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Complete Genome Sequence of a Salmonella enterica subsp. enterica Serovar Fresno Isolate Recovered from a Bovine Lymph Node

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GENOME SEQUENCES

Complete Genome Sequence of a *Salmonella enterica* subsp. *enterica* Serovar Fresno Isolate Recovered from a Bovine Lymph Node

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ABSTRACT *Salmonella enterica* serovar Fresno is an infrequently isolated serovar whose ecology and genomic characteristics have not yet been described. To further understand the genomic characteristics of this serovar, we sequenced the complete genome of a single isolate recovered from a bovine lymph node at harvest.

Salmonella enterica serovar Fresno is a rare serovar that has been occasionally isolated from humans, livestock, produce, and the environment in at least 10 countries (1). Although this serovar may be broadly distributed on a geographic scale, it is infrequently recovered in routine surveys of foods, food-producing animals, and human clinical cases, indicating that *S.* Fresno may be found at a higher frequency in other reservoirs, such as unmonitored wildlife, in regions where testing for *S. enterica* is not frequently conducted, or that it is relatively rare, in terms of abundance, compared to other serovars. However, *Salmonella* Fresno has been isolated from humans and a bovine lymph node, suggesting that it can cause disease and may cause invasive infections.

S. enterica isolate USMARC-69835 was recovered from a bovine lymph node collected at harvest in 2012 (2). The isolate was cultured on tryptic soy agar at 37°C for 18 to 20 h and subsequently grown overnight at 37°C in tryptic soy broth. Genomic DNA was extracted from this growth using the Genomic-tip 100/G columns and blood and cell culture DNA midi kits (Qiagen, Valencia, CA). Single-molecule real-time (SMRT) sequencing libraries were constructed using C4/P6 chemistry and sequenced on an RS II instrument (Pacific Biosciences [PB], Menlo Park, CA) following the manufacturer's instructions. A total of 78,613 reads with a mean length of 12,213 bp were sequenced. Sequencing reads were filtered for length and quality (minimum subread length, 500; minimum polymerase read quality, 0.80) using the PB SMRT Portal v.2.3.0.140893 analysis pipeline. *De novo* genome assembly was conducted using the Hierarchical Genome Assembly Process (HGAP) v.3.0 with a minimum seed length of 6,000 (3). A dot plot was constructed for every polished contig using Geneious v.11.1.3 (Biomatters Ltd., New Zealand) (4) to identify the overlapping region, which was subsequently trimmed from the 3′ end of the contig. The chromosomal origin of replication was identified using Ori-Finder (5). The trimmed and newly oriented sequences were validated using the PB RS_Resequencing pipeline to map the corresponding reads back to the new reference in order to generate closed, circular, consensus concordance assemblies (3). The final assembly yielded two contigs, consisting of one closed chromosome (GenBank accession number CP032444) and one plasmid (GenBank accession number CP032445). Genome sequencing statistics and genomic features are presented in Table 1.

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TABLE 1 Genome sequencing statistics, characteristics, and accession numbers

Strain or plasmid	Serovar	MLST ST	GenBank (SRA) accession no.	No. of reads (fold coverage [×])	Size (bp)	GC content (%)	Inc type[b]	Resistance gene[a]	SPI[d]	Yr of isolation
USMARC-69835	Fresno	649	CP032444 (SRX4742127)	77,242 (165.8)	4,732,430	52.2		*aac(6′)-Iaa*[c]	1, 2, 3, 4, 5, 6, 8, 9, 11, 13	2012
pSFR1-USMARC-69835			CP032445	3,392 (353)	89,737	50.2	IncI1			

[a] Antibiotic resistance genes were determined using ResFinder 3.1 (9).
[b] Plasmid Inc types were determined using PlasmidFinder 2.0 (10).
[c] May be cryptic and may not confer aminoglycoside resistance (11).
[d] SPI, *Salmonella* pathogenicity islands.

After genome sequencing and assembly, the isolate was identified *in silico* as *Salmonella* Fresno and typed as sequence type 649 (ST649) using SeqSero and MLST 2.0, respectively (6, 7). An IncI1 plasmid with a length of 89,737 bp was assembled among the sequencing reads. In a phylogenetic analysis of 457 *S. enterica* genomes, *S.* Fresno USMARC-69835 clustered with members of subclade A1 (8). Compared to 37 other *S.* Fresno genomes, *S.* Fresno USMARC-69835 was more closely related to isolates from livestock and produce in the United States and more distantly related to three other clades of *S.* Fresno isolated outside North America.

Data availability. The complete genome sequence of *S. enterica* USMARC-69835 has been deposited in GenBank under the accession numbers CP032444 (chromosome) and CP032445 (plasmid). The GenBank submission CP032444 represents a single complete genome. The SRA accession number is SRX4742127.

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