Genetic Characterization of a *Listeria monocytogenes* Serotype IVb Variant 1 Strain Isolated from Vegetal Matrix in Italy

**ABSTRACT** We report the chromosome and plasmid sequences of a strain of *Listeria monocytogenes* IVb variant 1, a recently emerging serotype, isolated in Italy from ready-to-eat vegetables.

*Listeria monocytogenes* IVb variant 1 (lineage I), characterized by a 6.3-kb cassette typical of lineage II strains, is often associated with recurring outbreaks and sporadic cases (1). We report the whole-genome sequence of *L. monocytogenes* IVb variant 1 strain 2017-TE-6913-1-1, which was isolated from an official radicchio sample from a catering company in northern Italy in February 2017.

The strain was isolated according to the ISO 11290-1:2005 procedure (2), which is based on two enrichment steps on Fraser broth and selection on Ottaviani-Agosti agar medium; a single typical colony was then plated onto blood agar and incubated at 37°C for 24 h. DNA was extracted using a Maxwell 16 tissue DNA purification kit (Promega Italia Srl, Milan, Italy) according to the manufacturer’s protocol. Species confirmation (3) and serogroup determination (4) returned the IVb variant 1 profile (5).

One nanogram of genomic DNA was used for library preparation with the Nextera XT DNA library preparation kit (Illumina, San Diego, CA) according to the manufacturer’s protocol. Sequencing was performed on the NextSeq 500 platform (Illumina) with the NextSeq 500/550 midoutput reagent cartridge v2 (300 cycles), which generated 2,666,952 read pairs (150 bp), corresponding to a theoretical coverage of 275×.

Quality control was performed with FastQC v0.52 (6), and trimming was conducted with a standalone command-line version of the FastQ positional and quality trimming tool available on the Orione platform (7), imposing a minimum quality score of 20 and a minimum length after trimming of 50 bp. The genome was *de novo* assembled using the SPAdes genome assembler v3.13 (8) with default parameters for the Illumina 2 × 150-bp sequencing strategy. A single pseudomolecule was generated by ordering scaffolds with abacas.pl v1.3 software (9), using the *L. monocytogenes* serogroup IVb strain F2365 genome sequence (GenBank accession number AE017262) as a guide.

Gaps were filled through several iteration cycles of GapFiller v1.10 (10) and Pilon v1.23 (11) software, which finally returned a complete genome sequence.Circularity was confirmed by treating the tail-to-head sequence as a gap, which was submitted to GapFiller v1.10 for filling. The final assembly, a single molecule of 2,953,624 bp (GC content, 37.9%), was reordered starting from the region potentially containing the *dnaA* gene. Among the contigs not included in the final sequence of the chromosome, only one (52,825 bp [GC content, 35.0%]) contained an origin of replication, as identified by PlasmidFinder v2.0.1 (12); it showed a significant match (99.98% similarity and 95%...
horizontal coverage) with an L. monocytogenes plasmid sequence isolated in the United States in 2015 (GenBank accession number CP044433), with similarity against the nonredundant database determined using BLASTn (13). The sequence was refined and annotated using Pilon v1.23 and Prokka v1.12 (14), respectively. Default parameters were used for all software unless otherwise specified. The remaining contigs, which returned matches against other Listeria plasmids but did not show any origin of replication, were discarded.

Both the chromosome and plasmid ascribable sequences were submitted to GenBank; for the former, PGAP annotation was requested (15) and returned 2,956 genes (2,858 protein-coding genes, 5 rRNA operons, 57 tRNAs, and 22 pseudogenes). Among them, the presence of the region containing the characteristic L. monocytogenes serogroup Ibv variant 1 genes lmo0737, ORF2110, and ORF2819 (16), corresponding to HNT73_RS01375, HNT73_07170, and HNT73_07960 loci, respectively, was confirmed.

The plasmid sequence annotation returned 58 coding sequences, including genes conferring resistance to heavy metals (mainly copper and cadmium). In particular, the cadmium resistance genes were located between an IS6 family transposase and an ISNCY transposase protein, thus suggesting their potential mobility.

**Data availability.** The whole-genome assembly of L. monocytogenes strain 2017-TE-6913-1 was deposited in GenBank under the accession number CP053357 and of the plasmid p2017-TE-6913-1 under the accession number MT459813. Raw reads have been deposited in the NCBI Sequence Read Archive (SRA) under the accession number SRR11922747. All of the records are linked to the BioProject number PRJNA261392.

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**REFERENCES**

1. Burll LS, Grim CJ, Mammel MK, Datta AR. 2017. A comprehensive evaluation of the genetic relatedness of Listeria monocytogenes serotype 4b variant strains. Front Public Health 5:241. https://doi.org/10.3389/fpubh.2017.00241.
2. Anonymous. 2005. ISO 11290-1:2005. Microbiology of food and animal feeding stuffs—horizontal method for detection and enumeration of Listeria monocytogenes. Part 1: detection method. International Organization for Standardization, Geneva, Switzerland.
3. Doumith M, Buchrieser C, Glaser P, Jacquet C, Martin P. 2004. Differentiation of the major Listeria monocytogenes serovars by multiplex PCR. J Clin Microbiol 42:3819–3822. https://doi.org/10.1128/CM.42 .3819-3822.2004.
4. Kérouanton A, Marault M, Petit L, Groult J, Dao TT, Brisabois A. 2010. Evaluation of a multiplex PCR assay as an alternative method for Listeria monocytogenes serotyping. J Microbiol Methods 80:134–137. https://doi.org/10.1016/j.mimet.2009.11.008.
5. Leclercq A, Chenal-Francisque V, Dieye H, Cantinelli T, Drali R, Brisse S, Lecuit M. 2011. Characterization of the novel Listeria monocytogenes PCR serogrouping profile IbV-v1. Int J Food Microbiol 147:74–77. https://doi.org/10.1016/j.ijfoodmicro.2011.03.010.
6. Andrews S. 2010. FastQC: a quality control tool for high throughput sequence data. http://www.bioinformatics.babraham.ac.uk/projects/fastqc.
7. Cucucu G, Orsini M, Pinna A, Sbardellati A, Soranzo N, Travaglione A, Uva P, Zanietti G, Fottia G. 2014. Orione, a Web-based framework for NGS analysis in microbiology. Bioinformatics 30:1928–1929. https://doi.org/10.1093/bioinformatics/btu135.
8. 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.
9. Assefa S, Keane TM, Otto TD, Newbold C, Berriman M. 2009. ABACAS: algorithm-based automatic configuration of assembled sequences. Bioinformatics 25:1968–1969. https://doi.org/10.1093/bioinformatics/btp347.
10. Nadalin F, Vezzi F, Policriti A. 2012. GapFiller: a de novo assembly approach to fill the gap within paired reads. BMC Bioinformatics 13(Suppl 14):S8. https://doi.org/10.1186/1471-2105-13-S14:58.
11. Walker BJ, Abeel T, Shea T, Priest M, Abouelliel S, Sakkthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS One 9:e112963. https://doi.org/10.1371/journal .pone.0112963.
12. Carattoli A, Hasman H. 2020. PlasmidFinder and in silico pMLST: identification and typing of plasmid replicons in whole-genome sequencing (WGS). Methods Mol Biol 2075:285–294. https://doi.org/10.1007/978-1 -4939-9877-7_20.
13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
14. Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics 30:2068–2069. https://doi.org/10.1093/bioinformatics/btu153.
15. Tatusova T, DiCuccio M, Badredin A, Chetverin V, Nawrocki EP, Zaslavsky L, Lomsadsie 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.
16. Lee S, Ward TJ, Graves LM, Wolf LA, Spery K, Siletzky RM, Kathariou S. 2012. Atypical Listeria monocytogenes serotype 4b strains harboring a lineage II-specific gene cassette. Appl Environ Microbiol 78:660–667. https://doi.org/10.1128/AEM.06378-11.