



Draft Genome Sequences of *Campylobacter jejuni* Strains Isolated from Poultry

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ABSTRACT Four wild-type *Campylobacter jejuni* strains isolated from the cecal contents of broiler chickens were sequenced. The average genome size was 1,622,170 bp, with 1,667 to 1,761 coding sequences and 47 to 51 RNAs. Multiple genes encoding motility, intestinal colonization, toxin production, stress tolerance, and multidrug resistance were present in all the strains.

ampylobacter jejuni is a major foodborne pathogen that is responsible for approximately 90% of the reported campylobacteriosis cases in humans (1, 2). Poultry, chickens in particular, act as the reservoir host for C. jejuni. The pathogen colonizes the ceca of poultry, thereby leading to contamination of the carcass during slaughter and to subsequent human infections through handling or consumption of contaminated poultry products (3, 4). Little is known about how this pathogen is able to colonize chickens while competing with specialist microbiota in the gut (5). Whole-genome sequencing and comprehensive characterization of wild-type C. jejuni strains could facilitate better understanding of their pathophysiology and development of effective intervention strategies. Therefore, we isolated four strains of C. jejuni from the cecal contents of commercial poultry in Fayetteville, AR. The cecal contents were serially diluted in Butterfield's phosphate diluent (BPD; 0.625 mM potassium dihydrogen phosphate [pH 6.67]), and each dilution was plated on Campylobacter line agar (6). The genus of pure culture isolates was confirmed as Campylobacter based on various biochemical tests (e.g., catalase, oxidase, hippurate hydrolysis, and nitrate/nitrite reduction) and the species as C. jejuni based on PCR using species-specific primers (7).

Each strain of *C. jejuni* was cultured separately in *Campylobacter* enrichment broth (International Diagnostics Group, Bury, Lancashire, UK) for 48 h microaerobically at 42°C, and the genomic DNA was isolated using a PureLink genomic DNA kit (Invitrogen, Carlsbad, CA). Next-generation sequencing was performed on a MiSeq platform using the Illumina v2 reagent kit with 2×250 cycles (Illumina, Inc., San Diego, CA) with a coverage greater than $100 \times$. The sequencing library was constructed using a Nextera XT sample preparation kit (Illumina) according to the manufacturer's protocol. FASTQ data sets generated with the MiSeq platform were trimmed and assembled using the *de novo* assembly algorithm in CLC Genomics Workbench version 11 (Qiagen, Inc., Redwood City, CA). Default parameters were used for all software unless otherwise noted. The assembled sequences were annotated and specific genes were identified using the NCBI Prokaryotic Genome Annotation Pipeline (8).

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	Genome	No. of	Avg	No. of	Contig	Contig	No. of	No. of	GenBank
Strain	size (bp)	reads	coverage (×)	contigs	N ₅₀ (bp)	L ₅₀	CDS	RNAs	accession no.
KADAMBIS1	1,620,512	2,278,000	351	149	45,811	11	1,715	47	SFCE00000000
KADAMBIS3	1,616,860	1,686,400	261	72	145,785	4	1,667	51	SFCF0000000
KADAMBIS4	1,628,567	2,196,400	337	105	86,266	6	1,694	50	SFCG0000000
KADAMBIS8	1,622,740	2,296,400	354	213	92,994	6	1,761	51	SFCH0000000

TABLE 1 Overview of genomic features of C. jejuni strains^a

^a Each strain was obtained from chickens.

The sizes of the genomes were between 1,616,860 and 1,628,567 bp, and the average size was 1,622,170 bp (Table 1). The GC content was 37% in each of the genomes. We found 72 to 213 contigs with an N_{50} range from 45,811 to 145,785 bp and L_{50} values ranging from 4 to 11. In addition, 1,667 to 1,761 coding sequences (CDS) and 47 to 51 RNAs were observed in the genome. Multiple genes encoding virulence factors, such as those for motility, intestinal colonization, toxin production, and stress tolerance, were observed in all the sequenced strains. Several genes encoding flagellar biosynthesis and motility were common among the strains. The cytolethal distending toxin production genes (*cdtA*, *cdtB*, and *cdtC*) were present in all four strains (9). Similarly, a peroxide stress response gene (*yaaA*) involved in tolerance to oxidative damage to cells was also detected in all strains. In addition, several multidrug efflux pump transporter genes (*cmeA*, *cmeB*, *cmeC*, *cmeD*, *cmeE*, *cmeF*, and *cmeR*) were identified in all four strains (10).

Data availability. All sequences have been published in GenBank under accession no. SFCE00000000 (KADAMBIS1), SFCF00000000 (KADAMBIS3), SFCG00000000 (KADAMBIS4), and SFCH00000000 (KADAMBIS8). The raw sequence reads have been deposited in the Sequence Read Archive (SRA) under accession no. SRR9960540 (KADAMBIS1), SRR9960539 (KADAMBIS3), SRR9960542 (KADAMBIS4), and SRR9960541 (KADAMBIS8). The versions described in this paper are the first versions.

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REFERENCES

- Rosner BM, Schielke A, Didelot X, Kops F, Breidenbach J, Willrich N, Gölz G, Alter T, Stingl K, Josenhans C, Suerbaum S, Stark K. 2017. A combined case-control and molecular source attribution study of human *Campylobacter* infections in Germany, 2011–2014. Sci Rep 7:5139. https://doi .org/10.1038/s41598-017-05227-x.
- Cody AJ, McCarthy ND, van Rensburg MJ, Isinkaye T, Bentley S, Parkhill J, Dingle KE, Bowler IC, Jolley KA, Maiden MC. 2013. Real-time genomic epidemiological evaluation of human *Campylobacter* isolates by use of whole-genome multilocus sequence typing. J Clin Microbiol 51: 2526–2534. https://doi.org/10.1128/JCM.00066-13.
- Humphrey TJ, Henley A, Lanning DG. 1993. The colonization of broiler chickens with *Campylobacter jejuni*: some epidemiological investigations. Epidemiol Infect 110:601–607. https://doi.org/10.1017/s0950268800051025.
- Allen VM, Bull SA, Corry JEL, Domingue G, Jørgensen F, Frost JA, Whyte R, Gonzalez A, Elviss N, Humphrey TJ. 2007. *Campylobacter* spp. contamination of chicken carcasses during processing in relation to flock colonisation. Int J Food Microbiol 113:54–61. https://doi.org/10.1016/j .ijfoodmicro.2006.07.011.
- Hermans D, Van Deun K, Martel A, Van Immerseel F, Messens W, Heyndrickx M, Haesebrouck F, Pasmans F. 2011. Colonization factors of

Campylobacter jejuni in the chicken gut. Vet Res 42:82. https://doi.org/ 10.1186/1297-9716-42-82.

- Line JE. 2001. Development of a selective differential agar for isolation and enumeration of *Campylobacter* spp. J Food Prot 64:1711–1715. https://doi.org/10.4315/0362-028x-64.11.1711.
- Winters DK, Slavik MF. 1995. Evaluation of a PCR based assay for specific detection of *Campylobacter jejuni* in chicken washes. Mol Cell Probes 9:307–310. https://doi.org/10.1016/s0890-8508(95)91556-7.
- 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.
- Young KT, Davis LM, DiRita VJ. 2007. Campylobacter jejuni: molecular biology and pathogenesis. Nat Rev Microbiol 5:665–679. https://doi.org/ 10.1038/nrmicro1718.
- Lin J, Sahin O, Michel LO, Zhang Q. 2003. Critical role of multidrug efflux pump CmeABC in bile resistance and *in vivo* colonization of *Campylo-bacter jejuni*. Infect Immun 71:4250–4259. https://doi.org/10.1128/iai.71 .8.4250-4259.2003.