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Draft Genome Sequence of a Divergent Anaerobic Member of the *Chloroflexi* Class *Ardenticatenia* from a Sulfidic Hot Spring

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**ABSTRACT** Here, we present a draft genome sequence of Nak82, the second genome sequence available for the *Chloroflexi* class *Ardenticatenia* and the first from a sulfidic terrestrial hot spring. Nak82 is genetically and metabolically distinct from *Ardenticatena maritima* and likely represents a new genus- or family-level lineage lacking high-potential respiratory pathways.

*Ardenticatenia* is a curious class in the *Chloroflexi* phylum; it is currently known only as a single isolate from an iron-rich hydrothermal field in Japan (1). *Ardenticatena maritima* is unique among the *Chloroflexi* for its capacity for iron reduction and complete denitrification (2). Here, we report the first genome sequence available from a second *Ardenticatenia* lineage strain, Nak82, recovered from Nakabusa Onsen in Japan. Nak82 is most closely related to *Ardenticatena maritima* but is genetically distinct at the genus or family level and does not share the diverse respiratory pathways that distinguish *Ardenticatena maritima* from other *Chloroflexi* species.

The Nak82 metagenome-assembled genome (MAG) was recovered from sequencing of Nakabusa Onsen, a moderately sulfidic hot spring in Japan. The site and metagenomic sequencing were described previously (3, 4). In brief, the site is a moderately sulfidic and alkaline (pH 8.5 to 9) hot spring with source water near 70°C and containing ~0.1 mM sulfide (5). Samples were collected from microbial mats, and DNA was extracted and submitted to SeqMatic LLC (Fremont, CA) for sequencing with an Illumina HiSeq instrument. Sequences from four samples were coassembled with MEGAHIT v. 1.1.2 (6), and genome bins were constructed based on differential coverage using MetaBAT (7). Genome bins were assessed for completeness and contamination using CheckM (8) and uploaded to the RAST server for overall characterization (9).

The Nak82 MAG totals 3.49 Mb and consists of 2,942 protein-coding sequences across 195 contigs. The genome has a 58.7% GC content and is estimated by CheckM to be 91.74% complete, with 0.64% contamination. Forty-four tRNAs were recovered. Phylogenetic analysis of Nak82 and other *Chloroflexi* using the RpoB protein—a valuable single-copy marker (10)—robustly places this organism as a sister taxon to *Ardenticatena maritima*; however, the RpoB sequences of these strains are only 72% similar, suggesting divergence to at least the genus level.

Nak82 does not have genes that encode the pathways for aerobic respiration and denitrification found in *Ardenticatena maritima*. The only dioxygen reductase recovered in the Nak82 genome is a bd oxidase, which may be used for oxygen detoxification, as it appears in obligate anaerobes, including some members of the phylum *Chloroflexi* class *Anaerolineae* (4, 11–14). This distribution of respiration genes is consistent with the acquisition of aerobic respiration and denitrification by *Ardenticatena maritima* via horizontal gene transfer after its divergence with Nak82, a pattern consistent with broader trends in the evolution of metabolic traits in the *Chloroflexi* (4, 15).
Genes involved in the synthesis of lipopolysaccharides and outer membrane proteins (e.g., lpxB, ompB5, and bamA) were not recovered from Nak82. This is consistent with other evidence that members of the *Chloroflexi* lack an outer membrane, in contrast to members of their sister phylum *Armatimonadetes* (4, 16, 17).

**Accession number(s).** This whole-genome shotgun project was deposited in DDBJ/EMBL/GenBank under the accession number QEXY00000000.

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**REFERENCES**

1. Kawauchi S, Ito N, Kamikawa R, Sugawara T, Yoshida T, Sako Y. 2013. *Ardenticatenia maritima* gen. nov., sp. nov., a ferric iron-and nitrate-reducing bacterium of the phylum “Chloroflexi” isolated from an iron-rich coastal hydrothermal field, and description of *Ardenticatenia* classis nov. Int J Syst Evol Microbiol 63:2992–3002. https://doi.org/10.1099/ijs.0.046532-0.

2. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Ardenticatenia maritima* 110S, a thermophilic nitrate- and iron-reducing member of the *Chloroflexi* class *Ardenticatenia*. Genome Announc 3:e01347-15. https://doi.org/10.1128/genomeA.01347-15.

3. Ward LM. 2017. Microbial evolution and the rise of oxygen: the roles of contingency and context in shaping the biosphere through time. PhD dissertation. California Institute of Technology, Pasadena, CA. https://doi.org/10.7907/29BZ642S.

4. Ward LM, Hemp J, Shihi PM, McGlynn SE, Fischer WW. 2018. Evolution of phototrophy in the *Chloroflexi* phylum driven by horizontal gene transfer. Front Microbiol 9:260. https://doi.org/10.3389/fmicb.2018.00260.

5. Kuro K, Knittel K, Amann R, Fukui M, Matsuura K. 2011. Sulphur-metabolizing bacterial populations in microbial mats of the Nakabusa hot spring, Japan. Syst Appl Microbiol 34:293–302. https://doi.org/10.1016/j.syapm.2010.12.002.

6. Li D, Luo R, Liu C-M, Leung C-M, Ting H-F, Sadakane K, Yamashita H, Lam T-W. 2016. MEGAHIT v1.0: a fast and scalable metagenome assembler driven by advanced methodologies and community practices. Methods 102:3–11. https://doi.org/10.1016/j.ymeth.2016.02.020.

7. Kang DD, Froula J, Egan R, Wang Z. 2015. MetaBAT, an efficient tool for accurately reconstructing single genomes from complex microbial communities. PeerJ 3:e1165. https://doi.org/10.7717/peerj.1165.

8. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. 2015. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. Genome Res 25:1043–1055. https://doi.org/10.1101/gr.186072.114.

9. Aziz RK, Bartels D, Best AA, DeLongh M, Diz S, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil JK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagamitoko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.

10. Hansmann S, Martin W. 2000. Phylogeny of 33 ribosomal and six other proteins encoded in an ancient gene cluster that is conserved across prokaryotic genomes: influence of excluding poorly alignable sites from analysis. Int J Syst Evol Microbiol 50:1655–1663. https://doi.org/10.1099/00207713-50-4-1655.

11. Giuffré A, Borisov VB, Arese M, Sarti P, Forte E. 2014. Cytochrome bd oxidase and bacterial tolerance to oxidative and nitrosative stress. Biochim Biophys Acta 1837:1178–1187. https://doi.org/10.1016/j.bbabio.2014.01.016.

12. Ward LM, Hemp J, Pace LA, Fischer WW. 2015. Draft genome sequence of *Leptolinea tardivitalis* YMTK-2, a mesophilic anaerobe from the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01356-15. https://doi.org/10.1128/genomeA.01356-15.

13. Pace LA, Hemp J, Ward LM, Fischer WW. 2015. Draft genome sequence of *Thermanaerovox daxensis* GNS-1, a thermophilic facultative anaerobe from the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01354-15. https://doi.org/10.1128/genomeA.01354-15.

14. Hemp J, Ward LM, Pace LA, Fischer WW. 2015. Draft genome sequence of *Levilinea saccharolytica* KIBI-1, a member of the *Chloroflexi* class *Anaerolineae*. Genome Announc 3:e01353-15. https://doi.org/10.1128/genomeA.01353-15.

15. Shihi PM, Ward LM, Fischer WW. 2017. Evolution of the 3-hydroxypropionate bicycle and recent transfer of anoxygenic photosynthesis into the *Chloroflexi* phylum driven by horizontal gene transfer. Front Microbiol 8:2. https://doi.org/10.3389/fmicb.2017.00574.

16. Sutcliffe IC. 2011. Cell envelope architecture in the *Chloroflexi*: a shifting frontline in a phylogenetic turf war. Environ Microbiol 13:279–282. https://doi.org/10.1111/j.1462-2920.2010.02339.x.

17. Ward LM, McGlynn SE, Fischer WW. 2017. Draft genome sequences of a novel lineage of *Armatimonadetes* recovered from Japanese hot springs. Genome Announc 5:e00820-17. https://doi.org/10.1128/genomeA.00820-17.