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Draft Genome Sequences of Two Basal Members of the Anaerolineae Class of Chloroflexi from a Sulfidic Hot Spring

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ABSTRACT Here, we describe the first genome sequences of the Anaerolineae from a sulfidic environment, expanding the environmental distribution of sequenced Anaerolineae. These genomes represent basal Anaerolineae lineages, branching soon after the divergence of the sister class “Candidatus Thermofonsia,” expanding our understanding of the metabolic evolution of this group.

Although members of the Anaerolineae class of the Chloroflexi phylum appear in diverse environmental 16S sequence data sets (e.g., carbonate tidal flats [1] and iron-rich hot springs [2]), the environmental distribution of previously sequenced Anaerolineae is primarily limited to groundwater and wastewater systems (e.g., references 3 and 4). Here, we present the first genomes of Anaerolineae from sulfidic environments, Nak19 and Nak57, expanding the genetic and environmental distribution of sequenced representatives of this clade.

The metagenome-assembled genomes (MAGs) were recovered from metagenomic sequencing of microbial communities from a sulfidic hot spring in Japan, as described previously (5, 6). In brief, samples were collected from microbial mats at Nakabusa Onsen in Nagano Prefecture, Japan, and DNA was extracted and sequenced via Illumina HiSeq. Sequences from four samples were coassembled with MegaHit version 1.1.2 (7) and genome bins constructed based on differential coverage using Metabat (8). Genome bins were assessed for completeness and contamination using CheckM (9) and uploaded to RAST for overall characterization (10).

MAG Nak19 totals 3.45 Mb recovered as 158 contigs, encoding 3,163 coding sequences and 44 tRNAs. The Nak19 genome has 51.2% GC content and was estimated by CheckM to be 90% complete, with 6.09% contamination. Nak57 is 3.77 Mb over 159 contigs, encoding 45 tRNAs and 3,391 coding sequences. Its GC content is 51.2%. CheckM estimates Nak57 to be 95.45% complete, with 4.85% contamination.

Neither Nak19 nor Nak57 recovered a 16S gene, but phylogenies based on RpoB and concatenated ribosomal proteins robustly place these MAGs as basal Anaerolineae, with Nak19 being the basal-most Anaerolineae and Nak57 being more closely related to cultured Anaerolineae, such as Thermaanaerobacter xanxensus (11) and Omatilinae apprima (12).

Nak19 and Nak57 both encode aerobic respiration via an A-family heme-copper oxidoeducase (HCO) complex, a bd oxidase, and a bc complex. Nak57 also encodes an alternative complex III (ACIII), while Nak19 encodes NirS for nitrite reduction. Phylogenetic analysis of the respiration genes from Nak57 show gene relationships congruent with organismal relationships, supporting the vertical inheritance of aerobic respiration in the Anaerolineae following acquisition at the base of the class, consistent with trends in other Chloroflexi classes (6, 13). ACIII was not recovered in Nak19, but this may be a false-negative result due to incompleteness of the MAG; however, as the basal-most Anaerolineae member, it may also be that ACIII had not yet been acquired by the
Anaerolineae when this clade diverged. ACIII may therefore have been acquired on the branch leading to Nak57 and the other Anaerolineae. The Anaerolineae have typically been described as obligately anaerobic fermenters (e.g., references 14 and 15), but genes for aerobic respiration appear to be widespread in this group (3, 4, 11). It remains uncertain whether these genes are used for aerobic respiration or only for O2 detoxification in the Anaerolineae whose genomes are described here and elsewhere.

Consistent with other reports from sequenced Chloroflexi, Nak19 and Nak57 do not appear to encode outer membrane proteins, supporting interpretations of a monoderm membrane architecture as distinct from that of the dierm sister phylum Armatimonadetes (6, 16, 17).

Accession number(s). These whole-genome shotgun projects were deposited in DDBJ/EMBL/GenBank under the accession numbers QEXX00000000 (Nak19) and QEXW00000000 (Nak57).

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Sequencing was performed at SeqMatic, Fremont, CA.

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