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GENOME SEQUENCES

Whole-Genome Sequencing of Four *Campylobacter* Strains Isolated from Gull Excreta Collected from Hobie Beach (Oxnard, CA, USA)

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ABSTRACT *Campylobacter* spp. are commensal organisms in avian species and are one of the leading causes of bacterial foodborne human diarrheal disease worldwide. We report the draft genome sequences of *Campylobacter volucris*, *C. lari*, and *C. jejuni* strains isolated from California gull (*Larus californicus*) excreta collected from a California beach.

Campylobacter species are Gram-negative spiral rods, non-spore-forming chemoorganotrophs, and members of the *Epsilonproteobacteria* class, which grow under microaerobic conditions (1). Several *Campylobacter* species are recognized as a leading cause of bacterial foodborne infection diseases worldwide and are common inhabitants of the intestine of many wild and domestic avian species (2–4). A previous study documented the presence of a diverse and abundant population of campylobacters in the excreta of California gulls (*Larus californicus*) from California beaches (5). Although the risk from water impacted by California gulls is low for the community, advances in genomic analysis of potentially human infectious *Campylobacter* spp. in gull excreta may provide additional information for estimating the risks posed by nonsewage fecal sources (6).

Four strains (CaG_5A, CaG_58BB, CaG_63A, and CaG_70BB) were isolated from gull excreta collected in the summer of 2012 from Hobie Beach (Oxnard, CA, USA) following Waldenström et al. (7). The four colonies were transferred to individual Bolton enrichment agar plates (without antibiotics) and incubated at 42°C under microaerophilic conditions (10% CO_2, 5% O_2, and 85% N_2) for 24 h. All colonies were isolated separately, and their genomic DNA was extracted from a single colony using the MasterPure DNA extraction kit (Epicentre, Madison, WI) and purified with the DNA Clean & Concentrator kit (Zymo Research, Irvine, CA) following the manufacturer's instructions. Genomic libraries were prepared using the TruSeq library kit followed by rapid mode sequencing (2 × 100 bp) on the HiSeq 2000 platform (Illumina, Inc., San Diego, CA).

A total of 39,770,374 reads were generated. Prior to assembly, the libraries were cleaned of adapters and phiX artifacts, error corrected, normalized (≤100×), and filtered to a minimum length of 80 nucleotides using the software package BBMap v38.22 (with the following settings: ktrim=r k=23 mink=11 hdist=1 tbo tpe maxns=0 trimq=10 qtrim=r maq=12 minlength=100 ecco=t eccc=t ecct=t target=100) (8). A reference-assisted *de novo* assembly approach was used to assemble the processed reads using Unicycler v0.4.7 (9). Average nucleotide identity (ANI), an index of similarity between two genomes (10), was calculated using FastANI v1.1 (11). The *in silico* multilocus sequence type (MLST) based on seven alleles (*aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, and *uncA*) was obtained using mlst v2.16.1 (12, 13), genes were assessed for antibiotic resistance with ResFinder v3.1 (14), and chromosomal point mutations were deter-

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TABLE 1 Summary statistics of whole-genome assemblies

Strain	Coverage (×)	No. of contigs	Assembly size (bp)	Contig N_{50} (bp)	G+C content (%)	Gene annotation data (no.)				ST[b]	Taxonomic affiliation		Reference genome	GenBank accession no.
						Genes	CDS[a]	rRNAs	tRNAs		Genus	Species		
CaG_5A	238	28	1,547,878	313,244	28.45	1,598	1,552	4	41	NA	*Campylobacter*	*volucris*	GCF_000816345	SMTU00000000
CaG_58BB	231	50	1,537,596	170,866	29.50	1,553	1,513	2	37	NA	*Campylobacter*	*lari*	GCA_000816385	SMTT00000000
CaG_63A	197	45	1,687,991	219,579	30.46	1,773	1,729	3	40	2654	*Campylobacter*	*jejuni*	GCA_000737085	SMTS00000000
CaG_70BB	228	19	1,569,087	436,706	28.41	1,631	1,585	4	41	NA	*Campylobacter*	*volucris*	GCA_000816345	SMTR00000000

[a] CDS, coding sequences.
[b] ST, sequence type (*in silico* MLST; *aspA*, *glnA*, *gltA*, *glyA*, *pgm*, *tkt*, *uncA*); NA, not assigned.

mined with PointFinder v3.1 (15). Default parameters were used for all software unless otherwise specified. The genome quality and statistics were estimated with BBMap and annotated with Prokka v1.13.1 (16) (Table 1).

ANI calculations revealed an average genome similarity of 98.38% between strains CaG_5A and CaG_70BB, which were both distantly related to CaG_58BB and CaG_63A with 79.00% and 81.36% similarity, respectively. Taxonomic affiliation analysis based on the ANI between genomes (17) shows that both CaG_5A and CaG_70BB were closely related to *Campylobacter volucris* LMG 24379 with 98.16% similarity, CaG_58BB to *C. lari* CCUG 22395 with 93.51% similarity, and CaG_63A to *C. jejuni* subsp. jejuni MTVDSCj20 with 98.10% similarity. Only strain CaG_63A was assigned to a sequence type (ST2654), which was previously detected in recreational beaches and environmental waters in France (18). Genome analysis using the Web tool PointFinder (15) confirmed the absence of known chromosomal point mutations or genes associated with antimicrobial resistance except for $bla_{\text{OXA-466}}$ in strain CaG_63A, potentially conferring resistance to β-lactams.

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The raw sequence reads have been submitted to the NCBI SRA under the accession numbers SRR8715499, SRR8715500, SRR8715501, and SRR8715502. The versions described in this paper are the first versions.

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