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Genome Sequence of Lineage III *Listeria monocytogenes* Strain HCC23▽

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More than 98% of reported human listeriosis cases are caused by *Listeria monocytogenes* serotypes within lineages I and II. Serotypes within lineage III (4a and 4c) are commonly isolated from environmental and food specimens. We report the first complete genome sequence of a lineage III isolate, HCC23, which will be used for comparative analysis.

Listeria monocytogenes strain HCC23 is a serotype 4a lineage III strain that was isolated from a healthy channel catfish (2). It is nonpathogenic in mice, even when given by injection (2, 4). Here we report the genome sequence of HCC23, which is the first strain to be sequenced from lineage III.

The complete genome sequence of HCC23 was determined using 454 pyrosequencing on a GS-FLX genome sequencer at 454 Life Sciences (Roche) to generate an assembly with 15× depth of coverage. Quality-filtered sequences from whole-genome shotgun sequencing were assembled using the 454 Newbler assembler. The assembly produced 23 contigs, each at least 500 bases in length. Scaffolding was done with Projector 2 software (7) using the *L. monocytogenes* strain F2365 genome sequence (NC_002973). Gaps were closed by direct Sanger sequencing of PCR templates with primer walking. Assembly of 454-generated contigs and Sanger reads was done using the SeqmanPro software program (Lasergene). Assembled bases had a Phred-equivalent quality score of 40 or above, which means that the level of accuracy was 99.99%. The DNA sequence was submitted to the JCVI Annotation Service, where it was run through JCVI's prokaryotic annotation pipeline using Annotation Engine. The manual annotation software tool Manatee was downloaded from SourceForge and used to manually review the output from the prokaryotic pipeline of the JCVI Annotation Service.

A homopolymer analysis was done to ensure quality of sequence. PCR amplification and sequencing were done over all homopolymer regions containing ≥9 bases. Only one insertion was detected in one 10-bp run. However, frameshift analysis using the Microbial Genome Submission Check at NCBI identified 38 potential frameshifts. PCR amplification and Sanger sequencing resulted in correction of 28 frameshifts; the remaining 10 potential frameshifts were shown to be authentic. Of the corrected frameshifts, 27 occurred in homopolymers of ≥5 bp in size (composed of A/Ts). Thirteen were single base insertions, and 14 were single base deletions.

The completed genome of strain HCC23 is 2,976,212 bp in length and has a total of 3,011 predicted coding regions. The sequence has an average G+C content of 38.2%. Similar to other *L. monocytogenes* strains, HCC23 has six ribosomal operons and 67 total tRNA genes. Synteny is highly conserved between the HCC23 genome and the genomes of EGDe (lineage I; NC_003210) and F2365 (lineage II). Many listerial virulence proteins are encoded by HCC23, including internalins A and B and proteins in the *prfA* locus (3). However, some virulence proteins are not encoded, such as InlC, InlH, and InlJ (1, 5, 6). Proteins involved in energy metabolism and transport in strains HCC23, F2365, and EGDe are almost identical, and carbohydrate metabolism in these three strains appears conserved.

The HCC23 genome sequence will allow whole-genomic comparisons across all three *L. monocytogenes* lineages for phylogenic classification and a better understanding of the evolution of *L. monocytogenes*. More significantly, HCC23 offers the opportunity to conduct comparative genomics between pathogenic and nonpathogenic *L. monocytogenes* isolates to identify potential genetic differences responsible for pathogenicity.

Nucleotide sequence accession number. The completed genome has been deposited in DDBJ/EMBL/GenBank under accession no. CP001175.

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REFERENCES

1. **Engelbrecht, F., et al.** 1996. A new PrfA-regulated gene of *Listeria monocytogenes* encoding a small, secreted protein which belongs to the family of internalins. Mol. Microbiol. **21:**823–837.
2. **Erdenlig, S., A. J. Ainsworth, and F. W. Austin.** 2000. Pathogenicity and production of virulence factors by *Listeria monocytogenes* isolates from channel catfish. J. Food Prot. **63:**613–619.
3. **Kathariou, S.** 2002. Listeria monocytogenes virulence and pathogenicity, a food safety perspective. J. Food Prot. **65:**1811–1829.

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4. **Liu, D., et al.** 2006. Listeria monocytogenes serotype 4b strains belonging to lineages I and III possess distinct molecular features. J. Clin. Microbiol. **44:**214–217.
5. **Raffelsbauer, D., et al.** 1998. The gene cluster inlC2DE of *Listeria monocytogenes* contains additional new internalin genes and is important for virulence in mice. Mol. Gen. Genet. **260:**144–158.
6. **Sabet, C., M. Lecuit, D. Cabanes, P. Cossart, and H. Bierne.** 2005. LPXTG protein InlJ, a newly identified internalin involved in *Listeria monocytogenes* virulence. Infect. Immun. **73:**6912–6922.
7. **van Hijum, S. A., A. L. Zomer, O. P. Kuipers, and J. Kok.** 2005. Projector 2: contig mapping for efficient gap-closure of prokaryotic genome sequence assemblies. Nucleic Acids Res. **33:**W560–W566.