTTCACYGCT) and 516uF (TGBCAGCMGCCGCGG-TAA) to obtain overlapping reads. Reads were trimmed to remove bases with quality scores of less than 20, aligned against the mothur-provided SILVA alignment in the program mothur v1.20.2 (Schloss *et al.*, 2009) and trimmed to the shortest sequence, resulting in an alignment of the near-full-length 16S rRNA gene sequences of all five strains 1355 nt in length.

The nucleotide sequence of the near-full-length 16S rRNA gene of strain JKG1^T was aligned with reference sequences of members of the phylum *Chloroflexi* using the mothur-provided SILVA alignment in the program mothur. The sequences of *Heliothrix oregonensis* and *Chloronema giganteum* were excluded due to poor sequence quality. BioEdit v7.0.5.3 was used to manually curate the alignment and calculate the pairwise 16S rRNA gene sequence identities (Hall, 1999). The mothur-provided SILVA-compatible 1349-position Lane mask was applied (Lane, 1991). PHYLIP v3.69 was used to produce neighbour-joining, maximum-parsimony and maximum-likelihood phylogenetic trees with 100 bootstraps per tree (Felsenstein, 2005). The following parameters in PHYLIP were modified as indicated: input order of species was randomized, out-group root was set to *Bacillus subtilis*, jumbles were 1, global rearrangements on, speedier but rougher analysis off, and more thorough search on. Trees were visualized and exported using Dendroscope v2 (Huson *et al.*, 2007).

Strain JKG1^T formed small, white, rhizoid colonies and gliding motility was observed. Dense liquid cultures were red and cells stained Gram-negative (Leboffe & Pierce, 2006). Phase-contrast microscopy revealed JKG1^T to be a non-branched, multicellular and filamentous bacterium with a diameter of 0.7–0.9 µm (Fig. 1a). Filaments were flexible and composed of individual cells of ~2.0 µm in length. Spores were not observed. Transparent sections resembling the sleeves observed in species of the genus *Herpetosiphon* were sometimes observed between cells or at the ends of filaments (Lee & Reichenbach, 2006). Filaments in VNG medium typically ranged from 10–40 µm; in R2A medium filaments were longer, reaching >300 µm. SEM and TEM showed the filaments to be cylindrical with constrictions at cell junctions within the filaments (Fig. 1b–d). TEM of thin sections did not show the presence of chlorosomes or cellular inclusions (Fig. 1e, f). The cell envelope was similar to those of other members of the phylum *Chloroflexi* (Lee & Reichenbach, 2006; Yamada *et al.*, 2006), composed of two electron-dense layers approximately 10 nm apart. The layers were present at the periphery of each cell, including the tip of the cells that terminated each filament (Fig. 1e), but neither layer was resolvable at the junctions of the cells within the filaments (Fig. 1f). The exact structure and composition of the cellular envelopes of members of the phylum *Chloroflexi* is

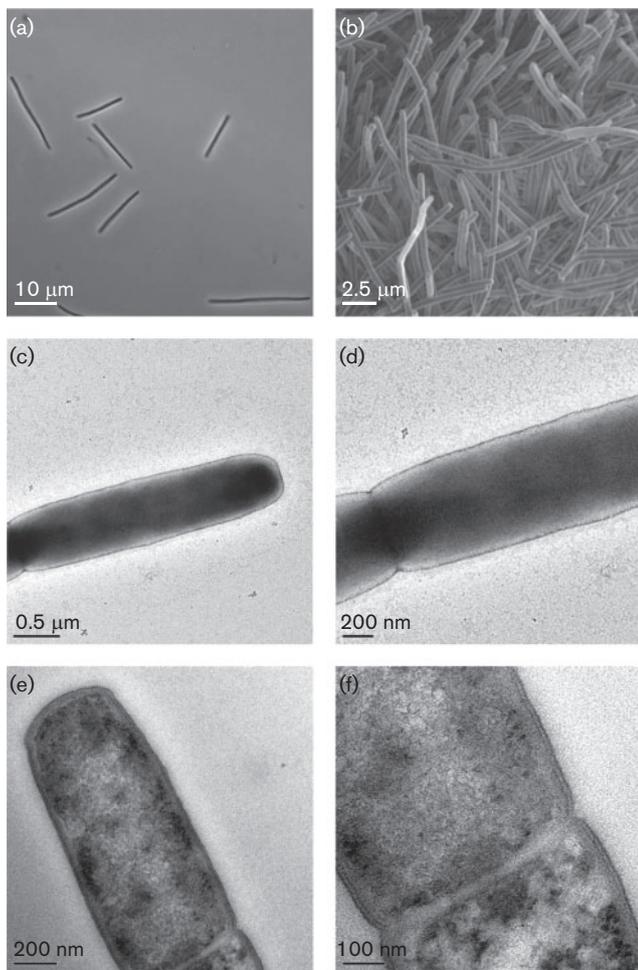


Fig. 1. Micrographs of strain JKG1^T. (a) Phase-contrast micrograph of whole filaments. (b) SEM image of whole filaments. (c) TEM image of a whole mount focused on a single cell and (d) the junction between cells in a filament. (e) TEM image of a single cell thin section and (f) the junction between cells in a filament.

currently unknown, but genomic evidence has failed to reveal components necessary for synthesis of an outer membrane and protein secretion to or across an outer membrane, suggesting a monoderm cell structure (Sutcliffe, 2010, 2011).

Strain JKG1^T was able to grow heterotrophically under aerobic conditions utilizing glucose, sucrose, xylose, arabinose, cellobiose, CM-cellulose, filter paper, microcrystalline cellulose, xylan, starch, Casamino acids, tryptone, peptone, yeast extract, acetate, citrate, lactate, pyruvate and glycerol as sole carbon sources. Optimal growth occurred at 55 °C and pH 7.5. Growth occurred from 45 to 65 °C and pH 6.5–9.5. Photoheterotrophic, photoautotrophic, fermentative and anaerobic respiration were not observed.

The major cellular fatty acids of strain JKG1^T were C_{18:0} (26.3% of total fatty acids), anteiso-C_{17:0} (15.0%), iso-C_{18:0} (12.7%), iso-C_{17:0} (11.5%), C_{16:0} (8.9%),

iso-C_{16:0} (6.2%) and C_{17:0} (5.0%). The minor fatty acids were anteiso-C_{19:0} (3.9%), anteiso-C_{15:0} (1.9%), iso-C_{19:0} (1.4%), C_{18:1 ω 9c} (1.4%), C_{19:0} (1.0%), C_{17:0} 3-OH (0.9%), C_{18:1 ω 7c} (0.8%), iso-C_{15:0} (0.7%), iso-C_{17:1 ω 9c} (0.6%), anteiso-C_{17:1 ω 9c} (0.5%), anteiso-C_{13:0} (0.5%), C_{15:0} (0.5%) and C_{14:0} (0.4%).

The major quinone was MK-9(H6) (84.1% relative abundance), with a smaller proportion of MK-8(H2) (15.9%). The peptidoglycan amino acids were alanine, ornithine, glutamic acid, serine and asparagine. *meso*-Diaminopimelic acid was not present and peptide cross-links are likely formed between ornithine and D-alanine, as in other members of the phylum *Chloroflexi* (Cavaletti *et al.*, 2006; Jürgens *et al.*, 1987, 1989; Yabe *et al.*, 2010, 2011). In particular, ornithine has been found to replace diaminopimelic acid as the diamino acid that forms the cross-peptide bonds (Garrity & Holt, 2001). Whole-cell sugars were mannose, rhamnose, glucose, galactose, ribose, arabinose xylose, and two unknown compounds. The DNA G+C content was 72.4 mol% and was determined by HPLC (Mesbah *et al.*, 1989).

The 16S rRNA gene sequences of all five strains were identical over the length of the alignment shared by all five sequences (1355 nt). Phylogenetic analysis of JKG1^T using the 16S rRNA gene sequence placed the strain in the class *Chloroflexia*, with bootstrap values of at least 90% at the node defining the class in all three phylogenetic methods utilized (Fig. 2). Members of the class *Chloroflexia* share an unbranched filamentous morphology, with the majority of the isolates capable of aerobic respiration (Table 1). However, strain JKG1^T is distinguishable from these other members of the class as the only thermophilic chemo-organotroph not capable of photosynthesis. In addition, strain JKG1^T harbours distinct profiles of fatty acids, quinones, whole-cell sugars and cell wall amino acids. Finally, the 16S rRNA gene sequence identity between strain JKG1^T and other members of the class *Chloroflexia* was \leq 82.5%, which is well below the mean pairwise 16S rRNA gene sequence identity between taxa whose lowest shared rank is class, \sim 87% (Lasher *et al.*, 2009; Konstantinidis & Tiedje, 2005), and the 16S rRNA gene sequence identity commonly used to circumscribe microbial orders in cultivation-independent studies, 85% (Cole *et al.*, 2013; Peacock *et al.*, 2013; Schmitt *et al.*, 2012; Webster *et al.*, 2010). Altogether, the unique phylogenetic, phenotypic and chemotaxonomic features of strain JKG1^T suggest that it is representative of a novel genus, family and order within the class *Chloroflexia*. The designations *Kallotenue* gen. nov., *Kallotenuaceae* fam. nov. and *Kallotenuales* ord. nov., are proposed.

Description of *Kallotenue* gen. nov.

Kallotenue (Kal.lo.te'nu.e. Gr. neut. n. *kallos* beauty, grace; L. adj. *tenuis* -is -e slender, fine, thin; N.L. neut. n. *Kallotenue* a thin beauty).

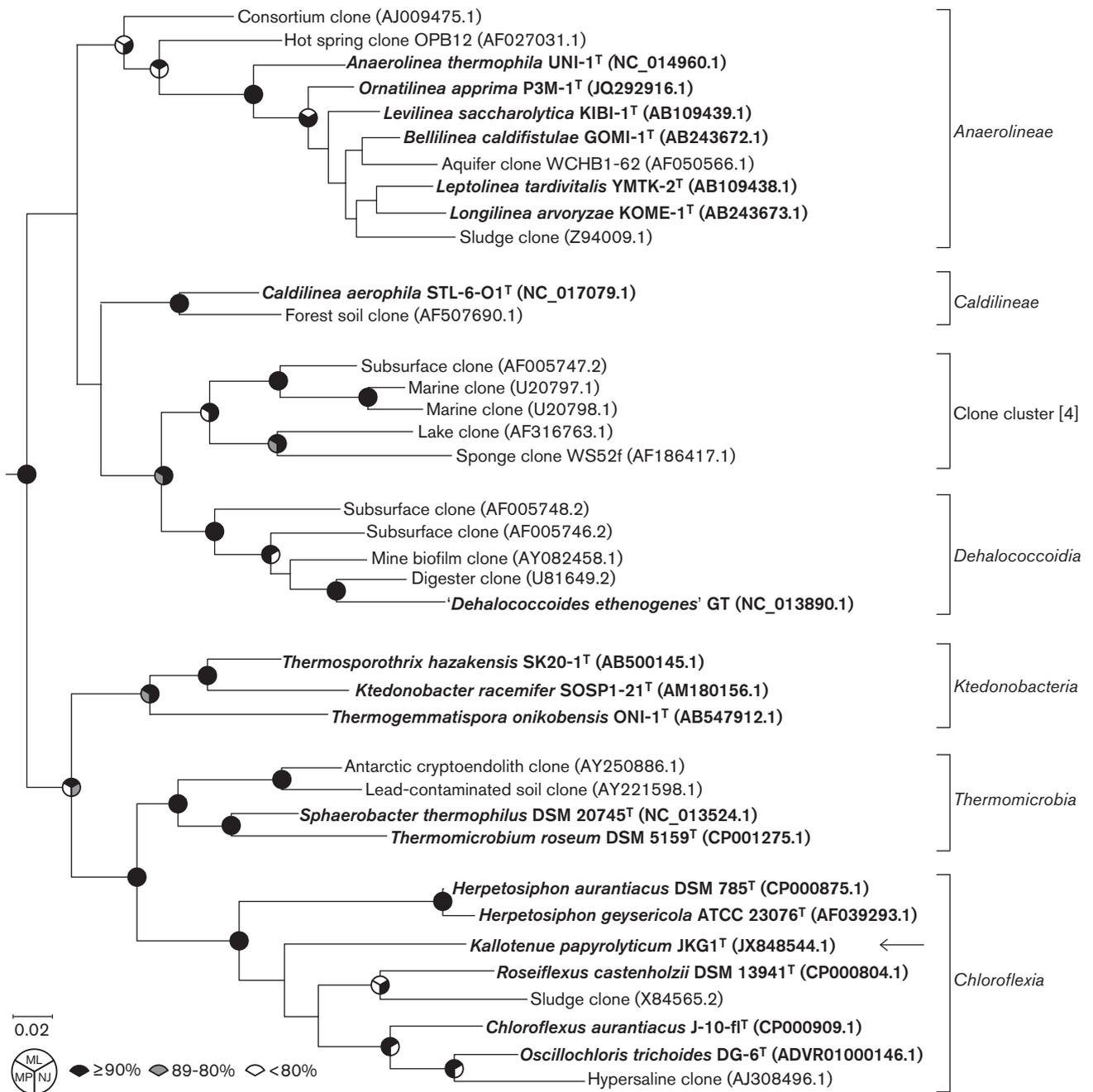


Fig. 2. Maximum-likelihood phylogenetic tree of the phylum *Chloroflexi*, with strain JKG1^T indicated by an arrow. The tree was reconstructed based upon 1183 aligned positions that remained after the application of the Lane mask to the 16S rRNA gene sequences. Bootstrap values are from 100 replicates for each tree-building method represented [maximum-likelihood (ML), maximum-parsimony (MP), and neighbour-joining (NJ)] and are indicated at each node using black ($\geq 90\%$ recovery), grey (89–80% recovery) or no shading ($< 80\%$ recovery). Bootstrap support for nodes supported by $< 80\%$ recovery from all three methods is not shown. Bar, 0.02 changes per nucleotide. Accession numbers are included in parentheses and strains are included for cultivated representatives. Outgroups were *Bacillus subtilis* 168^T (D26185.1), *Escherichia coli* ATCC 11775^T (X80725.1) and *Corynebacterium diphtheriae* NCTC 11397^T (X84248.1).

Filamentous, non-branching and non-spore-forming bacteria. Gram-stain negative. Thermophilic, aerobic chemotrophs that grow at 45–65 °C, pH 5.6–9.0 and with

0–2.0 % (w/v) NaCl. Peptidoglycan amino acids include alanine, ornithine, glutamine, serine, and asparagine. Major whole-cell sugars comprise mannose, rhamnose

Table 1. Phenotypic characteristics of strain JKG1^T and related members of the class *Chloroflexia*

+, Positive; −, negative; ±, variable; ND, not determined; Unk, unknown.

Characteristic	Aerobic chemotrophs			Chlorosome-less phototrophs	Chlorosome-containing phototrophs	
	JKG1 ^T	<i>Herpetosiphon aurantiacus</i> DSM 785 ^T	<i>Herpetosiphon geysericola</i> ATCC 23076 ^T	<i>Roseiflexus castenholzii</i> DSM 13941 ^T	<i>Chloroflexus aurantiacus</i> J-10-fl ^T	<i>Oscillochloris trichoides</i> DG-6 ^T
Cell diameter (µm)	0.7–0.9	1.0–1.5	1.0	0.8–1.0	0.7–1.2	1.0–1.5
Sleeves	+	+	+	−	±	±
Gas vesicles	−	−	−	−	−	+
Gram stain results	Negative	Negative	Negative	Negative	Negative	Variable
Optimum growth temperature (°C)	55	30–37	30–37	55	55	28–30
Metabolism						
Photoheterotrophy	−	−	−	+	+	+
Photoautotrophy	−	−	−	−	±	+
Aerobic respiration	+	+	+	+	+	−
Fermentation	−	ND	ND	−	ND	ND
Cellulolytic ability						
CM-cellulose	+	−	ND	ND	ND	ND
Insoluble cellulose	+	−	+	ND	ND	ND
Major cellular fatty acids (>5%)	C _{18:0} , anteiso-C _{17:0} , iso-C _{18:0} , iso-C _{17:0} , C _{16:0} , iso-C _{16:0} , C _{17:0}	C _{16:1} , C _{16:0} , C _{18:1}	C _{16:1} , C _{16:0} , C _{18:1}	C _{16:0} , C _{14:0} , C _{15:0}	C _{18:0} , C _{16:0} , C _{18:1} , C _{17:0}	C _{18:1} , C _{16:0} , C _{16:1}
Predominant menaquinone	MK-9	MK-6	ND	MK-11	MK-10	MK-10
DNA G+C content	72.4	50.9	51.8	62.0	56.9–57.1	59.2
Whole-cell or [cell wall] sugars	Man, Rham, Glc, Ara, Xyl, Unk (×2), Gal, Rib	[Man, Gal, Rham, Ara, Xyl]	ND	ND	[Rham, Man, Glc, Gal, Xyl, Ara]	ND
Cell wall amino acids	Ala, Orn, Glu, Ser, Asn	Glu, Ala, Orn, Gly, His	ND	ND	Glu, Ala, Orn, Gly, His	ND
Isolation environment	Lignocellulosic enrichment in terrestrial hot spring	Slimy coating of <i>Chara</i> sp. in freshwater lake	Rock surface beside hot spring	Bacterial mat in hot spring	Hot spring	Hydrogen sulfide-containing spring
References	Present study	Holt & Lewin (1968); Jürgens <i>et al.</i> (1989); Kiss <i>et al.</i> (2011)	Lewin (1970)	Hanada <i>et al.</i> (2002)	Jürgens <i>et al.</i> (1987); Knudsen <i>et al.</i> (1982); Pierson & Castenholz (1974)	Berg <i>et al.</i> (2005); Keppen <i>et al.</i> (1994)

and glucose. The major quinone is menaquinone-9. The type species is *Kallotenue papyrolyticum*.

Description of *Kallotenue papyrolyticum* sp. nov.

Kallotenue papyrolyticum [pa.py.ro.ly'ti.cum. Gr. n. *papyros* papyrus, paper; N.L. neut. adj. *lyticum* (from Gr. neut. adj. *lytikon*) able to loose, able to dissolve; N.L. neut. adj. *papyrolyticum* paper-dissolving].

The following properties are displayed, in addition to those specified for the genus. Growth occurs at an optimum temperature of 55 °C and optimum pH of 7.5. Colonies are small, 1–2 mm diameter, white and rhizoid. The following substrates support growth: glucose, sucrose, xylose, arabinose, cellobiose, CM-cellulose, filter paper, microcrystalline cellulose, xylan, starch, Casamino acids, tryptone, peptone, yeast extract, acetate, citrate, lactate, pyruvate and glycerol. The major cellular fatty acids are C_{18:0}, anteiso-C_{17:0}, iso-C_{18:0}, iso-C_{17:0}, C_{16:0}, iso-C_{16:0}, C_{17:0} and anteiso-C_{19:0}.

The type strain is JKG1^T (=DSM 26889^T=JCM 19132^T), isolated from a lignocellulosic enrichment incubated in a hot spring. The G+C content of the type strain is 72.4 mol%.

Description of *Kallotenaceae* fam. nov.

Kallotenuaceae (Kal.lo.te.nu.a'ce.a.e. N.L. n. *Kallotenue* type genus of the family; suff. *-aceae* ending denoting a family; N.L. fem. pl. n. *Kallotenuaceae* the family of the genus *Kallotenue*).

The description is the same as for the genus *Kallotenue*. The family is a member of the order *Kallotenuales*. The type genus is *Kallotenue*.

Description of *Kallotenuales* ord. nov.

Kallotenuales (Kal.lo.te.nu.a'les. N.L. n. *Kallotenue* type genus of the order; suff. *-ales* ending denoting an order; N.L. fem. pl. n. *Kallotenuales* the order of the genus *Kallotenue*).

The description is the same as for the genus *Kallotenue*. The order is a member of the class *Chloroflexia*. The type genus is *Kallotenue*.

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