

## AMERICAN SOCIETY FOR MICROBIOLOGY

## Complete Genome Sequence of *Listeria monocytogenes* Strain MR310, Isolated from a Pastured-Flock Poultry Farm System

Michael J. Rothrock, Jr.,<sup>a</sup> Peixin Fan,<sup>b,c</sup> Kwangcheol C. Jeong,<sup>b,c</sup> Sun Ae Kim,<sup>d</sup> Steven C. Ricke,<sup>e</sup> Si Hong Park<sup>f</sup>

<sup>a</sup>Egg Safety and Quality Research Unit, United States Department and Agriculture, Agricultural Research

Service, U.S. National Poultry Research Center, Athens, Georgia, USA

<sup>b</sup>Emerging Pathogens Institute, University of Florida, Gainesville, Florida, USA

<sup>c</sup>Department of Animal Sciences, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida, USA

<sup>d</sup>Department of Food Science and Engineering, Ewha Womans University, Seoul, South Korea

eDepartment of Food Science and Centers for Food Safety, University of Arkansas, Fayetteville, Arkansas, USA

<sup>f</sup>Department of Food Science and Technology, Oregon State University, Corvallis, Oregon, USA

**ABSTRACT** Investigation of *Listeria monocytogenes* transmission from environmental sources associated with pasture-raised chickens to poultry products is needed to determine ways to prevent potential foodborne illness. Here, we report the complete genome sequence of *Listeria monocytogenes* MR310, one of the isolates from a pastured-flock poultry management system.

Due to the increased demands for natural and organic poultry products by consumers, high-quality and safe poultry products without antibiotic treatments are now more commonly found in grocery markets. Foodborne pathogens from the environment, such as *Listeria monocytogenes*, may be associated with pasture-raised poultry and thus are potential threats (1–3). The goal of the study was to initiate an investigation of the presence, abundance, and diversity of *Listeria monocytogenes* in pastured-flock poultry management systems. In the overall study, *Listeria* organisms were isolated from live production (feces and soil), processing (ceca and whole-carcass rinses), and final products (whole-carcass rinses) to determine which *Listeria* species were present, the sites along the farm-to-fork continuum where they were present, and how the diversity of *Listeria* species varies based on the different farms and management stages investigated in the study. Achieving a better understanding of the ecology of *Listeria* species through their genotypes may elucidate potential intervention targets for future studies.

Pure genomic DNA from overnight-grown culture was extracted using a Qiagen DNeasy blood and tissue kit according to the manufacturer's protocol. DNA was subsequently diluted to 0.2 ng/ $\mu$ l for construction of tagmented DNA with a Nextera XT DNA library prep kit (Illumina). Libraries were loaded on a cartridge in the MiSeq reagent kit v2 and sequenced with an Illumina MiSeq instrument. In total, 1,003,393 reads of 250 bp were obtained from the MiSeq run. The reads were trimmed using the sickle tool to remove low-quality nucleotides that may have occurred in the 5' or 3' ends (https://github.com/najoshi/sickle). Reads with less than 50 nucleotides and Phred quality scores lower than 30 were discarded. The trimmed reads were assembled *de novo* using SPAdes 3.5.0 (4), with k-mer sizes set at 21, 33, 55, 77, 99, and 127. The resulting assembly showed 86× coverage in 20 contigs. The estimated genome size was 2.88 Mb, with a G+C content of 37.93%. There were 2,842 coding sequences (CDSs) and 52 tRNA and 7 rRNA sequences predicted by the Pathosystems Resource Integration Center (PATRIC) database (5). The multilocus sequence type (MLST) of the MR310 strain was ST-389 according to analysis by the Center for Genomic Epidemiology (CGE)

Received 7 February 2018 Accepted 13 February 2018 Published 8 March 2018

**Citation** Rothrock MJ, Jr, Fan P, Jeong KC, Kim SA, Ricke SC, Park SH. 2018. Complete genome sequence of *Listeria monocytogenes* strain MR310, isolated from a pastured-flock poultry farm system. Genome Announc 6:e00171-18. https://doi.org/10.1128/genomeA.00171-18. This is a work of the U.S. Government and is not subject to copyright protection in the

not subject to copyright protection in the United States. Foreign copyrights may apply. Address correspondence to Steven C. Ricke, sricke@uark.edu, or Si Hong Park, sihong.park@oregonstate.edu. (6). A total of 33 virulence factors were predicted by performing BLASTn analysis of the MR310 genome against the Virulence Factors Database (VFDB). The predicted virulence profiles included genes for immune modulation, intracellular growth, toxins, invasion, bile resistance, immune evasion, stress resistance, adherence, and actin-based motility. The predicted proteins were analyzed by BLASTp against the Comprehensive Antibiotic Resistance Database (CARD) (7) to predict potential antibiotic resistance. Two antimicrobial resistance genes were identified in the MR310 genome, an antibiotic target-modifying enzyme (*mprF*) that changes cell wall charge, and an antibiotic target-modifying enzyme (*fosX*) that is a determinant of fosfomycine resistance. The identification of antibiotic resistance gene prevalence in the genome will contribute to a better understanding of horizontal gene transmission from environmental sources to poultry.

**Accession number(s).** The sequence and annotation information of the *L. mono-cytogenes* strain MR310 genome has been deposited at DDBJ/EMBL/GenBank under the accession no. PPHC00000000.

## **ACKNOWLEDGMENTS**

We acknowledge Laura Lee Rutherford and Cheryl Pearson Gresham for their assistance in sample acquisition and processing as well as Tori McIntosh and Aude Locatelli for the molecular analyses of *Listeria* isolates.

This work was funded under USDA ARS CRIS 6040-32000-011-00-D, entitled "Reduction of Invasive Salmonella enterica in Poultry through Genomics, Phenomics, and Field Investigations of Small Multi-Species Farm Environments."

## REFERENCE

- Locatelli A, Lewis MA, Rothrock MJ. Jr. 2017. The distribution of *Listeria* in pasture-raised broiler farm soils are potentially related to university of Vermont medium enrichment bias toward *Listeria innocua* over *Listeria monocytogenes*. Front Vet Sci 4:227. https://doi.org/10.3389/fvets.2017 .00227.
- Milillo SR, Stout JC, Hanning IB, Clement A, Fortes ED, den Bakker HC, Wiedemann M, Ricke SC. 2012. *Listeria monocytogenes* and hemolytic *Listeria innocua* in poultry. Poult Sci 91:2158–2163. https://doi.org/10 .3382/ps.2012-02292.
- Rothrock MJ, Davis ML, Locatelli A, Bodie A, McIntosh TG, Donaldson JR, Ricke SC. 2017. *Listeria* occurrence in poultry flocks: detection and potential implications. Front Vet Sci 4:125. https://doi.org/10.3389/fvets.2017 .00125.
- 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.
- 5. Wattam AR, Davis JJ, Assaf R, Boisvert S, Brettin T, Bun C, Conrad N,

Dietrich EM, Disz T, Gabbard JL, Gerdes S, Henry CS, Kenyon RW, Machi D, Mao C, Nordberg EK, Olsen GJ, Murphy-Olson DE, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Vonstein V, Warren A, Xia F, Yoo H, Stevens RL. 2017. Improvements to PATRIC, the all-bacterial bioinformatics database and analysis resource center. Nucleic Acids Res 45: D535–D542. https://doi.org/10.1093/nar/gkw1017.

- Bartual SG, Seifert H, Hippler C, Luzon MAD, Wisplinghoff H, Rodriguez-Valera F. 2005. Development of a multilocus sequence typing scheme for characterization of clinical isolates of *Acinetobacter baumannii*. J Clin Microbiol 43:4382–4390. https://doi.org/10.1128/JCM.43.9.4382-4390 .2005.
- Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. 2017. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res 45:D566–D573. https://doi.org/10.1093/ nar/gkw1004.