

























Draft Genome Sequences of *Bacillus glennii* V44-8, *Bacillus saganii* V47-23a, *Bacillus* sp. Strain V59.32b, *Bacillus* sp. Strain MER_TA_151, and *Paenibacillus* sp. Strain MER_111, Isolated from Cleanrooms Where the Viking and Mars Exploration Rover Spacecraft Were Assembled

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ABSTRACT We report the draft genome sequences of *Bacillus glennii* V44-8, *Bacillus saganii* V47-23a, and *Bacillus* sp. strain V59.32b, isolated from the Viking spacecraft assembly cleanroom, and *Bacillus* sp. strain MER_TA_151 and *Paenibacillus* sp. strain MER_111, isolated from the Mars Exploration Rover (MER) assembly cleanroom.

Three strains used in this study, *Bacillus glennii* V44-8, *Bacillus saganii* V47-23a, and *Bacillus* sp. strain V59.32b, were isolated from the vehicle assembly building (VAB) at Cape Canaveral, Florida, where the Viking spacecraft were assembled (1). Teflon ribbons were left out for 7 days to collect airborne microorganisms and then exposed to a total of 6 different heat treatments at 3 different time cycles (2). The other 2 isolates, *Bacillus* sp. strain MER_TA_151 and *Paenibacillus* sp. strain MER_111, were isolated from the Mars Exploration Rover (MER) cleanroom.

All 5 strains were cultured in tryptic soy agar (TSA) medium at 32°C for 48 h, and the DNA was extracted using an automated DNA extraction instrument (Maxwell 16, Promega, USA). An Illumina TruSeq DNA PCR-free library preparation kit (350-bp insert size) was used following the manufacturer's instructions, and paired-end Illumina sequencing was performed on the HiSeq 2500 platform at Psomagen (Rockville, MD, USA). The raw reads were processed with CLC Genomics Workbench v10.1.1, using the default parameters for performing filtering and trimming of adapters and ambiguous nucleotides. The assembly k-mer size was optimized based on the N_{50} scores. The quality of the assembled genomes was assessed using QUAST v4.0 (3). The genome statistics were analyzed using Bioinformatic Tools v1.4.71 (4), and the estimated completeness and contamination were evaluated using CheckM v1.1.2 (5). The genomes were subsequently annotated using the NCBI PGAP pipeline v4.6 (V44-8, V47-23a, and V59.32b) and v4.9 (MER_TA_151 and

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TABLE 1 Sequencing and assembly metrics and NCBI PGAP annotation data for bacterial strains

| Statistic | Data for strain: | | | | |
|---|--------------------------------|--------------------------------|------------------------------|------------------------------|------------------------------|
| | V44-8 | V47-23a | V59.32b | MER_TA_151 | MER_111 |
| Project accession no. | QVTD00000000.1 | QVTE00000000.1 | QVTC00000000 | VYKL00000000 | VYKK00000000 |
| No. of raw read pairs | 6,707,121 | 8,523,831 | 6,008,123 | 6,558,078 | 5,667,551 |
| Assembly size (bp) | 4,469,041 | 4,356,520 | 3,830,155 | 5,743,622 | 4,305,989 |
| No. of contigs >1,000 bp | 27 | 84 | 123 | 85 | 45 |
| N_{50} (bp) | 242,250 | 81,582 | 57,552 | 146,989 | 224,736 |
| L_{50} | 4 | 15 | 23 | 11 | 6 |
| GC content (%) | 42.26 | 40.58 | 41.71 | 37.86 | 56.99 |
| Estimated completeness (%) | 98.91 | 98.09 | 98.36 | 99.33 | 99.07 |
| Estimated contamination (%) | 0.96 | 1.81 | 1.73 | 6.62 | 1.18 |
| No. of identified genes (total) | 4,358 | 4,109 | 3,803 | 5,427 | 3,905 |
| No. of identified CDSs ^a (total) | 4,286 | 4,028 | 3,722 | 5,326 | 3,828 |
| No. of complete rRNAs (5S, 16S, 23S) | 4, 1, 1 | 2, 1, 0 | 0, 1, 0 | 1, 0, 0 | 2, 1, 0 |
| No. of predicted tRNAs | 60 | 71 | 73 | 83 | 69 |
| No. of predicted ncRNAs ^b | 6 | 6 | 6 | 12 | 4 |

^a CDSs, coding DNA sequences.

^b ncRNAs, noncoding RNAs.

MER_111) (6). See Table 1 for information on the assemblies and for the annotation summaries of the five strains.

The taxonomic assignments of *B. glennii* and *B. saganii* were determined based on a polyphasic study, including the biochemical, phylogenetic, and phenotypic characteristics (1). GToTree v1.4.11 (7) was used to create a phylogenomic tree with NCBI-designated representative genomes (as accessed on 14 February 2020) of *Bacillus* and *Paenibacillus* based on the concatenated alignments of 119 single-copy core genes specific to the *Firmicutes* phylum (default settings used other than “-H Firmicutes”) (8–14). The genus-level taxonomies of the *Paenibacillus* isolate, *Bacillus* sp. strain V59.32b, and *Bacillus* sp. strain MER_TA_151 were determined by their positions in the phylogenetic tree (as shown in <https://doi.org/10.6084/m9.figshare.12245441>). We were unable to assign species-level taxonomy to these isolates due to the known discrepancies between phylogeny and taxonomy in these genera.

Data availability. The whole-genome shotgun sequencing projects were deposited in GenBank and the raw sequencing reads in the NCBI Sequence Read Archive under the accession numbers [QVTD00000000.1](#) and [SRR11096019](#) (*Bacillus glennii* V44-8), [QVTE00000000.1](#) and [SRR11096037](#) (*Bacillus saganii* V47-23a), [QVTC00000000](#) and [SRR11097317](#) (*Bacillus* sp. strain V59.32b), [VYKL00000000](#) and [SRR11096322](#) (*Bacillus* sp. strain MER_TA_151), and [VYKK00000000](#) and [SRR11097201](#) (*Paenibacillus* sp. strain MER_111), respectively.

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REFERENCES

- Seuylemezian A, Ott L, Wolf S, Fragante J, Yip O, Pukall R, Schumann P, Vaishampayan P. 2020. *Bacillus glennii* sp. nov. and *Bacillus saganii* sp. nov., isolated from the vehicle assembly building at Kennedy Space Center where the Viking spacecraft were assembled. *Int J Syst Evol Microbiol* 70:71–76. <https://doi.org/10.1099/ijsem.0.003714>.
- Puleo JR, Bergstrom SL, Peeler JT, Oxborrow GS. 1978. Thermal resistance of naturally occurring airborne bacterial spores. *Appl Environ Microbiol* 36:473–479. <https://doi.org/10.1128/AEM.36.3.473-479.1978>.
- Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUASt: quality assess-

- ment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
4. Lee MD. 2018. Bioinformatics Tools (bit). <https://doi.org/10.5281/zenodo.3633827>.
 5. 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>.
 6. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44: 6614–6624. <https://doi.org/10.1093/nar/gkw569>.
 7. Lee MD. 2019. GToTree: a user-friendly workflow for phylogenomics. *Bioinformatics* 35:4162–4164. <https://doi.org/10.1093/bioinformatics/btz188>.
 8. Eddy SR. 2011. Accelerated profile HMM searches. *PLoS Comput Biol* 7:e1002195. <https://doi.org/10.1371/journal.pcbi.1002195>.
 9. Edgar RC. 2004. MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinformatics* 5:113. <https://doi.org/10.1186/1471-2105-5-113>.
 10. Capella-Gutierrez S, Silla-Martínez JM, Gabaldón T. 2009. trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25:1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>.
 11. Hyatt D, Chen G-L, LoCascio PF, Land ML, Larimer FW, Hauser LJ. 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. *BMC Bioinformatics* 11:119. <https://doi.org/10.1186/1471-2105-11-119>.
 12. Shen W, Xiong J. 2019. TaxonKit: a cross-platform and efficient NCBI taxonomy toolkit. *bioRxiv* <https://doi.org/10.1101/513523>.
 13. Price MN, Dehal PS, Arkin AP. 2010. FastTree 2—approximately maximum-likelihood trees for large alignments. *PLoS One* 5:e9490. <https://doi.org/10.1371/journal.pone.0009490>.
 14. Tange O. 2018. GNU Parallel. <https://doi.org/10.5281/zenodo.1146014>.
 15. Towns J, Cockerill T, Dahan M, Foster I, Gaither K, Grimshaw A, Hazlewood V, Lathrop S, Lifka D, Peterson GD, Roskies R, Scott JR, Wilkins-Diehr N. 2014. XSEDE: accelerating scientific discovery. *Comput Sci Eng* 16:62–74. <https://doi.org/10.1109/MCSE.2014.80>.