

High-Quality Draft Genome Sequence of *Kallotenua papyrolyticum* JKG1^T Reveals Broad Heterotrophic Capacity Focused on Carbohydrate and Amino Acid Metabolism

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The draft genome of *Kallotenua papyrolyticum* JKG1^T, a member of the order *Kallotenuales*, class *Chloroflexia*, consists of 4,475,263 bp in 4 contigs and encodes 4,010 predicted genes, 49 tRNA-encoding genes, and 3 rRNA operons. The genome is consistent with a heterotrophic lifestyle including catabolism of polysaccharides and amino acids.

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Strain JKG1^T was isolated from a cellulolytic enrichment in Great Boiling Spring, Nevada (1), using optical tweezers and a microfluidic device due to its capacity to grow aerobically on filter paper (optimum 55 °C) (2). Strain JKG1^T was described as a member of a new genus and species, *Kallotenua papyrolyticum*, and a new order, *Kallotenuales*, of the phylum *Chloroflexi* (2). It has broad heterotrophic activity, including the capacity to hydrolyze polysaccharides such as carboxymethylcellulose, microcrystalline cellulose, filter paper, xylan, and starch, and proteinaceous substrates such as casamino acids, tryptone, and peptone. *K. papyrolyticum* is one of several, new high-level taxonomic groups of thermophilic *Chloroflexi* isolated from Great Boiling spring (3).

The draft genome of strain JKG1^T was generated at the DOE Joint genome Institute (JGI) using Pacific Biosciences (PacBio) technology. A PacBio SMRTbell library was constructed and sequenced on the PacBio RS platform, which generated 304,235 filtered subreads totaling 854.0 Mbp. All general aspects of library construction and sequencing performed at the JGI can be found at <http://www.jgi.doe.gov>. The raw reads were assembled using HGAP (version 2.0.0) (4). The genome was annotated using Prodigal version 2.5 (5), as part of the JGI microbial annotation pipeline (6). The *K. papyrolyticum* draft genome is 4,475,263 bp in 4 contigs, encoding 4,010 predicted genes, including 49 tRNA-encoding genes, and three rRNA operons. The genome encodes enzymes for complete glycolysis, tricarboxylic acid cycle, and pentose phosphate pathways and dedicates a large amount of its genome to carbohydrate (10.1%; clusters of orthologous groups [COG] category G) and amino acid (8.9%; COG category E) metabolism. Analysis of the genome for carbohydrate-active enzymes (CAZymes) (7) revealed 171 total CAZymes, 55 of which are glyco-

side hydrolases (GHs), including GHs putatively involved in chitin (GH18), woody plant (GH53), and cellulose (GH5,6,9,10,51) depolymerization. This number of CAZymes and GH domains is similar to other cellulolytic thermophiles, such as *Thermotoga maritima* (8), *Caldicellulosiruptor* species (9), and *Dictyoglomus turgidum* (10).

Catabolism of organic compounds can be coupled to aerobic respiration. Similar to *Chloroflexus* (11), strain JKG1^T has a prototypical respiratory complex I and II to transfer electrons into the quinone pool; however, quinols are likely oxidized by an alternative complex III (ACIII) (12) rather than a *bc₁/b_f* complex. The presence of lactate and ethanol dehydrogenases, acetate-CoA ligase, and an iron-only hydrogenase suggest capacity for fermentation, however, fermentation of glucose, casamino acids, or yeast extract was not observed in JKG1^T cultures (2). Strain JKG1^T may be able to oxidize carbon monoxide through a putative type II carbon monoxide dehydrogenase (CODH) encoded by a *cox* gene cluster, although the functions of type II CODH are not firmly established (13). Known pathways for phototrophy, autotrophy, and anaerobic respiration are absent, although putative molybdopterin oxidoreductases and multicopper oxidases may be involved in anaerobic respiration.

PFAM domains representing proteins involved in outer membrane biogenesis and transport are not present, suggesting JKG1^T has a monoderm cell envelope structure similar to other *Chloroflexi* (14).

Nucleotide sequence accession numbers. This whole-genome shotgun project has been deposited in GenBank under accession numbers JAGA01000001 to JAGA01000004.

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REFERENCES

1. Peacock JP, Cole JK, Murugapiran SK, Dodsworth JA, Fisher JC, Moser DP, Hedlund BP. 2013. Pyrosequencing reveals high-temperature cellulolytic microbial consortia in great boiling spring after *in situ* lignocellulose enrichment. *PLoS One* 8:e59927. <http://dx.doi.org/10.1371/journal.pone.0059927>.
2. Cole JK, Gieler BA, Heisler DL, Palisoc MM, Williams AJ, Dohnalkova AC, Ming H, Yu TT, Dodsworth JA, Li W-J, Hedlund BP. 2013. *Kallotenua 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*. *Int J Syst Evol Microbiol* 63:4675–4682. <http://dx.doi.org/10.1099/ijs.0.053348-0>.
3. Dodsworth JA, Gevorkian J, Despujos F, Cole JK, Murugapiran SK, Ming H, Li W-, Zhang G, Dohnalkova A, Hedlund BP. 2014. *Thermoflexus hugenholtzii* gen. nov., sp. nov., a thermophilic, microaerophilic, filamentous bacterium representing a novel class in the *Chloroflexi*, *Thermoflexia* classis nov, and description of *Thermoflexaceae* fam. nov. and *Thermoflexales* ord. nov. *Int J Syst Evol Microbiol* 64:2119–2127. <http://dx.doi.org/10.1099/ijs.0.055855-0>.
4. Chin C, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <http://dx.doi.org/10.1038/nmeth.2474>.
5. Hyatt D, Chen G, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <http://dx.doi.org/10.1186/1471-2105-11-119>.
6. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen IM-A, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The standard operating procedure of the DOE-JGI microbial genome annotation pipeline (MGAP v.4). *Stand Genomic Sci*, in press.
7. Nelson KE, Clayton RA, Gill SR, Gwinn ML, Dodson RJ, Haft DH, Hickey EK, Peterson JD, Nelson WC, Ketchum KA, McDonald L, Utterback TR, Malek JA, Linher KD, Garrett MM, Stewart AM, Cotton MD, Pratt MS, Phillips CA, Richardson D, Heidelberg J, Sutton GG, Fleischmann RD, Eisen JA, White O, Salzberg SL, Smith HO, Venter JC, Fraser CM. 1999. Evidence for lateral gene transfer between Archaea and bacteria from genome sequence of *Thermotoga maritima*. *Nature* 399:323–329. <http://dx.doi.org/10.1038/20601>.
8. Blumer-Schuette SE, Ozdemir I, Mistry D, Lucas S, Lapidus A, Cheng J-, Goodwin LA, Pitluck S, Land ML, Hauser LJ, Woyke T, Mikhailova N, Pati A, Kyrpides NC, Ivanova N, Detter JC, Walston-Davenport K, Han S, Adams MWW, Kelly RM. 2011. Complete genome sequences for the anaerobic, extremely thermophilic plant biomass-degrading bacteria *Caldicellulosiruptor hydrothermalis*, *Caldicellulosiruptor kristjanssonii*, *Caldicellulosiruptor kronotskyensis*, *Caldicellulosiruptor owensensis*, and *Caldicellulosiruptor lactoaceticus*. *J Bacteriol* 193:1483–1484. <http://dx.doi.org/10.1128/JB.01515-10>.
9. Brumm P, Hermanson S, Hochstein B, Boyum J, Hermersmann N, Gowda K, Mead D. 2011. Mining *Dictyoglomus turgidum* for enzymatically active carbohydrases. *Appl Biochem Biotechnol* 163:205–214. <http://dx.doi.org/10.1007/s12010-010-9029-6>.
10. Cantarel BL, Coutinho PM, Rancurel C, Bernard T, Lombard V, Henrissat B. 2009. The carbohydrate-active EnZymes database (CAZy): an expert resource for glycogenomics. *Nucleic Acids Res* 37:D233–D238. <http://dx.doi.org/10.1093/nar/gkn663>.
11. Yanyushin MF, del Rosario MC, Brune DC, Blankenship RE. 2005. New class of bacterial membrane oxidoreductases. *Biochemistry* 44:10037–10045. <http://dx.doi.org/10.1021/bi047267l>.
12. Refojo PN, Teixeira M, Pereira MM. 2012. The alternative complex III: Properties and possible mechanisms for electron transfer and energy conservation. *Biochim Biophys Acta* 1817:1852–1859. <http://dx.doi.org/10.1016/j.bbabi.2012.05.003>.
13. King GM, Weber CF. 2007. Distribution, diversity and ecology of aerobic CO-oxidizing bacteria. *Nat Rev Microbiol* 5:107–118. <http://dx.doi.org/10.1038/nrmicro1595>.
14. Sutcliffe IC. 2011. Cell envelope architecture in the *Chloroflexi*: a shifting frontline in a phylogenetic turf war. *Environ Microbiol* 13:279–282. <http://dx.doi.org/10.1111/j.1462-2920.2010.02339.x>.