Metagenome-Assembled Genome of USCα AHI, a Potential High-Affinity Methanotroph from Axel Heiberg Island, Canadian High Arctic

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ABSTRACT Metagenomic sequencing of active-layer cryosols from the Canadian High Arctic has yielded a nearly complete genome for an atmospheric CH4-oxidizing bacterium belonging to upland soil cluster α (USCα). This genome contains genes involved in CH4 metabolism, H2 metabolism, and multiple carbon assimilation pathways.

Recent studies have shown that mineral cryosols from the Canadian High Arctic Axel Heiberg Island (AHI) act as CH4 sinks during the summer (1), drawing CH4 from both the atmosphere and underlying hypoxic cryosols (2, 3), and harbor metabolically active upland soil cluster α (USCα) proteobacteria (1). Twenty-one metagenomic data sets of active-layer cryosols (4) from long-term core incubation experiments were used to construct the draft genome of this USCα. Sequencing and sample collection methods were published by Chauhan et al. (4).

Raw reads were filtered using the Princeton University Galaxy server using “filter by quality” to keep reads having 90% of the bases with a Phred score of >30. Nextera transposase adaptor sequences and the last five bases at the 3’ end were removed using Trim Galore. IDBA-UD v1.1.1 (with the settings mink = 20, makk = 100, and step = 20) was used to create 21 individual assemblies and 1 coassembly from reads longer than 50 nucleotides (nt) (5). Bins were created using MetaBAT v0.32.4 (6) (–very sensitive option), evaluated using CheckM v1.0.6 (7), and annotated using PROKKA v1.12-beta (8) and BLAST v2.2.29+ (9). Default parameters were used for all software unless otherwise specified. The coassembly yielded a 90.56% complete genome with 0.31% contamination, containing a USCα-like particulate methane monoxygenase β-subunit (pmoA) gene. CheckM assigned this genome as an unknown species within the Beijerinckiaceae.

As CheckM analysis indicated that 4 of the 21 individual assemblies had unknown Beijerinckiaceae bins (6.43 to 36.49% complete), we extracted Beijerinckiaceae reads from these 4 metagenomes (SRA accession numbers SRR1586250, SRR1586265, SRR1586287, and SRR1586310). We then mapped the quality-filtered reads onto the USCα bin and four Beijerinckiaceae genomes having different phylogenetic distances from USCα (10), namely, Methylocapsa acidiphila B2 (NZ_ATYA01000001), Methylocella silvestris BL2 (NC_011666), Methyllophilus sp. strain SC2 (NC_018485), and Methylosinus trichosporium OB3b (NZ_ADVE02000003), using Bowtie2 v2.3.2 (11). All mapped reads were pooled and reassembled using SPAdes v3.10.1 (12). Binning using MetaBAT v0.32.4 (–very sensitive option) yielded a single bin. Evaluated by CheckM v1.0.6, this final genome had slightly improved completeness and less contamination (Table 1). This genome was annotated using PROKKA v1.12-beta (8), BLAST v2.2.29+ (9) against

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the SILVA SSU v128 and NCBI databases, and the Kyoto Encyclopedia of Genes and Genomes (KEGG) automatic annotation server v2.1 (13). A phylogenetic tree using single-copy genes (14) was created using Anvi’o v5.2 (15) phylogenomic analysis for Beijerinckiaceae genomes selected by referencing Tveit et al. (10). Average nucleotide identity (ANI) and average amino acid identity (AAI) values were calculated using the scripts ani.rb (with the options –win, 1,000; –step, 200; –len, 700; –id, 70) and aai.rb (with the options –len-fraction, 0.8; –id, 20), respectively, from the enveomics package v1.4.4 (16).

The USC\(\alpha\) AHI genome belongs within the Beijerinckiaceae (Fig. 1) and possesses a 416-nt-long 16S rRNA gene that is 98.1 to 98.6% similar to published USC\(\alpha\) 16S rRNA genes (10, 17). Its pmoA and pmoB genes match 99.7 to 100% with DNA and RNA sequences previously reported from AHI that were phylogenetically determined as the high-affinity form for CH\(_4\) oxidation (1). USC\(\alpha\) AHI is able to assimilate C from CH\(_4\) and from CO\(_2\) via the serine cycle, the reductive glycine pathway, and the Calvin-Benson-Bassham cycle. USC\(\alpha\) AHI can utilize various carbon sources via the pentose phosphate and Entner-Doudoroff pathways, including acetate in its tricarboxylic acid (TCA) cycle, although the acetate transporter gene (actP) is absent. The [NiFe] group 1h hydrogenase for H\(_2\) metabolism is also present.

**Data availability.** The draft genome sequence of USC\(\alpha\) AHI has been deposited at NCBI GenBank under the accession number VDMG000000000 (BioSample number SAMN11877018 and BioProject number PRJNA545288). The version described in this

| CheckM output | Beijerinckiaceae bin from coassembly | USC\(\alpha\) AHI genome from reassembly |
|---------------|-------------------------------------|--------------------------------------|
| Marker lineage | o__Rhizobiales (UID3654) | o__Rhizobiales (UID3654) |
| No. of genomes | 92 | 92 |
| No. of markers | 481 | 481 |
| No. of marker sets | 319 | 319 |
| 0 copies (missing) | 36 | 32 |
| 1 copy | 444 | 449 |
| 2 copies | 1 | 0 |
| 3 copies | 0 | 0 |
| 4 copies | 0 | 0 |
| ≥5 copies | 0 | 0 |
| Completeness (%) | 90.56 | 91.64 |
| Contamination (%) | 0.31 | 0.00 |
| Strain heterogeneity (%) | 0.00 | 0.00 |
| No. of unique markers (of 43) | 42 | 42 |
| No. of multicity markers | 0 | 0 |
| Insertion branch UID | UJD3666 | UJD3666 |
| Taxonomy (contained) | k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Beijerinckiaceae | k__Bacteria; p__Proteobacteria; c__Alphaproteobacteria; o__Rhizobiales; f__Beijerinckiaceae |
| Taxonomy (sister) | Unresolved | Unresolved |
| GC content (%) | 59.1 | 59 |
| Genome size (Mbp) | 3.03 | 3.26 |
| Gene count | 3,388 | 3,928 |
| Coding density (fraction) | 0.82 | 0.81 |
| Translation table | 11 | 11 |
| No. of descendant genomes | 3 | 3 |
| Lineage | | |
| GC content (%) | | |
| Mean | 60.6 | 60.6 |
| SD | 2.6 | 2.6 |
| Genome size (Mbp) | | |
| Mean | 4.28 | 4.28 |
| SD | 0.13 | 0.13 |
| Gene count | | |
| Mean | 3,861 | 3,861 |
| SD | 86 | 86 |

*Values that are different between the two draft genomes are marked in bold font.*
The raw reads of 21 metagenomes have been deposited at the NCBI Sequence Read Archive under the accession number SRP047512.

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M.C.Y.L. conceived the analysis. T.A.V. performed the total DNA extraction and submitted it to A.L. and A.C. for sequencing. A.C. and A.L. performed the initial quality filtering. C.R. and M.C.Y.L. assembled the sequenced reads. C.R. performed the mapping, binning, reassembly, gene prediction, and annotation with consultation from M.C.Y.L., and C.R., M.C.Y.L., and T.C.O. contributed to the interpretation of the data and production of the manuscript.

We declare no conflict of interest.

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