



Draft Genome Sequence of *Listeria innocua* Strain MEZLIS29, Isolated from a Cow in South Africa

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ABSTRACT Here, we report the draft genome sequence of *Listeria innocua* strain MEZLIS29, which was isolated from a healthy cow in Flagstaff, Eastern Cape Province, South Africa. The genome was sequenced using the Illumina MiSeq platform and had a length of 2,805,865 bp, with a G+C content of 37.5% and 2,783 coding DNA sequences, 58 tRNAs, 4 noncoding RNAs, and 8 rRNA genes.

L *isteria* species are small, motile, catalase-positive, non-spore-forming, rod-shaped, Gram-positive bacteria. The genus *Listeria* currently consists of 26 species (1), of which *Listeria monocytogenes* is a significant foodborne and invasive opportunistic pathogen causing serious epidemics and sporadic listeriosis in humans (2, 3). *Listeria innocua* is a nonpathogenic species that is closely related to *L. monocytogenes* (4). Although rare, atypical hemolytic *L. innocua* can actively cross the intestinal epithelium and spread systemically within the host (5). In addition to its ecological and clinical relevance (5–8), *L. innocua* genomes provide important insight into the evolution of pathogenicity of *L. monocytogenes* (4). Here, we report the draft genome sequence of *L. innocua* strain MEZLIS29, which was isolated from fecal material from a cow (*Bos taurus*) in Flagstaff, Eastern Cape, South Africa, in May 2018. The sample was analyzed as reported previously (9). The sample was collected in 10 mL of 0.1% buffered peptone water and incubated for 24 h. Following enrichment in *Listeria* broth (Oxoid, England), the sample was streaked onto *Listeria* selective agar (Oxoid) and incubated at 37°C for 18 h. A slant of the bacterial culture was shipped to the College of Veterinary Medicine, North Carolina State University, for further analysis. Colony PCR for the hemolysin (*hly*) gene was performed (10). An aliquot of overnight culture in brain heart infusion (BHI) broth was submitted to the Clinical Sciences Department at North Carolina State University for matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) using the VITEK MS identification system (bioMérieux, Marcy L’Etoile, France) and the associated software Knowledge Base v3.2 clinical US for further confirmation, as reported previously (9). The isolate was cultured on sheep blood agar for 18 to 24 h at 37°C in the presence of 5% CO₂, and a single CFU was cultured in tryptic soy broth (TSB) to an inoculum density of a 0.5 McFarland turbidity standard. An aliquot of the culture in TSB was diluted with 0.85% sterile saline solution to the desired inoculum density of 1 × 10⁶ CFU/mL using a Thermo Fisher Scientific Sensititre nephelometer, and the chilled culture tube was submitted to the University of Minnesota Genomics Center for DNA extraction and whole-genome sequencing (WGS). DNA isolation was performed using a Qiagen DNeasy blood and tissue kit (Lucigen, WI, USA) according to the manufacturer’s protocol. The DNA sample was quantified using a fluorimetric PicoGreen assay using a Synergy 2 plate reader (BioTech). The purity of the DNA sample was also assessed using a Nanodrop 8000 instrument (Thermo Fisher).

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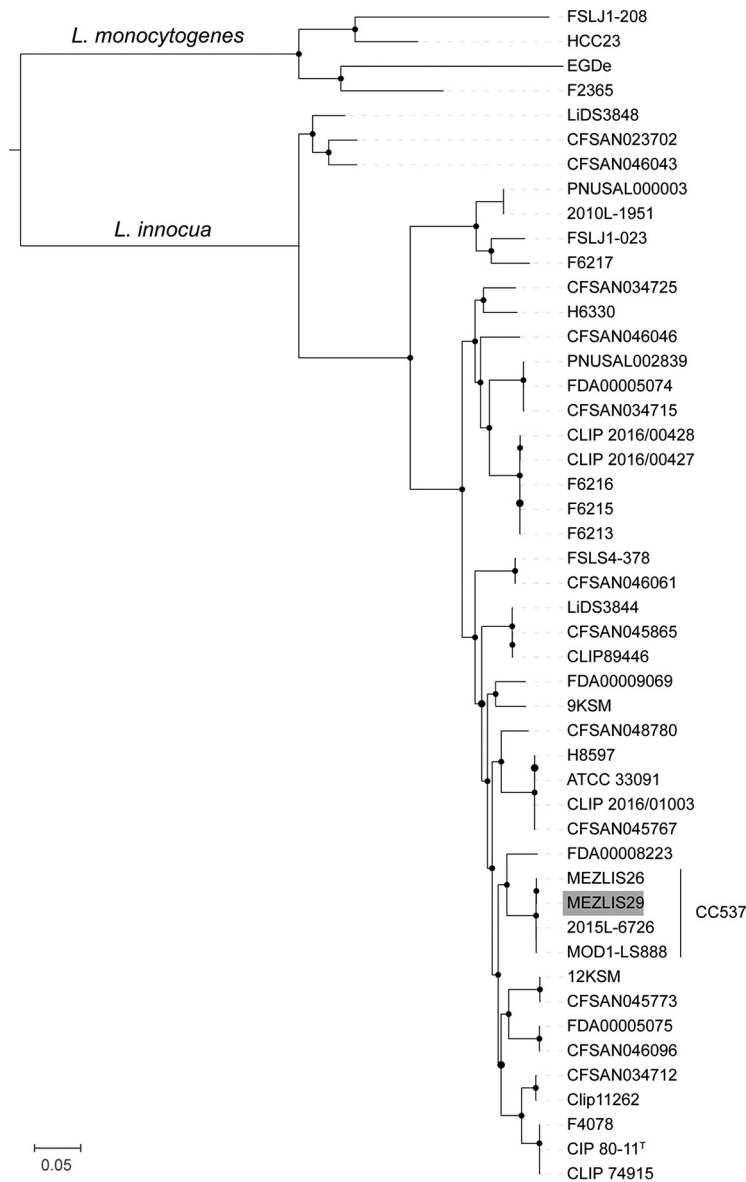


FIG 1 Positioning of isolate MEZLIS29 (highlighted in gray) within the *L. innocua* phylogeny. Representative genomes of *L. monocytogenes* were used as the outgroup. The maximum likelihood phylogeny was inferred from a core genome alignment of 632,995 nucleotide positions. Black circles represent bootstrap branch support values higher than 90%, based on 1,000 replicates.

Sequencing libraries were prepared using a Nextera XT library preparation kit (Illumina Inc., CA, USA). Sequencing was performed using the MiSeq platform (Illumina Inc., CA, USA) using the v2 reagent kit, which yielded 250-bp paired-end (PE) reads. Sequence analyses were performed at the National Reference Center and World Health Organization Collaborating Center for *Listeria* (Institut Pasteur, Paris, France). Raw PE reads (251,655 reads [54× coverage]) were trimmed for adaptors and low-quality bases with AlienTrimmer (11). Cleaned reads (247,781 reads) were assembled using SPAdes v.3.12.0 (12) using the automatic k-mer, --only-assembler, and --careful options. Contigs smaller than 300 nucleotides were discarded. Assembly quality was assessed using QUASt v.2.2 (13), yielding a total of 73 contigs and 2,805,865 bp, with a G+C content of 37.5% and an N_{50} value of 123,459 bp. The final sequence coverage of the assembled genome was 45×. The genome was annotated using the Prokaryotic Genome Annotation Pipeline (PGAP) v.5.1 (14) by the National Center for Biotechnology Information (NCBI), which identified 2,783 coding DNA sequences, 58 tRNAs, 4 noncoding RNAs, and 8 rRNA genes. A maximum likelihood phylogeny was inferred from the

core genome alignment of 43 *L. innocua* and 4 *L. monocytogenes* public genomes (5) using Parsnp, implemented in the Harvest suite v.1.1.2 (15), and was visualized with iTOL v.4.2 (16). Isolate MEZLIS29 clustered within clonal complex 537 (CC537) (nonhemolytic *L. innocua*) (Fig. 1) together with isolate MEZLIS26 (GenBank accession number [AADHQU000000000](https://www.ncbi.nlm.nih.gov/nuccore/AADHQU000000000)), which was previously isolated from a goat in the same region (9). The average nucleotide identity by BLAST of MEZLIS29 and MEZLIS26 was 100%, confirming the circulation of the same strain in different animal species.

Data availability. The Whole Genome Shotgun project has been deposited in DDBJ/ENA/GenBank under BioProject accession number [PRJNA716986](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA716986) (BioSample accession number [SAMN18492169](https://www.ncbi.nlm.nih.gov/biosample/SAMN18492169)) and GenBank accession number [JAGISK000000000](https://www.ncbi.nlm.nih.gov/nuccore/JAGISK000000000). The version described in this paper is the first version, [JAGISK000000000.1](https://www.ncbi.nlm.nih.gov/nuccore/JAGISK000000000.1). The sequences have been submitted to the Sequence Read Archive (SRA) under the accession numbers [SRX10515072](https://www.ncbi.nlm.nih.gov/sra/SRX10515072) and [SRR14145944](https://www.ncbi.nlm.nih.gov/sra/SRR14145944). All isolates used in this study are also publicly available at <https://bigsgdb.pasteur.fr/listeria>.

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REFERENCES

- Carlin CR, Liao J, Weller DL, Guo X, Orsi R, Wiedmann M. 2021. *Listeria costarcticae* sp. nov., *Listeria farberii* sp. nov., *Listeria immobilis* sp. nov., *Listeria portnoyi* sp. nov., and *Listeria rustica* sp. nov. isolated from agricultural water and natural environments. *Int J Syst Evol Microbiol* 71:e004795. <https://doi.org/10.1099/ijsem.0.004795>.
- Hoffman S, Maculloch B, Batz M. 2015. Economic burden of major foodborne illnesses acquired in the United States. U.S. Department of Agriculture, Economic Research Service, Washington, DC.
- Buchrieser C, Rusniok C, Kunst F, Cossart P, Glaser P, The *Listeria* Consortium. 2003. Comparison of the genome sequences of *Listeria monocytogenes* and *Listeria innocua*: clues for evolution and pathogenicity. *FEMS Immunol Med Microbiol* 35:207–213. [https://doi.org/10.1016/S0928-8244\(02\)00448-0](https://doi.org/10.1016/S0928-8244(02)00448-0).
- Glaser P, Frangeul L, Buchrieser C, Rusniok C, Amend A, Baquero F, Berche P, Bloecker H, Brandt P, Chakraborty T, Charbit A, Chetouani F, Couvé E, de Daruvar A, Dehoux P, Domann E, Domínguez-Bernal G, Duchaud E, Durant L, Dussurget O, Entian KD, Fsihi H, García-del Portillo F, Garrido P, Gautier L,

- Goebel W, Gómez-López N, Hain T, Hauf J, Jackson D, Jones LM, Kaerst U, Kreft J, Kuhn M, Kunst F, Kurapkat G, Madueno E, Maitournam A, Vicente JM, Ng E, Nedjari H, Nordsiek G, Novella S, de Pablos B, Pérez-Díaz JC, Purcell R, Rimmel B, Rose M, Schlueter T, Simoes N, Tierrez A, Vázquez-Boland JA, Voss H, Wehland J, Cossart P. 2001. Comparative genomics of *Listeria* species. *Science* 294:849–852. <https://doi.org/10.1126/science.1063447>.
5. Moura A, Disson O, Lavina M, Thouvenot P, Huang L, Leclercq A, Fredriksson-Ahomaa M, Eshwar AK, Stephan R, Lecuit M. 2019. Atypical hemolytic *Listeria innocua* isolates are virulent, albeit less than *Listeria monocytogenes*. *Infect Immun* 87:e00758-18. <https://doi.org/10.1128/IAI.00758-18>.
 6. Favaro M, Sarmati L, Sancesario G, Fontana C. 2014. First case of *Listeria innocua* meningitis in a patient on steroids and etanercept. *JMM Case Rep* <https://doi.org/10.1099/jmmcr.0.003103>.
 7. Perrin M, Bemer M, Delamare C. 2003. Fatal case of *Listeria innocua* bacteremia. *J Clin Microbiol* 41:5308–5309. <https://doi.org/10.1128/jcm.41.11.5308-5309.2003>.
 8. Rocha P, Dalmaso A, Grattarola C, Casalone C, Del Piero F, Bottero MT, Capucchio MT. 2013. Atypical cerebral listeriosis associated with *Listeria innocua* in a beef bull. *Res Vet Sci* 94:111–114. <https://doi.org/10.1016/j.rvsc.2012.07.017>.
 9. El Zowalaty ME, Hickman RA, Moura A, Lecuit M, Zishiri OT, Noyes N, Järhult JD. 2019. Genome sequence of *Listeria innocua* strain MEZLIS26, isolated from a goat in South Africa. *Microbiol Resour Announc* 8:e00991-19. <https://doi.org/10.1128/MRA.00991-19>.
 10. Kumar A, Grover S, Batish VK. 2015. Exploring specific primers targeted against different genes for a multiplex PCR for detection of *Listeria monocytogenes*. *3 Biotech* 5:261–269. <https://doi.org/10.1007/s13205-014-0225-x>.
 11. Criscuolo A, Brisse S. 2013. AlienTrimmer: a tool to quickly and accurately trim off multiple short contaminant sequences from high-throughput sequencing reads. *Genomics* 102:500–506. <https://doi.org/10.1016/j.ygeno.2013.07.011>.
 12. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
 13. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
 14. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.
 15. Treangen TJ, Ondov BD, Koren S, Phillippy AM. 2014. The Harvest suite for rapid core-genome alignment and visualization of thousands of intraspecific microbial genomes. *Genome Biol* 15:524. <https://doi.org/10.1186/s13059-014-0524-x>.
 16. Letunic I, Bork P. 2016. Interactive Tree of Life (iTOL) v3: an online tool for the display and annotation of phylogenetic and other trees. *Nucleic Acids Res* 44:W242–W245. <https://doi.org/10.1093/nar/gkw290>.