

Draft genome sequences of *Alicyclobacillus* strains isolated from the Chinoike-Jigoku hot spring, Oita, Japan

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ABSTRACT The genus *Alicyclobacillus* is a thermoacidophilic bacterial genus found in diverse environments. Here, we report the draft genome sequence of *Alicyclobacillus* sp. strains CIJ1 and CIJ2 isolated from the Chinoike-Jigoku hot spring in Oita, Japan. Phylogenomic analyses suggest that these strains represent a candidate novel species within the genus.

KEYWORDS draft genomes, *Alicyclobacillus*, isolate, Chinoike-Jigoku

Members of the genus *Alicyclobacillus* are spore-forming Gram-positive rods that are typically acidophilic to moderately acidophilic and often exhibit thermophilic or thermotolerant growth (1). *Alicyclobacillus* species were historically classified within the genus *Bacillus* before being reclassified based on phylogenetic and chemotaxonomic features, including ω -alicyclic fatty acids (e.g., ω -cyclohexane fatty acids) in the cell membrane (2). *Alicyclobacillus* spp. have been isolated from diverse environments, such as soils (3–5), acidic fruit juices, and other beverages (6–12), human clinical specimens (13, 14), and hot springs (13, 15–17).

Several *Alicyclobacillus* species are important in food microbiology because their heat-resistant spores can survive pasteurization and spoil acidic beverages, sometimes producing off-flavor compounds, such as guaiacol (10, 18). On the other hand, other members of the genus are adapted to geothermal environments, such as hot springs. In these habitats, low pH and elevated temperatures provide ecological conditions consistent with their physiological traits. These environments likely represent natural reservoirs and sources of unexplored diversity within the genus.

Here, we report the draft genome sequences of two *Alicyclobacillus* strains, CIJ1 and CIJ2, isolated from a hot spring water sample collected at the Chinoike-Jigoku hot spring (Beppu, Oita Prefecture, Japan; 33.32°N, 131.47°E) in January 2023. One milliliter of hot spring water was spread on DSMZ medium 88 (pH 2.0) and incubated aerobically at 55°C for 1 week. Single colonies were purified by repeated streaking and cultured in DSMZ medium 88 at 55°C.

Genomic DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). Short-read sequencing libraries were prepared using the MGIEasy FS DNA Library Prep Set (MGI Tech Co., Shenzhen, China). The DNA libraries were sequenced on the DNBSEQ-G400RS platform (MGI Tech Co.) to generate paired-end sequences (150 bp × 2). A total of 4,795,509 and 3,214,148 paired-end reads were obtained for CIJ1 and CIJ2, respectively. Reads were trimmed and quality filtered using Platanus_trim v1.0.7 and assembled using Platanus_b v1.3.2 (19). Assemblies were annotated using the DDBJ Fast Annotation and Submission Tool (DFAST) v1.3.1 (20). Genome completeness and contamination were estimated using CheckM v1.2.2 (21) in DFAST. CheckM automatically selected a lineage-specific marker set corresponding to the *Alicyclobacillus acidocaldarius* clade (six reference genomes). Average nucleotide identity (ANI) values were calculated using pyANI version 0.3.0-alpha (22). The digital

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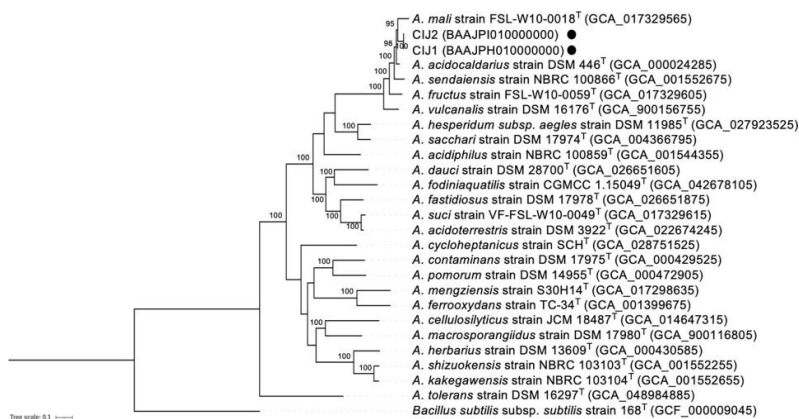


FIG 1 Maximum-likelihood tree based on 81 core genes among strains of *Alicyclobacillus* species. Labels for nodes with less than 80% bootstrap support were removed. The isolates in this study are highlighted with filled circles. The scale bar represents nucleotide substitutions per site. *Bacillus subtilis* subsp. *subtilis* strain 168^T was used as the outgroup. Scale bar, 0.1 substitutions per site.

DNA-DNA hybridization (dDDH) values were estimated using the Genome-to-Genome Distance Calculator (GGDC) (23). Default parameters were used for all software unless otherwise specified.

The details of draft genomes for *Alicyclobacillus* sp. strains CIJ1 and CIJ2 are summarized in Table 1. Phylogenetic analysis was performed based on the concatenation of 81 core genes from each genome using UBCG2 (24). The two strains formed a distinct clade within the genus *Alicyclobacillus* (Fig. 1). The ANI and dDDH values between the genomes of strains CIJ1 and CIJ2 were 99.9% and 100%, respectively, indicating that CIJ1 and CIJ2 belong to the same species. The closest species were *A. mali* and *A. acidocaldarius*. ANI values with *A. mali* and *A. acidocaldarius* were 90.0% and 90.9%, respectively. In addition, the dDDH values with *A. mali* and *A. acidocaldarius* were 36.9% and 43.6%,

TABLE 1 Information on genome sequences of strains in this study

Strain	CIJ1	CIJ2
Assembly length (bp)	3,015,628	3,017,064
No. of contigs	147	160
G + C (%)	61.8	61.8
Contigs N50 (bp)	187,884	172,877
CDS	2,973	2,984
rRNA	3	3
tRNA	64	64
CRISPR	4	4
Coding ratio (%)	87.6	87.7
CheckM completeness (%)	95.31	94.99
CheckM contamination (%)	4.46	4.46
SRA accession no.	DRX879802	DRX879803
GenBank accession no.	BAAJPH000000000.1	BAAJPI000000000.1

respectively. These values are clearly below the accepted species delineation thresholds of 95%–96% ANI and 70% dDDH (23, 25), indicating that strains CIJ1 and CIJ2 represent a candidate for a novel species within the genus *Alicyclobacillus*.

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Yu Sato, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Supervision, Writing – original draft, Writing – review and editing | Eriko Miyamoto, Formal analysis, Investigation, Methodology, Resources | Yuna Sato, Formal analysis, Investigation, Methodology, Resources, Writing – review and editing | Shintaro Maeno, Formal analysis, Investigation, Methodology, Writing – review and editing

DATA AVAILABILITY

The draft genome sequences of strains CIJ1 and CIJ2 are deposited at DDBJ/EMBL/GenBank under accession numbers [BAAJPH010000000](#) and [BAAJPI010000000](#), respectively. The raw sequencing reads are available in the Sequence Read Archive (SRA) under accession numbers [DRX879802](#) and [DRX879803](#), respectively.

REFERENCES

1. Ciuffreda E, Bevilacqua A, Sinigaglia M, Corbo MR. 2015. *Alicyclobacillus* spp.: new insights on ecology and preserving food quality through new approaches. *Microorganisms* 3:625–640. <https://doi.org/10.3390/microorganisms3040625>
2. Wisotzkey JD, Jurtshuk P, Fox GE, Deinhard G, Poralla K. 1992. Comparative sequence analyses on the 16S rRNA (rDNA) of *Bacillus acidocaldarius*, *Bacillus acidoterrestris*, and *Bacillus cycloheptanicus* and proposal for creation of a new genus, *Alicyclobacillus* gen. nov. *Int J Syst Bacteriol* 42:263–269. <https://doi.org/10.1099/00207713-42-2-263>
3. Groenewald WH, Gouws PA, Witthuhn RC. 2009. Isolation, identification and typification of *Alicyclobacillus acidoterrestris* and *Alicyclobacillus acidocaldarius* strains from orchard soil and the fruit processing environment in South Africa. *Food Microbiol* 26:71–76. <https://doi.org/10.1016/j.fm.2008.07.008>
4. Goto K, Mochida K, Kato Y, Asahara M, Fujita R, An S-Y, Kasai H, Yokota A. 2007. Proposal of six species of moderately thermophilic, acidophilic, endospore-forming bacteria: *Alicyclobacillus contaminans* sp. nov., *Alicyclobacillus fastidiosus* sp. nov., *Alicyclobacillus kakegawensis* sp. nov., *Alicyclobacillus macrosporangioides* sp. nov., *Alicyclobacillus sacchari* sp. nov. and *Alicyclobacillus shizuokensis* sp. nov. *Int J Syst Evol Microbiol* 57:1276–1285. <https://doi.org/10.1099/ijs.0.64692-0>
5. Deinhard G, Blanz P, Poralla K, Altan E. 1987. *Bacillus acidoterrestris* sp. nov., a new thermotolerant acidophile isolated from different soils. *Syst Appl Microbiol* 10:47–53. [https://doi.org/10.1016/S0723-2020\(87\)80009-7](https://doi.org/10.1016/S0723-2020(87)80009-7)
6. Walls I, Chuyate R. 2000. Isolation of *Alicyclobacillus acidoterrestris* from fruit juices. *J AOAC Int* 83:1115–1120. <https://doi.org/10.1093/jaoac/83.5.1115>
7. Goto K, Matsubara H, Mochida K, Matsumura T, Hara Y, Niwa M, Yamasato K. 2002. *Alicyclobacillus herbarius* sp. nov., a novel bacterium containing omega-cycloheptane fatty acids, isolated from herbal tea. *Int J Syst Evol Microbiol* 52:109–113. <https://doi.org/10.1099/00207713-52-1-109>
8. Roth K, Rana YS, Daeschel D, Kovac J, Worobo R, Snyder AB. 2021. *Alicyclobacillus mali* sp. nov., *Alicyclobacillus suci* sp. nov. and *Alicyclobacillus fructus* sp. nov., thermoacidophilic sporeforming bacteria isolated from fruit beverages. *Int J Syst Evol Microbiol* 71:005016. <https://doi.org/10.1099/ijsem.0.005016>
9. Matsubara H, Goto K, Matsumura T, Mochida K, Iwaki M, Niwa M, Yamasato K. 2002. *Alicyclobacillus acidiphilus* sp. nov., a novel thermoacidophilic, omega-alicyclic fatty acid-containing bacterium isolated from acidic beverages. *Int J Syst Evol Microbiol* 52:1681–1685. <https://doi.org/10.1099/00207713-52-5-1681>

10. Luong TSV, Moir C, Bowman JP, Chandry PS. 2021. Heat resistance and genomics of spoilage *Alicyclobacillus* spp. Isolated from fruit juice and fruit-based beverages. Food Microbiol 94:103662. <https://doi.org/10.1016/j.fm.2020.103662>
11. Oh S. 2017. Heat resistance of *Alicyclobacillus* isolated from fruit juices. Trends Agric Life Sci 55:23–27. <https://doi.org/10.29335/tals.2017.55.23>
12. Groenewald WH, Gouws PA, Witthuhn RC. 2013. Thermal inactivation of *Alicyclobacillus acidoterrestris* spores isolated from a fruit processing plant and grape juice concentrate in South Africa. Afr J Microbiol Res 7:2736–2740. <https://doi.org/10.5897/AJMR12.1789>
13. Kim MG, Lee J-C, Park D-J, Li W-J, Kim C-J. 2014. *Alicyclobacillus tengchongensis* sp. nov., a thermo-acidophilic bacterium isolated from hot spring soil. J Microbiol 52:884–889. <https://doi.org/10.1007/s12275-014-3625-z>
14. Glaeser SP, Falsen E, Martin K, Kämpfer P. 2013. *Alicyclobacillus consociatus* sp. nov., isolated from a human clinical specimen. Int J Syst Evol Microbiol 63:3623–3627. <https://doi.org/10.1099/ijs.0.048173-0>
15. Aulitto M, Gallo G, Puopolo R, Mormone A, Limauro D, Contursi P, Piochi M, Bartolucci S, Fiorentino G. 2021. Genomic insight of *Alicyclobacillus mali* FL18 isolated from an arsenic-rich hot spring. Front Microbiol 12:639697. <https://doi.org/10.3389/fmicb.2021.639697>
16. López G, Díaz-Cárdenas C, David Alzate J, Gonzalez LN, Shapiro N, Woyke T, Kyrpides NC, Restrepo S, Baena S. 2018. Description of *Alicyclobacillus montanus* sp. nov., a mixotrophic bacterium isolated from acidic hot springs. Int J Syst Evol Microbiol 68:1608–1615. <https://doi.org/10.1099/ijs.0.002718>
17. Simbahan J, Drijber R, Blum P. 2004. *Alicyclobacillus vulcanalis* sp. nov., a thermophilic, acidophilic bacterium isolated from Coso hot springs, California, USA. Int J Syst Evol Microbiol 54:1703–1707. <https://doi.org/10.1099/ijs.0.03012-0>
18. Neggazi I, Colás-Medà P, Viñas I, Garza S, Alegre I. 2023. Occurrence of *Alicyclobacillus acidoterrestris* in pasteurized and high hydrostatic pressure-treated fruit juices and isolates' characterization. Int J Food Microbiol 396:110197. <https://doi.org/10.1016/j.jifoodmicro.2023.110197>
19. Kajitani R, Yoshimura D, Ogura Y, Gotoh Y, Hayashi T, Itoh T. 2020. Platanus_B: an accurate *de novo* assembler for bacterial genomes using an iterative error-removal process. DNA Res 27:dsaa014. <https://doi.org/10.1093/dnares/dsaa014>
20. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. <https://doi.org/10.1093/bioinformatics/btx713>
21. 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>
22. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. 2016. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. Anal Methods 8:12–24. <https://doi.org/10.1039/C5AY02550H>
23. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. 2022. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res 50:D801–D807. <https://doi.org/10.1093/nar/gkab902>
24. Kim J, Na S-I, Kim D, Chun J. 2021. UBCG2: up-to-date bacterial core genes and pipeline for phylogenomic analysis. J Microbiol 59:609–615. <https://doi.org/10.1007/s12275-021-1231-4>
25. Meier-Kolthoff JP, Göker M, Spröer C, Klenk H-P. 2013. When should a DDH experiment be mandatory in microbial taxonomy? Arch Microbiol 195:413–418. <https://doi.org/10.1007/s00203-013-0888-4>