Complete Genome Sequence of *Campylobacter jejuni* Strain NADC 20827, Isolated from Commercial Turkeys

Daniel W. Nielsen,[a,c] Joel J. Maki,[a,b,c] Torey Looft,[a] (ID) Nicole Ricker,[a,c] (ID) Matthew J. Sylte[a]

[a]Food Safety and Enteric Pathogens Research Unit, National Animal Disease Center (NADC), Agricultural Research Service (ARS), U.S. Department of Agriculture (USDA), Ames, Iowa, USA
[b]Interdepartmental Microbiology Graduate Program, Iowa State University, Ames, Iowa, USA
[c]Oak Ridge Institute for Science and Education, ARS Research Participation Program, Oak Ridge, Tennessee, USA

ABSTRACT *Campylobacter jejuni* is the main cause of bacterial foodborne disease in humans, who are exposed mostly by consumption of contaminated poultry products. *C. jejuni* strain NADC 20827 was isolated from the feces of turkeys naturally colonized with *Campylobacter* spp. We present the complete annotated genome and plasmid sequences of strain NADC 20827.

Campylobacteriosis is the most prevalent bacterial foodborne disease in humans worldwide, with over 90% of cases caused by *Campylobacter jejuni* subsp. *jejuni* (*C. jejuni*). Consumption of contaminated poultry is the main source of human exposure (1). We recently inoculated turkeys with *C. jejuni* strain NADC 20827, which was isolated in 2005 from feces at a hybrid breed turkey farm in Iowa, and asymptomatically colonized the ceca ($>10^8$ CFU/g of cecal contents) for at least 21 days postinoculation (2). In order to better understand how *C. jejuni* colonizes turkeys, the genome of strain NADC 20827 was fully sequenced.

Campylobacter jejuni strain NADC 20827 has been minimally passaged since its isolation in 2005 and was stored at $-80°C$ in glycerol prior to sequencing. The strain was cultured on Campy-Line agar containing 25 μg/ml sulfamethoxazole (3) and grown at 42°C in a microaerophilic environment (5% O_2, 10% CO_2, and 85% N_2). A single colony was picked and grown statically for 18 h in biphasic Mueller-Hinton broth at 42°C in a microaerophilic environment (2). Four milliliters of the broth phase was adjusted to an optical density at 600 nm (OD_{600}) of 0.4 and centrifuged at 13,000 × g for 10 min at 4°C. The DNA was extracted using the PureLink genomic DNA minikit (Life Technologies, Carlsbad, CA) for Nanopore and Illumina sequencing. The quality of the extracted DNA was assessed using a 2200 TapeStation apparatus and genomic DNA ScreenTape analysis (Agilent, Santa Clara, CA). Approximately 95% of the DNA was >100 kb, demonstrating the isolation of high-quality genomic DNA. The DNA yields were quantified using a Qubit fluorimeter and the double-stranded DNA (dsDNA) BR kit (Life Technologies). The genomic library for Nanopore sequencing was prepared with the rapid barcoding kit (SQK-RBK004; Oxford Nanopore, Oxford, UK), following the manufacturer's instructions. The genomic library for MiSeq sequencing was prepared with the Nextera Flex kit (Illumina, San Diego, CA).

Genomic sequencing was performed on a MinION instrument (Oxford Nanopore), using a FLO-MIN106 R9.4.1 flow cell, and a MiSeq (Illumina) instrument. The MinION flow cell was run for 48 h, and the resultant reads with a quality (Q) score greater than 7 were demultiplexed and trimmed with Guppy v. 3.1.5 (4). After filtering, there were 178,208 MinION reads with a mean length of 11,006 bp and a maximum length of 148,446 bp. The Illumina data were trimmed using Trimmomatic v. 0.36 (5). Trimmed Illumina and Nanopore reads were assembled with Unicycler v. 0.4.7 (6). The genome,

including tRNA and antibiotic resistance genes, was annotated using Prokka v. 1.13 (7). Default parameters were used for all software unless otherwise noted.

The genome of *C. jejuni* strain NADC 20827 consists of a single chromosome and two plasmids with short-read coverage of 570.5, 352.8, and 3,607.6×, respectively. The chromosome consists of 1,806,805 bp and has a 30.3% GC content. It contains 1,853 coding sequences and encodes 44 tRNAs, as well as 1 CRISPR region with four spacers. The larger plasmid, p20827L, has a 28.7% GC content and consists of 47,087 bp with 54 coding sequences, including the *tetO* antibiotic resistance gene. The smaller plasmid, p20827S, has a 30.8% GC content and consists of 4,366 bp with 5 coding sequences.

Data availability. The plasmids and chromosome have been deposited in GenBank under the accession numbers CP045046, CP045047, and CP045048. The Nanopore and Illumina reads are available in the NCBI Sequence Read Archive (accession numbers SRR10239225 and SRR10239224).

REFERENCES

1. Humphrey T, O'Brien S, Madsen M. 2007. Campylobacters as zoonotic pathogens: a food production perspective. Int J Food Microbiol 117:237–257. https://doi.org/10.1016/j.ijfoodmicro.2007.01.006.
2. Sylte MJ, Inbody MH, Johnson TA, Looft T, Line JE. 2018. Evaluation of different Campylobacter jejuni isolates to colonize the intestinal tract of commercial turkey poults and selective media for enumeration. Poult Sci 97:1689–1698. https://doi.org/10.3382/ps/pex384.
3. Line JE, Bailey JS, Berrang ME. 2008. Addition of sulfamethoxazole to selective media aids in the recovery of *Campylobacter* spp. from broiler rinses. J Rapid Methods Autom Microbiol 16:2–12. https://doi.org/10.1111/j.1745-4581.2008.00111.x.
4. Wick RR, Judd LM, Holt KE. 2019. Performance of neural network base-calling tools for Oxford Nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y.
5. 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.
6. 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.
7. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.