

8 | Human Microbiome | Announcement

# Draft *Turicibacter* sp. genome isolated from a spore-forming community in mice

Kendra A. Klag,<sup>1</sup> Allison M. Weis,<sup>1</sup> W. Zac Stephens,<sup>1</sup> June L. Round<sup>1</sup>

AUTHOR AFFILIATION See affiliation list on p. 2.

**ABSTRACT** *Turicibacter* is a common mammalian gut commensal; however, very few genomes have been sequenced, and little is understood regarding its importance for host health. Here, we add a complete *Turicibacter* sp. genome isolated from a spore-forming community in mice.

KEYWORDS gut microbiota, bacteria, metabolism, spore forming bacteria, genomics

*T* uricibacter is a genus of Bacilli within phylum Bacillota (1). This Gram-positive spore-forming bacterium lives in the intestines of humans and other animals (2–4). Recent studies point to important roles for *Turicibacter* in intestinal health (5). *Turicibacter* has two established species: *Turicibacter sanguinis*, isolated from the blood of a febrile patient (3), and *Turicibacter bilis*, isolated from chicken eggs and pig ileum (4). Other species of *Turicibacter* likely exist and have been vastly understudied.

We present a draft *Turicibacter* genome, strain KK003, isolated from mice from a spore-forming (SF) community. To isolate SF bacteria, feces from C57BL/6 mice (IACUC Protocol 00001562) were incubated anaerobically with 0.1% cysteine and 3% chloro-form at 37°C for 1 h to kill vegetative bacteria. Chloroform was removed by bubbling CO<sub>2</sub> through the sample for 30 s. To propagate SF, the sample was gavaged into a breeder pair of germ-free C57BL/6 mice. To isolate *Turicibacter*, feces from SF animals were chloroform treated again, serial diluted, and plated on Schaedler agar (Thermo Scientific) at 37°C anaerobically. Individual colonies were picked after 48 h, streaked to isolation, and DNA was extracted from overnight cultures in Schaedler broth using a Purelink Microbiome DNA purification kit according the manufacturer (Invitrogen) for all sequencing. One of the colonies picked was identified as *Turicibacter* by Sanger 16S rRNA gene sequencing using primers 16S\_F:AGAGTTTGATCMTGGC and 16S\_R:TACCTTGTTACG ACTT and the Silva classifier (6).

The KK003 genome was sequenced using Illumina NovaSeq (paired-end 150) and Oxford Nanopore Technologies (ONT) minION reads. Illumina libraries were prepared with NEBNext Ultra II FS DNA kit (NEB, E7805S), and the resulting 8M reads were adapter and quality-trimmed with cutadapt (v2.10) (7) in the trim\_galore (v0.6.6) wrapper using default parameters. ONT libraries were prepared without DNA shearing or size selection using rapid barcoding kit R9.4.1 chemistry (ONT, SQK-RBK004). The resulting 41.8K long reads (120.7M total bases) were basecalled, demultiplexed, adapter, barcode-trimmed with guppy (v6.0.1\_gpu), quality-filtered with NanoFilt v2.8 (8) for a minimum average read-quality of 10 and minimum length of 200. The genome was assembled using SPAdes v3.15.5 within Unicycler v0.5.0 "normal mode" (9, 10), annotated with PGAP v6.6 (11). The KK003 assembly contains 2,503,176 bp, 37% GC content, and 2,447 predicted genes, which are 66.23% complete and 100th percentile by Genbank standards using CheckM v1.2.2 (12). The single contig was circularized and rotated within Unicycler by identification of linked ends and a DnaA gene that was put on the forward strand.

**Editor** Vanja Klepac-Ceraj, Wellesley College, Wellesley, Massachusetts, USA

Address correspondence to June L. Round, june.round@path.utah.edu.

Kendra A. Klag and Allison M. Weis contributed equally to this article. Author order was determined by descending height.

The authors declare no conflict of interest.

See the funding table on p. 3.

Received 12 April 2024 Accepted 3 June 2024 Published 5 July 2024

Copyright © 2024 Klag et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.



TABLE 1 Key Turicibacter sp. K003 genomic elements

Genes	Туре	Protein name and function	
bsh	Bile salt metabolism	Choloylglycine hydrolase 1	
bsh	Bile salt metabolism	Choloylglycine hydrolase 2	
unnamed	Bile salt metabolism	Conjugated bile salt MFS transporter	
unnamed	Metabolism	Putative mucin/carbohydrate-binding domain-containing protein	
unnamed	Metabolism	Aminotransferase class I/II-fold pyridoxal phosphate-depend- ent enzyme	
unnamed	Metabolism	DegT/DnrJ/EryC1/StrS family aminotransferase	
unnamed	Sulfur metabolism	Desulfoferrodoxin family protein	
sufB	Sulfur metabolism	sufB Fe-S cluster assembly protein	
sufC	Sulfur metabolism	sufC Fe-S cluster assembly ATPase	
sufD	Sulfur metabolism	sufD Fe-S cluster assembly protein	
unnamed	Sulfur metabolism	TauE/SafE family protein sulfite exporter	
unnamed	Sulfur metabolism	Radical SAM/SPASM domain-containing protein	
cotE	Sporulation	CotE outer spore coat protein	
gerQ	Sporulation	GerQ spore coat protein	
spo0A	Sporulation	Spo0A sporulation transcription factor	
ffh	Secretion system	Signal recognition particle protein	
ftsY	Secretion system	FtsY signal recognition particle-docking protein	
IspA	Secretion system	Signal peptidase II	
lepB	Secretion system	Signal peptidase I	
unnamed	Secretion system	Type II secretion system F family protein	
unnamed	Secretion system	GspE/PulE family protein	
yajC	Secretion system	YajC preprotein translocase subunit	
yihY	Virulence	YihY/virulence factor BrkB family protein	
unnamed	Toxin-antitoxin	Type II system antitoxin SocA domain-containing protein	
unnamed	Toxin-antitoxin	Type II system PemK/MazF family toxin	

The taxonomic classification KK003 is genus *Turicibacter*, species unclassified (GTDB-Tk - v1.7.0). KK003 is not a member of *T. sanguinis* or *T. bilis* because it only shares 80% sequence identity with either species (using FastANI v0.1.3) (13). The nearest genome is *Turicibacter* sp. TS3 (GCF\_009935875.1), which shares 99% sequence identity, indicating that *Turicibacter* should be their own species. The KK003 genome contains *bsh*, which codes for choloylglycine hydrolase, the enzyme for bile salt metabolism (5). KK003 contains a bile salt transporter, sulfur metabolism genes, sporulation genes, and elements of a Type II secretion system (Table 1).

#### ACKNOWLEDGMENTS

We thank the Round lab for help with mouse work, bacteria isolation, and editing this manuscript.

#### **AUTHOR AFFILIATION**

<sup>1</sup>Department of Pathology, Division of Microbiology and Immunology, University of Utah School of Medicine, Salt Lake City, Utah, USA

### **AUTHOR ORCIDs**

Allison M. Weis http://orcid.org/0000-0001-9991-1661 W. Zac Stephens https://orcid.org/0000-0002-8072-9023 June L. Round http://orcid.org/0000-0002-7158-9874

# FUNDING

Funder	Grant(s)	Author(s)
W. M. Keck Foundation (WMKF)	N/A	June L. Round
Burroughs Wellcome Fund (BWF)	N/S	June L. Round
HHS   NIH   National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)	R01AT011423 June L. Round	

## DATA AVAILABILITY

The genome is under GCF\_037014675.1; reads are SRR28014011, SRR28014012.

### REFERENCES

- Oren A, Garrity GM. 2021. Valid publication of the names of forty-two phyla of prokaryotes. Int J Syst Evol Microbiol 71. https://doi.org/10. 1099/ijsem.0.005056
- Auchtung TA, Holder ME, Gesell JR, Ajami NJ, Duarte RTD, Itoh K, Caspi RR, Petrosino JF, Horai R, Zárate-Bladés CR. 2016. Complete genome sequence of *Turicibacter* sp strain H121, isolated from the feces of a contaminated germ-free mouse. Genome Announc 4:e00114-16. https:// doi.org/10.1128/genomeA.00114-16
- Bosshard PP, Zbinden R, Altwegg M. 2002. Turicibacter sanguinis gen. nov., sp. nov., a novel anaerobic, Gram-positive bacterium. Int J Syst Evol Microbiol 52:1263–1266. https://doi.org/10.1099/00207713-52-4-1263
- Maki JJ, Looft T. 2022. Turicibacter bilis sp. nov., a novel bacterium isolated from the chicken eggshell and swine ileum. Int J Syst Evol Microbiol 72:005153. https://doi.org/10.1099/ijsem.0.005153
- Lynch JB, Gonzalez EL, Choy K, Faull KF, Jewell T, Arellano A, Liang J, Yu KB, Paramo J, Hsiao EY. 2023. Gut microbiota *Turicibacter* strains differentially modify bile acids and host lipids. Nat Commun 14:3669. https://doi.org/10.1038/s41467-023-39403-7
- Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res 41:D590–D596. https://doi.org/10.1093/nar/gks1219
- Martin M. 2011. Cutadapt removes adapter sequences from highthroughput sequencing reads. EMBnet J 17:10. https://doi.org/10.14806/ ej.17.1.200

- De Coster W, D'Hert S, Schultz DT, Cruts M, Van Broeckhoven C. 2018. NanoPack: visualizing and processing long-read sequencing data. Bioinformatics 34:2666–2669. https://doi.org/10.1093/bioinformatics/ bty149
- 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
- Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595
- 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
- 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
- Chaumeil PA, Mussig AJ, Hugenholtz P, Parks DH. 2019. GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. Bioinformatics 36:1925–1927. https://doi.org/10.1093/bioinformatics/ btz848