Title: Whole Genome Sequence of *Alantibacter subterranea* Isolated from Uranium-contaminated Sediment

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ABSTRACT We report the whole genome sequence of an antibiotic resistance strain of *Atlantibacter subterranea* that was isolated from Uranium-contaminated sediment in Tennessee. The whole genome sequence of this strain was 4,717,064 bp in length, contained 34 contigs and 29 scaffolds, and had a GC content of 55.17%.

Atlantibacter subterranea is a rod-shaped, gram-negative bacterium, found in aquatic areas in North America (1). It is facultatively anaerobic, motile, with potential to spread in animals by consuming contaminated food (2). The bacterium can reduce hexavalent uranium to tetravalent uranium, later precipitating to mineral uraninite, effectively immobilizing uranium from radioactive waste (3).This strain was isolated from Uranium (VI)-contaminated subsurface sediment in 2001 in Tennessee, USA (3). It was isolated using sediment sampling, enrichment, and serial dilutions plated on aerobic agar containing acetate. (3). Analysis of *A. subterranea* is important for identifying similarities in specific extracellular polymeric substance secretions (EPS) across bacterial species related to its significance in uranium reduction (3). It was taxonomically identified prior to genome sequencing and the 16S rRNA gene sequence is found in the NCBI database (accession number AY373829) (3).

Details on organism growth and DNA isolation to be provided by DSMZ. The bacterium was sequenced at the JGI with the Illumina HiSeq 2000 platform by creating an Illumina std shotgun library, TSPS, with a read type of 2x150 bp. It resulted in 2,999,762 raw sequence reads and 450 mb DNA sequences. This data was then filtered through a filtering program, DUK, by getting rid of Illumina sequencing and library preparation artifacts that were already known (4) Genome assembly used Velvet (v1.02.07) initially (parameters: contig length 500, coverage cutoff 10) (5). Then the final assembly used Allpaths-LG (v46652) (parameters: PHRED 64, PLOIDY=1, COVERAGE=125) (6). Annotation followed the JGI Microbial Genome Annotation Pipeline (7). The Genome is 100% complete and 0.11% contaminated (8).

Table 1 - Genomic features of Atlantibacter subterranea DSM 16208	
Feature	**Finding**
length (bp)	4,717,064
status	complete
No. of contigs	34
GC content (%)	55.17%
No. of scaffolds	29
Scaffold N50 (bp)	434618
Average fold coverage	0.635938
No. of rRNAs	17
No. of tRNAs	67
No. of genes	4504
No. of coding sequences	4351

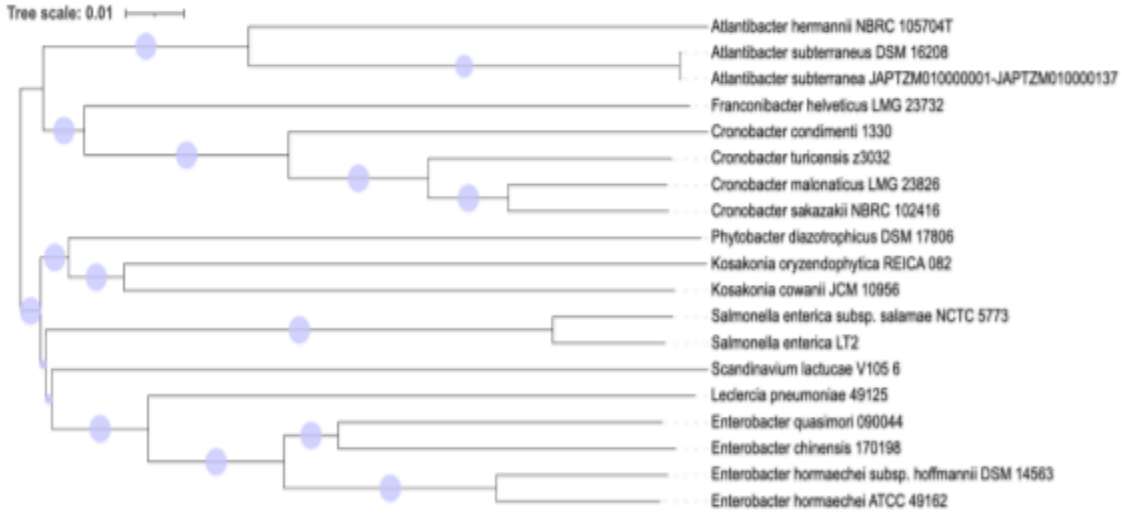

FIG 1 Whole-genome-based phylogenetic classification of *Atlantibacter subterranea*. The genome BLAST distance phylogeny (GBDP) tree was generated with the Type Strain Genome Server accessed 14 March 2025. The tree was inferred with FastME 2.1.6.1 from GBDP distances calculated from genome sequences. The branch lengths are scaled in terms of the GBDP distance formula d_5. The numbers at the nodes are GBDP pseudobootstrap support values of >60% from 100 replications. The average branch support was 93.7%. The tree was midpoint rooted .

The probability of *Atlantibacter subterranea* being a human pathogen is 0.702, as determined by PathogenFinder v1.1 (9). The genome of *Alantibacter subterranea* consists of one chromosome that is 4,717.064 bp with a G+C content of 55.17% (Table 1). The Whole-genome-based phylogenetic classification of *Atlantibacter subterranea* was generated using the Type Strain Genome Server and inferred with FastME 2.1.6.1 (Figure 1).

Using the Comprehensive Antibiotic Resistance Database (CARD 4.0.0), we confirmed that *Atlantibacter subterranea* is resistant to various types of antibiotics, such as cephalosporin, penicillin betalactam, fluoroquinolone, and macrolide (10). CRISPR-Cas Finder version 1.1.2 -I2BC identified 1 CRISPR region for the final version (11). There are six secondary metabolite regions found in CP100494.1 and no secondary metabolite regions found in CP100495 and CP100496.1, as identified using the antiSMASH 7.0 software. The secondary metabolites included: arylpolyene, NRP metallophore, terpene, thiopeptide, butyrolactone and RiPP like (12).

Data Availability

This Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under the accession no. LC126283. The version described in this paper is the first version, LC126283.1. The data was deposited under the BioProject accession no. PRJDB2388, the BioSample accession no. SAMD00010876, and the Sequence Read Archive accession no. DRR015979.

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References

1. Reimer LC, Sarda Carbasse J, Schober I, Koblitz J, Podstawka A, Overmann J. 2024. Salmonella subterranea Shelobolina et al. 2005 (9.2). DSMZ.

2. Hata H, Natori T, Mizuno T, Kanazawa I, Eldesouky I, Hayashi M, Miyata M, Fukunaga H, Ohji S, Hosoyama A, Aono E, Yamazoe A, Tsuchikane K, Fujita N, Ezaki T. 2016. Phylogenetics of family Enterobacteriaceae and proposal to reclassify Escherichia hermannii and Salmonella subterranea as Atlantibacter hermannii and Atlantibacter subterranea gen. nov., comb. nov. Microbiol Immunol 60:303–311.

3. Shelobolina ES, Sullivan SA, O'Neill KR, Nevin KP, Lovley DR. 2004. Isolation, characterization, and U(VI)-reducing potential of a facultatively anaerobic, acid-resistant Bacterium from Low-pH, nitrate- and U(VI)-contaminated subsurface sediment and description of Salmonella subterranea sp. nov. Appl Environ Microbiol 70:2959–2965.

4. Sharp CE, Smirnova AV, Kalyuzhnaya MG, Bringel F, Hirayama H, Jetten MSM, Khmelenina VN, Klotz MG, Knief C, Kyrpides N, Camp HJMO den, Reshetnikov AS, Sakai Y, Shapiro N, Trotsenko YA, Vuilleumier S, Woyke T, Dunfield PF. 2015. Draft Genome Sequence of the Moderately Halophilic Methanotroph Methylohalobius crimeensis Strain 10Ki. Genome Announcements 3:e00644.

5. Zerbino DR, Birney E. 2008. Velvet: Algorithms for de novo short read assembly using de Bruijn graphs. Genome Res 18:821–829.

6. Gnerre S, MacCallum I, Przybylski D, Ribeiro FJ, Burton JN, Walker BJ, Sharpe T, Hall G, Shea TP, Sykes S, Berlin AM, Aird D, Costello M, Daza R, Williams L, Nicol R, Gnirke A, Nusbaum C, Lander ES, Jaffe DB. 2011. High-quality draft assemblies of mammalian genomes from massively parallel sequence data. Proceedings of the National Academy of Sciences 108:1513–1518.

7. Huntemann M, Ivanova NN, Mavromatis K, Tripp HJ, Paez-Espino D, Palaniappan K, Szeto E, Pillay M, Chen I-MA, Pati A, Nielsen T, Markowitz VM, Kyrpides NC. 2015. The standard operating procedure of the DOE-JGI Microbial Genome Annotation Pipeline (MGAP v.4). Standards in Genomic Sciences 10:86.

8. Chklovski A, Parks DH, Woodcroft BJ, Tyson GW. 2023. CheckM2: a rapid, scalable and accurate tool for assessing microbial genome quality using machine learning. Nat Methods 20:1203–1212.

9. Cosentino S, Larsen MV, Aarestrup FM, Lund

O. 2013. PathogenFinder - Distinguishing Friend from Foe Using Bacterial Whole Genome Sequence Data. PLOS ONE 8:e77302.

10. Alcock BP, Huynh W, Chalil R, Smith KW, Raphenya AR, Wlodarski MA, Edalatmand A, Petkau A, Syed SA, Tsang KK, Baker SJC, Dave M, McCarthy MC, Mukiri KM, Nasir JA, Golbon B, Imtiaz H, Jiang X, Kaur K, Kwong M, Liang ZC, Niu KC, Shan P, Yang JYJ, Gray KL, Hoad GR, Jia B, Bhando T, Carfrae LA, Farha MA, French S, Gordzevich R, Rachwalski K, Tu MM, Bordeleau E, Dooley D, Griffiths E, Zubyk HL, Brown ED, Maguire F, Beiko RG, Hsiao WWL, Brinkman FSL, Van Domselaar G, McArthur AG. 2023. CARD 2023: expanded curation, support for machine learning, and resistome prediction at the Comprehensive Antibiotic Resistance Database. Nucleic Acids Res 51:D690–D699.

11. Couvin D, Bernheim A, Toffano-Nioche C, Touchon M, Michalik J, Néron B, Rocha EPC, Vergnaud G, Gautheret D, Pourcel C. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins.

12. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, Fetter A, Terlouw BR, Metcalf WW, Helfrich EJN, van Wezel GP, Medema MH, Weber T. 2023. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research 51:W46–W50.