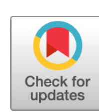
GENOME SEQUENCES


Complete Genomic Sequences of *Campylobacter coli* Strains Isolated from Poultry Sold in Pennsylvania Farmers' Markets

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ABSTRACT *Campylobacter* strains were collected in a survey of fresh chicken carcasses in Pennsylvania farmers' markets. Three *Campylobacter coli* strains were observed to have unique sequence variations in their gyrase subunit B genes, compared with other *Campylobacter* strains. The strains were sequenced and analyzed, producing genome sequences consisting of single closed chromosomes.

Campylobacter strains are responsible for the largest number of annual cases of bacterial foodborne gastrointestinal disease in the developed world (1–3). Poultry raised for human consumption is a primary source of the *Campylobacter* strains causing human disease through transmission by undercooked poultry products or cross contamination of other foods (4, 5). *Campylobacter jejuni* strains are responsible for roughly 90% of human cases of disease, with *Campylobacter coli* causing about 10% (1, 6).

A large number of presumptive *Campylobacter* isolates were derived from a survey of fresh chicken carcasses sold by vendors at farmers' markets in Pennsylvania during the summer of 2011 (7). The DNA gyrase subunit B gene (*gyrB*) of 176 presumptive *Campylobacter* strains isolated from the survey was sequenced to identify the species of the *Campylobacter* strains using a previously described method (8). The species of three isolates (PSU-29, PSU-31, and PSU-32) could not be clearly determined from their *gyrB* sequences, and it was decided to sequence those strains completely.

The three presumptive *C. coli* strains were streaked from freezer stock (−80°C) onto *Brucella* agar for individual colonies. Single colonies were selected from the agar plates and subsequently grown in *Brucella* broth in a microaerobic environment (5% O_2/10% CO_2/85% N_2). Genomic DNA for sequencing was prepared using a MagAttract high-molecular-weight (HMW) DNA kit (Qiagen, Germany) and quantitated with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). The genomic DNA was fragmented with g-TUBE units (Covaris, Woburn, MA), and the fragmented DNA was size selected using a BluePippin system (Sage Scientific, Beverly, MA). Standard manufacturer's protocol was used to prepare SMRTBell sequencing libraries for the three genomes, and sequencing was performed using a PacBio RS II system (Pacific Biosciences, Menlo Park, CA). During the genome assembly process, all software systems were used with their default parameters unless otherwise specified. Assembly was performed with the Celera Assembler v8.1, with the subread filtering derived from the single-molecule real-time (SMRT) Analysis software suite (9, 10). Quiver was used to polish the assembled contigs, and Geneious v7.1.5 (Biomatters, Auckland, New Zealand) performed a reorientation after trimming the overlapping contig ends. The trimming and reorientation steps were verified using a second application of Quiver (9). The resulting three individual assemblies produced consensus accuracies of at least 99.9999% with ≥190× coverage (Table 1).

The genomes were annotated through the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) using default parameters (11). The tRNA was predicted using tRNAscan-SE v2.0 (12). Antimicrobial resistance genes (class D β-lactamase, OXA-61

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TABLE 1 *Campylobacter coli* whole-genome sequencing results

Strain name	BioSample no.	GenBank accession no.	SRA accession no.	Total length sequenced (Mb)	No. of reads	Read N_{50} (bp)	Genome coverage (×)	Genome size (bp)	GC content (%)	OrthoANI (%)	No. of genes	No. of coding sequences	No. of RNAs	No. of tRNAs
PSU-29	SAMN16083344	CP066487	SRR13175776	1,108.44	96,291	6,115	225	1,670,501	31.45	100	1,732	1,241	56	44
PSU-31	SAMN16083724	CP066486	SRR13175987	1,106.02	97,138	5,323	196	1,672,436	31.44	99.89	1,725	1,250	56	44
PSU-32	SAMN16083725	CP061537	SRR13142778	906.46	80,218	6,001	190	1,673,158	31.43	99.93	1,722	1,615	56	44

family) were identified in all three genomes using ResFinder v3.2 software (13). Average nucleotide identity (ANI) values were determined according to reference 14. All sequences were examined for the presence of phage in the genome using PHASTER software (15), and no phage were detected. Additionally, virulence genes for cytolethal distending toxin (*cdtABC*), the multidrug efflux system (*cmeABCDEF*), flagella (*flaABCG*, *flgABCDEFGHIKLQS*, *fliDEFGHIKLMNPQRSW*, *flhABF*, *motAB*, and *fleN*), chemotaxis (*cheBRVY*), and invasion (*ciaB* and *phlA*) were found in each of the three strains.

Finally, Jaccard similarity coefficients were calculated for the gene contents of the three presumptive *C. coli* strains, compared to each other and to reference *C. coli* and *C. jejuni* strains. Strains PSU-29, PSU-31, and PSU-32 were observed to be significantly more similar (>80% similarity) to the reference *C. coli* strains than to the reference *C. jejuni* strains (<21% similarity). Therefore, despite their *gyrB* sequences not grouping as expected with *C. coli gyrB* sequences, these *Campylobacter* isolates from poultry belong to the species *C. coli*.

Data availability. The genome sequences and sequencing reads for the three *C. coli* strains were deposited in GenBank under the accession numbers listed in Table 1.

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