AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

PROKARYOTES

Whole-Genome Shotgun Sequencing of Three *Listeria monocytogenes* Strains Isolated from a Ready-to-Eat Salad-Producing Facility in Switzerland

Matthias Ziegler,[a] Hyein Jang,[b] Gopal Gopinath,[b] Jule Anna Horlbog,[a] Roger Stephan,[a] Claudia Guldimann[a]

[a]Institute for Food Safety and Hygiene, Vetsuisse Faculty, University of Zürich, Zürich, Switzerland
[b]Center of Food Safety and Applied Nutrition, U.S. Food and Drug Administration, Laurel, Maryland, USA

ABSTRACT Ready-to-eat (RTE) raw foods harbor the risk of transmitting *Listeria monocytogenes* from the environment to the consumer. We isolated three strains from a facility producing RTE salad. These strains were used to perform challenge tests on different RTE salad products. Here, we present the shotgun genome sequences of all three of these strains.

Genomic DNA from *Listeria monocytogenes* strains N16-0716, N16-0855, and N16-1125 was extracted from a brain heart infusion culture using a DNA blood and tissue kit (Qiagen) and subjected to whole-genome sequencing using the MiSeq platform (Illumina) and a Nextera XT library kit utilizing either 500 or 600 cycles of paired-end reads (Illumina). The paired-end libraries were generated and sequenced in conjunction with the Nextera XT DNA sample preparation guide on the Illumina MiSeq instrument (1). The reads were *de novo* assembled with SPAdes version 3.11 (2) into genomes of 2,968,449 bp with 37.9% GC content (N16-0716), 3,138,349 bp with 38.8% GC content (N16-0855), and 3,124,080 bp with 38.6% GC content (N16-1125). The genomes were annotated using the Rapid Annotations using Subsystems Technology (RAST) annotation server (3), and 2,931 (N16-0716), 3,072 (N16-0855), and 3,078 (N16-1125) coding sequences were identified.

Multilocus sequence type (MLST) analysis using the BIGSdb database (http://bigsdb.pasteur.fr/listeria) confirmed our earlier PCR-based analysis, which found that N16-0716 was sequence type 517 (ST517) clonal complex 517 (CC517), N16-0855 was ST6 CC6, and N16-1125 was ST91 CC14. An analysis of known virulence genes in comparison to the laboratory strain 10403S revealed that all genes associated with the LIPI-1 island were present in all three strains. In all three strains, full-length *plcB* was present with several amino acid (aa) substitutions between the strains. The alternative start codon GTG was used in *plcB* of all three strains (as well as in 10403S). All other full-length genes on LIPI-1 were present, and none of the known *prfA** mutations were detected. Two different alleles of *hly* were present between the strains, separated by four aa substitutions. In *plcA*, *mpl*, and *actA*, several aa substitutions were detected between the strains, and *ilsA* coding for listeriolysin S on LIPI-3 was found only in N16-0855. Full-length InlA was present in all sequences, with several aa substitutions between the strains.

Screening of the genomes against the ARG-ANNOT (4) and MEGARes (5) databases of antimicrobial resistance genes using the method described by Carroll et al. (6) and implemented in BTyper version 2.2.0 (7) revealed the presence of the multidrug efflux pumps *norB, msrA*, and *mepA* in all three strains.

PHASTER (8) identified two intact phages in N16-0716, two intact and one incomplete phage in N16-0855, and four intact phages in N16-1125.

Received 14 May 2018 **Accepted** 14 May 2018 **Published** 28 June 2018

Citation Ziegler M, Jang H, Gopinath G, Horlbog JA, Stephan R, Guldimann C. 2018. Whole-genome shotgun sequencing of three *Listeria monocytogenes* strains isolated from a ready-to-eat salad-producing facility in Switzerland. Genome Announc 6:e00547-18. https://doi.org/10.1128/genomeA.00547-18.

Address correspondence to Claudia Guldimann, claudia.guldimann@uzh.ch.

Accession number(s). This whole-genome shotgun project has been deposited in GenBank under the accession no. QELV00000000 (N16-0716), QELU00000000 (N16-0855), and QELT00000000 (N16-1125).

ACKNOWLEDGMENT

We thank Denise Althaus for initial serotyping of the three strains used in this project.

REFERENCES

1. Allard MW, Strain E, Melka D, Bunning K, Musser SM, Brown EW, Timme R. 2016. Practical value of food pathogen traceability through building a whole-genome sequencing network and database. J Clin Microbiol 54:1975–1983. https://doi.org/10.1128/JCM.00081-16.
2. 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.
3. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365.
4. Gupta SK, Padmanabhan BR, Diene SM, Lopez-Rojas R, Kempf M, Landraud L, Rolain J-M. 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. Antimicrob Agents Chemother 58:212–220. https://doi.org/10.1128/AAC.01310-13.
5. Lakin SM, Dean C, Noyes NR, Dettenwanger A, Ross AS, Doster E, Rovira P, Abdo Z, Jones KL, Ruiz J, Belk KE, Morley PS, Boucher C. 2017. MEGARes: an antimicrobial resistance database for high throughput sequencing. Nucleic Acids Res 45:D574–D580. https://doi.org/10.1093/nar/gkw1009.
6. Carroll LM, Wiedmann M, Bakker den H, Siler J, Warchocki S, Kent D, Lyalina S, Davis M, Sischo W, Besser T, Warnick LD, Pereira RV. 2017. Whole-genome sequencing of drug-resistant *Salmonella enterica* isolates from dairy cattle and humans in New York and Washington states reveals source and geographic associations. Appl Environ Microbiol 83:e00140-17. https://doi.org/10.1128/AEM.00140-17.
7. Carroll LM, Kovac J, Miller RA, Wiedmann M. 2017. Rapid, high-throughput identification of anthrax-causing and emetic *Bacillus cereus* group genome assemblies via BTyper, a computational tool for virulence-based classification of *Bacillus cereus* group isolates by using nucleotide sequencing data. Appl Environ Microbiol 83:e01096-17. https://doi.org/10.1128/AEM.01096-17.
8. Arndt D, Grant JR, Marcu A, Sajed T, Pon A, Liang Y, Wishart DS. 2016. PHASTER: a better, faster version of the PHAST phage search tool. Nucleic Acids Res 44:W16–W21. https://doi.org/10.1093/nar/gkw387.