



Genome Sequence of the Thermophilic Soil Bacterium Ureibacillus terrenus ATCC BAA- 384^{T}

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ABSTRACT Ureibacillus terrenus TH9A^T (=ATCC BAA-384^T) was isolated from uncultivated soil in Italy in 1995. We present a draft genome sequence for the type strain, with a predicted genome length of 2,936,851 bp, containing 2,766 protein-coding genes, 82 RNA genes, and 5 CRISPR arrays, with a G+C content of 42.5%.

S trains from the thermophilic genus *Ureibacillus* have been identified in several different habitats, including compost, soil, landfill and waste treatment systems, and air (1–6). Unusually, *Ureibacillus* spp. are Gram-negative *Firmicutes* that do not grow anaerobically or catabolize sugars as a source of carbon and energy (2). *Ureibacillus terrenus* TH9A^T (=ATCC BAA-384^T = DSM 12654^T = LMG 19470^T) was isolated from uncultivated Italian soil in 1995 (1, 2). *U. terrenus* is a rod-shaped, motile species displaying terminal or subterminal spherical endospores under appropriate conditions and is differentiated from the type species, *Ureibacillus thermosphaericus*, based on its isoprenoid quinone composition and ability to grow at higher temperatures (up to 65°C) and pH (up to 9.0) (2, 3, 5, 7). *U. terrenus* and other members of its genus have been noted as important players in compost and waste decomposition (8–10) and have been investigated for biotechnology applications, given their thermophilic enzymes (11–16). Additionally, given recent emendations of the *Ureibacillus* genus within the family *Caryophanaceae* (17, 18), the completion of the genome sequence for *U. terrenus* will contribute to further discussions of the taxonomic structure of this family.

Freeze-dried U. terrenus ATCC BAA-384^T cells were obtained from ATCC (Manassas, VA, USA) and then rehydrated in Trypticase soy broth (TSB) and incubated at 55°C for 24 h at 1 atm. After streaking onto Trypticase soy agar, a single colony of U. terrenus was grown to log phase at 50°C in 2 ml TSB before its genomic DNA (gDNA) was isolated using the QIAamp DNA minikit (Qiagen, Valencia, CA, USA). gDNA fragmentation and adapter attachment were performed using a KAPA HyperPlus kit v.3.16 (KR1145; Wilmington, MA, USA). Sequencing followed on an Illumina HiSeq 2500 instrument (Hubbard Center for Genome Studies, Durham, NH, USA). Paired-end 250-bp reads were trimmed using Trimmomatic v.0.38 (settings: paired-end mode with a window size of 4, quality requirement of 15, and minimum read length of 36); then, 6,355,970 trimmed reads were assembled using SPAdes v.3.13.0 (19, 20) with default bacterial assembly parameters. Small contigs (<500 bp) were removed, along with any containing containing contaminants flagged during PGAP (below). QUAST (21) analysis of this assembly showed 80 contigs-the largest 392,574 bp-with an N_{so} value of 158,024 bp. A genome coverage of 984× was calculated. The NCBI Prokaryotic Genome Assembly Pipeline (PGAP) (22) was used for gene identification and annotation. The assembled genome was 2,936,851 bp long, and PGAP revealed a total of 2,910 genes, 2,766 protein-coding sequences, 62 pseudogenes, 53 tRNAs, 24 partial or complete copies of the rRNA genes (including 1 complete 16S rRNA gene), 5 noncoding RNAs (ncRNAs), and a G+C content of 42.5%, close to the published values for the species (39.6 to 41.5%) and

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Received 27 October 2021 Accepted 15 November 2021 Published 2 December 2021 genus (35.7 to 41.5%) (2). Five CRISPR arrays were identified, as well as the CRISPR-associated genes encoding the enzymes Cas1-3, Cas4a, and Cas6 (23).

Data availability. The Ureibacillus terrenus ATCC BAA-384^T whole-genome shotgun sequence (WGS) project has been deposited at DDBJ/ENA/GenBank under accession number VIGD00000000. The raw Illumina data were submitted to the NCBI Sequence Read Archive (SRA) under accession number SRX6431131 and BioSample accession number SAMN12147508.

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REFERENCES

- Mora D, Fortina MG, Nicastro G, Parini C, Manachini PL. 1998. Genotypic characterization of thermophilic bacilli: a study on new soil isolates and several reference strains. Res Microbiol 149:711–722. https://doi.org/10 .1016/S0923-2508(99)80018-7.
- Fortina MG, Pukall R, Schumann P, Mora D, Parini C, Manachini PL, Stackebrandt E. 2001. Ureibacillus gen. nov., a new genus to accommodate Bacillus thermosphaericus (Andersson et al. 1995), emendation of Ureibacillus thermosphaericus and description of Ureibacillus terrenus sp. nov. Int J Syst Evol Microbiol 51: 447–455. https://doi.org/10.1099/00207713-51-2-447.
- Kim B-Y, Lee S-Y, Weon H-Y, Kwon S-W, Go S-J, Park Y-K, Schumann P, Fritze D. 2006. Ureibacillus suwonensis sp. nov., isolated from cotton waste composts. Int J Syst Evol Microbiol 56:663–666. https://doi.org/10.1099/ijs.0.63703-0.
- Weon H-Y, Lee S-Y, Kim B-Y, Noh H-J, Schumann P, Kim J-S, Kwon S-W. 2007. Ureibacillus composti sp. nov. and Ureibacillus thermophilus sp. nov., isolated from livestock-manure composts. Int J Syst Evol Microbiol 57:2908–2911. https://doi.org/10.1099/ijs.0.65232-0.
- Ash C, Farrow JAE, Wallbanks S, Collins MD. 2008. Phylogenetic heterogeneity of the genus Bacillus revealed by comparative analysis of small-subunit-ribosomal RNA sequences. Lett Appl Microbiol 13:202–206. https:// doi.org/10.1111/j.1472-765X.1991.tb00608.x.
- Zhou S, Tang J, Qin D, Lu Q, Yang G. 2014. Ureibacillus defluvii sp. nov., isolated from a thermophilic microbial fuel cell. Int J Syst Evol Microbiol 64:1617–1621. https://doi.org/10.1099/ijs.0.056655-0.
- Demharter W, Hensel R. 1989. Bacillus thermocloaceae sp. nov., a new thermophilic species from sewage sludge. Syst Appl Microbiol 11:272–276. https://doi .org/10.1016/S0723-2020(89)80025-6.
- Wang C-M, Shyu C-L, Ho S-P, Chiou S-H. 2007. Species diversity and substrate utilization patterns of thermophilic bacterial communities in hot aerobic poultry and cattle manure composts. Microb Ecol 54:1–9. https:// doi.org/10.1007/s00248-006-9139-4.
- Li R, Li L, Huang R, Sun Y, Mei X, Shen B, Shen Q. 2014. Variations of culturable thermophilic microbe numbers and bacterial communities during the thermophilic phase of composting. World J Microbiol Biotechnol 30: 1737–1746. https://doi.org/10.1007/s11274-013-1593-9.
- Papale M, Romano I, Finore I, Lo Giudice A, Piccolo A, Cangemi S, Di Meo V, Nicolaus B, Poli A. 2021. Prokaryotic diversity of the composting thermophilic phase: the case of ground coffee compost. Microorganisms 9: 218. https://doi.org/10.3390/microorganisms9020218.
- Gagné A, Chicoine M, Morin A, Houde A. 2001. Phenotypic and genotypic characterization of esterase-producing Ureibacillus thermosphaericus isolated from an aerobic digestor of swine waste. Can J Microbiol 47:908–915. https://doi.org/10.1139/w01-096.
- Akita H, Fujino Y, Doi K, Ohshima T. 2011. Highly stable meso-diaminopimelate dehydrogenase from an Ureibacillus thermosphaericus strain A1

isolated from a Japanese compost: purification, characterization and sequencing. AMB Express 1:43. https://doi.org/10.1186/2191-0855-1-43.

- Charbonneau DM, Meddeb-Mouelhi F, Boissinot M, Sirois M, Beauregard M. 2012. Identification of thermophilic bacterial strains producing thermotolerant hydrolytic enzymes from manure compost. Indian J Microbiol 52:41–47. https://doi.org/10.1007/s12088-011-0156-8.
- Ting ASY, Tay H, Peh KL, Tan WS, Tee CS. 2013. Novel isolation of thermophilic Ureibacillus terrenus from compost of empty fruit bunches (EFB) of oil palm and its enzymatic activities. Biocatal Agric Biotechnol 2:162–164. https://doi.org/10.1016/j.bcab.2012.11.004.
- Samoylova YV, Sorokina KN, Romanenko MV, Parmon VN. 2018. Cloning, expression and characterization of the esterase estUT1 from Ureibacillus thermosphaericus which belongs to a new lipase family XVIII. Extremophiles 22:271–285. https://doi.org/10.1007/s00792-018-0996-9.
- Akita H, Hayashi J, Sakuraba H, Ohshima T. 2018. Artificial thermostable D-amino acid dehydrogenase: creation and application. Front Microbiol 9:1760. https://doi.org/10.3389/fmicb.2018.01760.
- Gupta RS, Patel S. 2019. Robust demarcation of the family Caryophanaceae (Planococcaceae) and its different genera including three novel genera based on phylogenomics and highly specific molecular signatures. Front Microbiol 10:2821. https://doi.org/10.3389/fmicb.2019.02821.
- Oren A, Garrity G. 2020. Notification of changes in taxonomic opinion previously published outside the IJSEM. Int J Syst Evol Microbiol 70:4061–4090. https://doi.org/10.1099/ijsem.0.004245.
- Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10 .1093/bioinformatics/btu170.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2013. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/ nar/gkw569.
- 23. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: Rapid Annotations using Subsystems Technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.