



Complete Genome Sequence of a Shiga Toxin-Producing *Escherichia coli* O26:H11 Strain (Sequence Type 21) and Two Draft Genome Sequences of *Listeria monocytogenes* Strains (Clonal Complex 1 [CC1] and CC59) Isolated from Fresh Produce in Germany

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ABSTRACT The complete genome sequence of a Shiga toxin-producing *Escherichia coli* (STEC) O26:H11 strain, MBT-5 (sequence type 21 [ST21], *stx*_{1a}, *stx*_{2a}, *eae*, *ehxA*), and two draft genome sequences of *Listeria monocytogenes* strains MBT-6 and MBT-7 belonging to the virulent sequence types 1 (ST1, clonal complex 1 [CC1]) and 59 (ST59, CC59), respectively, were determined. The strains were isolated in 2015 from ready-to-eat mixed greens in Germany.

Shiga toxin-producing *Escherichia coli* (STEC) serotype O26 can lead to life-threatening infections and is the second most common serotype (after O157) found in clinical and food samples in Europe (1, 2). *Listeria monocytogenes* primarily afflicts immunocompromised individuals and can cause listeriosis with a mortality rate of about 20% (3). Both pathogens may enter the food chain through raw animal and vegetable products (4). The strains in this study were isolated by enrichment and using selective agar from fresh salad greens as previously described in detail (5). In addition to the species and serotyping confirmation done previously (5), here, the genome sequence data were determined to aid in virulence assessment.

Cultures were grown overnight at 37°C in brain heart infusion (BHI) broth, and genomic DNA was extracted using the ZR fungal/bacterial DNA miniprep kit (Zymo Research, Freiburg, Germany) for *Listeria* and the Genomic Micro AX Bacteria+ kit (A&A Biotechnology, Gdynia, Poland) for *E. coli*. The AX Bacteria+ kit is suitable for getting high-molecular-weight DNA. DNA quantification and paired-end (MiSeq; Illumina) and long-read (MinION; Oxford Nanopore) sequencing were performed as previously described (6). For short-read sequencing, the Nextera XT library kit and the MiSeq reagent kit v3 were used, and for long-read sequencing, the PCR barcoding genomic DNA (SQK-LSK109) protocol vNBE_9065_v109_revR_14Aug2019 with a MinION MK1B instrument was used. Default parameters were used for all software. The raw reads were quality controlled using FastQC v0.11.7 (<https://github.com/s-andrews/FastQC>). Hybrid assembly of short and long sequence reads of STEC strain MBT-5, obtained from Illumina and MinION sequencing, respectively, was performed with Unicycler v0.4.8 (7). The assembly of *L. monocytogenes* strains MBT-6 and MBT-7 was done with SPAdes v3.10.0 (8), with Illumina-generated sequences only. Annotation was done on the assembled contigs using the NCBI Prokaryotic Genome Annotation Pipeline v4.11 (9).

The hybrid assembly of STEC strain MBT-5 generated a single circular chromosome of 5,705,580 bp, with 6 circular plasmids (pMBT-5.1, 92,578 bp; pMBT-5.2, 89,589 bp; pMBT-5.3, 34,196 bp; pMBT-5.4, 6,715 bp; pMBT-5.5, 4,742 bp; pMBT-5.6, 2,784 bp). The circular chromosome of MBT-5 was rotated to *dnaA* as the origin with Geneious v9.2.

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TABLE 1 Genome statistics of sequenced strains

Strain	Platform (no. of raw reads; avg length [bp])	Contig or no. of contigs	GC content (%)	Genome size (bp)	Contig type or N_{50} (bp)	Coverage (x)	No. of genes (CDS) ^a	ST (CC) ^b	Molecular Serotype ^c	Virulence genes determined by VirulenceFinder (total no.) ^d	GenBank accession no.	SRA accession no.	
<i>Escherichia coli</i> MBT-5	Paired end using MiSeq (1,482,454; 251); MinION (127,117; 7,911)	Chromosome	50.7	5,705,580	Circular	260	5,790	ST25 ^e	O26:H11	<i>astA, cif, eae, efa1, espB, espF, espJ, espP, fyuA, gad, iha, irp2, iss, iucC, iutA, lpfA, nleA, nleB, nleC, ompT, stx_{1a}, stx_{2a}, terC, tir, tra</i> (chromosome); <i>ehxA, espA, katP, toxB</i> (plasmid 5.1) (29)	CP058682 CP058683 CP058684 CP058685 CP058686 CP058687 CP058688	SRR12207194 (paired end), SRR12207193 (MinION)	
		Plasmid 5.1	47.8	92,578	Circular	281	93						
		Plasmid 5.2	47.6	89,589	Circular	192	106						
		Plasmid 5.3	39.7	34,196	Circular	386	36						
		Plasmid 5.4	45.6	6,715	Circular	1,918	9						
<i>Listeria monocytogenes</i> MBT-6	Paired end using MiSeq (669,662; 247)	Plasmid 5.5	41.2	4,742	Circular	1,151	4						
		Plasmid 5.6	42.2	2,784	Circular	3,621	3						
		21	37.93	2,879,121	515,847	44	2,874	ST1 (CC01)	4b	<i>agrA, flhA, flgC, flgE, gmar, lap, lapB, oatA, oppA, rli55, rli60, rsbv, bile, bsh, chiA, clpB, clpC, clpe, clpp, codY, ctap, ctsR, dal, degU, dltA, fbpA, fri, fur, hfg, hly, htrA, iap, inlA, inlB, inlC, inlH, inlJ, lgt, lhrC, lipA, lisR, lntA, lpeA, lplA1, lsp, mogR, mpl, mprf, murA, perR, prfA, prsA2, pycA, recA, relA, secA2, sigB, sipX, sipZ, sod, srtA, srtB, stp, svpA, tcsA, tig, ulpT, virR</i> (72)	JACBGM000000000 SRR12207196 (paired end)		
<i>Listeria monocytogenes</i> MBT-7	Paired end using MiSeq (616,456; 247)	24	37.83	2,975,327	482,161	45	2,985	ST59 (CC59)	1/2b	<i>agrA, ami, aut, flhA, flgC, flgE, gmar, lap, lapB, oatA, oppA, rli55, rli60, rsbv, bile, bsh, chiA, clpB, clpC, clpe, clpp, codY, ctap, ctsR, dal, degU, dltA, fbpA, fri, fur, gtcA, hfg, hly, htrA, iap, inlA, inlB, inlC, inlH, inlJ, inlK, lgt, lhrC, lipA, lisR, lntA, lpeA, lplA1, lsp, mogR, mpl, mprf, murA, perR, pgdA, ppl, plcA, plcB, prfA, prsA2, pycA, recA, relA, secA2, sigB, sipX, sipZ, sod, srtA, srtB, stp, svpA, tcsA, tig, uHpt, virR</i> (76)	JACBGL000000000 SRR12207195 (paired end)		

^a CDS, coding DNA sequences.
^b See reference 11.
^c See references 5 and 10.
^d The relevant species was selected for (13).
^e MLST scheme *E. coli*-1.

Plasmids were not rotated. Strain MBT-5 was identified as serotype O26:H11, sequence type 21 (ST21), and adhesin *fimH* type 440 using SerotypeFinder v2.0, MLST v2.0, and *FimTyper* v1.0, respectively (10–12). Virulence genes were found using *VirulenceFinder* v2.0 (Table 1) (13). Interestingly, plasmid pMBT-5.1 harbored the virulence genes *ehxA* (enterohemolysin), *espA* (extracellular serine protease), *toxB* (toxin B), and *katP* (catalase peroxidase), while all other virulence genes were located on the chromosome.

The assembly of *L. monocytogenes* strains MBT-6 and MBT-7 generated 21 and 24 contigs, respectively. No plasmid sequences could be determined. Genome characteristics are shown in Table 1. The molecular serotype and multilocus sequence type (MLST) were identified using MLST 2.0 and multiplex PCR as described earlier (5). Both *L. monocytogenes* strains showed genetic characteristics similar to those of previously reported clinical, food, and outbreak-associated populations (ST1 = clonal complex 1 [CC1] and ST59 = CC59) (14), indicating that virulent STEC and *L. monocytogenes* strains can be transmitted via fresh produce. However, these strains could be isolated only at low incidence in northern Germany in 2015 (5).

Data availability. The genome sequences have been deposited in GenBank under the BioProject number [PRJNA643697](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA643697). The sequences of STEC strain MBT-5 and *L. monocytogenes* strains MBT-6 and MBT-7 were deposited at DDBJ/ENA/GenBank under the accession numbers [CP058682](https://www.ncbi.nlm.nih.gov/nuccore/CP058682) (chromosome), [CP058683](https://www.ncbi.nlm.nih.gov/nuccore/CP058683) (plasmid 5.1), [CP058684](https://www.ncbi.nlm.nih.gov/nuccore/CP058684) (plasmid 5.2), [CP058685](https://www.ncbi.nlm.nih.gov/nuccore/CP058685) (plasmid 5.3), [CP058686](https://www.ncbi.nlm.nih.gov/nuccore/CP058686) (plasmid 5.4), [CP058687](https://www.ncbi.nlm.nih.gov/nuccore/CP058687) (plasmid 5.5), [CP058688](https://www.ncbi.nlm.nih.gov/nuccore/CP058688) (plasmid 5.6), [JACBGM000000000](https://www.ncbi.nlm.nih.gov/nuccore/JACBGM000000000), and [JACBGL000000000](https://www.ncbi.nlm.nih.gov/nuccore/JACBGL000000000). The raw sequencing data were also deposited at the SRA with the accession numbers shown in Table 1.

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