AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

GENOME SEQUENCES

Complete Genome Sequence of *Campylobacter coli* Strain P4581, a Hybrid Carrying *Campylobacter jejuni* Genomic Content, Isolated from Rhesus Monkey, *Macaca mulatta*

Sung Guk Kim,[a] Christine V. Summage-West,[a] Lillie M. Sims,[a] Leihong Wu,[b] JaeHyun Kim,[b] Seongwon Nho,[c] Steven L. Foley[c]

[a]Surveillance/Diagnostic Laboratory, Office of Scientific Coordination, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, USA
[b]Division of Bioinformatics and Biomathematics, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, USA
[c]Division of Microbiology, National Center for Toxicological Research, U.S. Food and Drug Administration, Jefferson, AR, USA

ABSTRACT *Campylobacter coli* is a leading bacterial cause of human gastroenteritis. We reported the circularized 1.8-Mbp complete genome of MLST type 1055 *C. coli* strain P4581 isolated from a rhesus monkey, *Macaca mulatta*, hybridizing Illumina short- and Nanopore long-reads.

Campylobacter coli and *C. jejuni* are two major pathogens causing gastroenteritis in humans and other animals. Horizontal gene transfer among *C. coli* and *C. jejuni* is an evolutionary force for generating large numbers of the new genomic types, "hybrids." with novel genes or genetic elements (1, 2). Recent evolutionary studies of the *Campylobacter* population revealed that some *C. coli* carries more than 10% of *C. jejuni* reference genomic contents. These *C. coli* strains containing *C. jenuni* genomic contents were designated "Hybrid (with >10%)" or "Half hybrid (with <10%)". Some of these hybrid *C. coli* strains display ambiguous diagnostic results (3). Though overwhelming genome data of *Campylobacter* isolates from humans and poultry are currently available, those from nonhuman primates are rare.

Rhesus monkey fecal samples, collected from a nonhuman primate colony at a research center in Arkansas, were screened for *Campylobacter* species as part of routine surveillance by culturing on *Campylobacter* Selective media (Remel, San Diego, CA) at 42°C for 4 days under microaerobic conditions (5% O_2, 10% CO_2, and 85% N_2). A typical curved Gram-negative rod-shaped isolate was identified as *C. coli* using a matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) Biotyper (Bruker, Billerica, MA).

Genomic DNA was isolated from an overnight culture under microaerobic conditions as described earlier using the Blood & Tissue kit (Qiagen, Redwood City, CA), and the quality and quantity of DNA were determined using a NanoDrop spectrophotometer and Qubit fluorometer (Fisher, Waltham, MA). The same genomic DNA was used for Illumina and Nanopore sequencing with no shearing. Following the manufacturer's protocols, for short reads, a genomic library was constructed using a Nextera XT Library Prep kit and sequenced using a MiSeq v2 Reagent kit in the 250 bp paired-end mode on MiSeq (Illumina, San Diego, CA). Long read sequencing was performed using a NEBNext Companion Module E7180 (New England BioLabs, Ipswich, MA), the ligation sequencing kit SQK-LAK109, and a flow cell FLO-MIN107 on Nanopore MinIon (Oxford Nanopore, Oxford, UK). Default parameters were used for all software unless otherwise noted. After quality checks and trimming with tools (CutAdapt v2.2 with Python v3.7.9) in PATRIC, short reads (n = 2,686,576) and long reads (n = 20,000; N_{50} = 5423) were assembled with 670× depth using Unicycler v. 0.4.8 (default normal bridging mode, rotation) in PATRIC (4). The complete genome of *C. coli* P4581 is 1,808,117 bp in length, containing one chromosome (1,693,479 bp) and one plasmid, pCC001, (114,638 bp). The G+C contents of the chromosome and plasmid were 31.43% and 26.3%, respectively. The strain P4581 genome was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP v4.11) (5). The annotated chromosome

Editor Steven R. Gill, University of Rochester School of Medicine and Dentistry

Address correspondence to Sung Guk Kim, SungGuk.Kim@fda.hhs.gov, or Steven L. Foley, Steven.Foley@fda.hhs.gov.

The authors declare no conflict of interest.

Received 15 August 2022
Accepted 22 August 2022
Published 1 September 2022

TABLE 1 Summary of genetic features of *C. coli* strain P4581

Genetic element	Size (bp)	G+C content (%)	No. Coding sequences	No. of rRNAs	No. of tRNAs	GenBank accession no.
Chromosome	1,693,479	31.43	1,777	9	44	CP086657
Plasmid	114,638	26.3	146	0	0	CP086658

contained 1,777 coding genes, 53 pseudogenes, 44 tRNAs, 9 rRNAs, and 2 antibiotic resistance genes, *tetO*, and *oxa-*$_{61}$. The Mash distance between *C. coli* strain P4581 and representative *C. coli* strain OR12 (CP013733) was 0.00973, which equated to an average nucleotide identity (ANI) of 99.03% as estimated using MinHash v2.3 (6). The annotated plasmid, pCC001, contained 146 coding genes, including transfer-related genes and 56 repeat regions. Plasmid pCC001 shared 97% sequence identity (query coverage, 78%) with *C. coli* strain YH503 plasmid pCO5503 (CP046318.1) and 87% sequence identity (query coverage, 87%) with *C. jejuni* CC19PF065 plasmid pPF065-186 (CP068568). The genomic features are presented in Table 1. Taxonomic computation of *C. coli* strain P4581 revealed "a chimeric genome of *C. coli* and *C. jejuni*" using the Kraken2 algorithm in the Taxonomic Classification Services in PATRIC (7).

Data availability. The genome sequences have been deposited in GenBank under the accession numbers CP086657 and CP086658. The raw reads have been deposited in the Sequence Read Archive (SRA) under the accession numbers SRR17194716 and SRR17194717.

ACKNOWLEDGMENTS

We appreciated the technical support from Joanna Deck, Heather Bogy, and Leah Rowe for the identification and characterization of *C. coli* P4581. This article reflects the views of the authors and does not necessarily reflect those of the U.S. Food and Drug Administration.

We declare no conflict of interest.

REFERENCES

1. Sheppard SK, Maiden MC. 2015. The evolution of Campylobacter jejuni and Campylobacter coli. Cold Spring Harb Perspect Biol 7:a018119. https://doi.org/10.1101/cshperspect.a018119.
2. Sheppard SK, Dallas JF, Wilson DJ, Strachan NJ, McCarthy ND, Jolley KA, Colles FM, Rotariu O, Ogden ID, Forbes KJ, Maiden MC. 2010. Evolution of an agriculture-associated disease causing Campylobacter coli clade: evidence from national surveillance data in Scotland. PLoS One 5:e15708. https://doi.org/10.1371/journal.pone.0015708.
3. Golz JC, Epping L, Knüver MT, Borowiak M, Hartkopf F, Deneke C, Malorny B, Semmler T, Stingl K. 2020. Whole genome sequencing reveals extended natural transformation in Campylobacter impacting diagnostics and the pathogens adaptive potential. Sci Rep 10:3686. https://doi.org/10.1038/s41598-020-60320-y.
4. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N, Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial Bioinformatics Database and Analysis Resource Center. Nucleic Acids Res 45:D535–D542. https://doi.org/10.1093/nar/gkw1017.
5. Li W, O'Neill KR, Haft DH, DiCuccio M, Chetvernin V, Badretdin A, Coulouris G, Chitsaz F, Derbyshire MK, Durkin AS, Gonzales NR, Gwadz M, Lanczycki CJ, Song JS, Thanki N, Wang J, Yamashita RA, Yang M, Zheng C, Marchler-Bauer A, Thibaud-Nissen F. 2021. RefSeq: expanding the Prokaryotic Genome Annotation Pipeline reach with protein family model curation. Nucleic Acids Res 49:D1020–D1028. https://doi.org/10.1093/nar/gkaa1105.
6. Ondov BD, Treangen TJ, Melsted P, Mallonee AB, Bergman NH, Koren S, Phillippy AM. 2016. Mash: fast genome and metagenome distance estimation using MinHash. Genome Biol 17:132. https://doi.org/10.1186/s13059-016-0997-x.
7. Wood DE, Salzberg SL. 2014. Kraken: ultrafast metagenomic sequence classification using exact alignments. Genome Biol 15:R46. https://doi.org/10.1186/gb-2014-15-3-r46.