Complete Genomic Sequence of the Clinical Isolate *Legionella pneumophila* Serogroup 1 Strain 80-045 from Japan

Masatomo Morita,[a] Nanno Harada,[b] Yuna Shinohara,[b] Miyo Murai,[b] Naoko Ishii,[a] (ORCID) Junko Amemura-Maekawa,[a] Yukihiro Akeda[a]

aDepartment of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan
bDepartment of Health Sciences, Saitama Prefectural University, Saitama, Japan

ABSTRACT   We report the complete genomic sequence of *Legionella pneumophila* serogroup 1 strain 80-045, isolated from autopsy lung tissue of the first patient diagnosed with Legionnaires' disease in Japan.

*L*egionella pneumophila inhabits natural and human-made water environments as a symbiotic organism in free-living amebae (1). *L. pneumophila* was identified as the human opportunistic pathogen in an outbreak of fatal pneumonia at an American Legion convention in Philadelphia in 1976 (2). The first genomic sequence of Philadelphia 1, which was the isolate responsible for the outbreak, was reported as belonging to *L. pneumophila* (3). The first case of Legionnaires' disease in Japan was reported as a fatal pneumonia case in 1980. The patient was a 64-year-old male with diabetes mellitus. The lung aspirates and lung tissue were inoculated onto buffered charcoal-yeast-extract agar plates; the colonies obtained were grayish-white and smooth, characteristic of *Legionella* spp., and were identified as *L. pneumophila* serogroup 1 using the direct fluorescent antibody method (4). Here, we report the complete genomic sequence of *L. pneumophila* strain 80-045.

The *L. pneumophila* isolate was grown on buffered charcoal-yeast-extract agar at 35℃ for 90 h. Genomic DNA was extracted using a DNeasy blood and tissue kit (Qiagen, Hilden, Germany). Whole-genome sequencing was performed using the MiSeq (Illumina, San Diego, CA) and MinION (Oxford Nanopore Technologies, Oxford, UK) platforms. A genomic library was prepared using a Nextera XT DNA library prep kit and sequenced using the MiSeq platform. The raw sequence reads were filtered to remove low-quality sequences and trimmed to remove adaptor sequences using MiSeq Control Software 2.6.2.1. A genomic library was also prepared using a rapid sequencing kit (SQK-RAD004), and the library was sequenced on a MinION sequencer using an R9.4.1 flow cell (5). The raw data were base called using Guppy 5.0.11, and adaptors were removed before assembly using Porechop 0.2.4 (https://github.com/rrwick/Porechop) (6, 7). As a result, 952,592 short reads (total read length, 233,150,299 bp) and 13,657 long reads (total read length, 78,646,486 bp; maximum read length, 62,146 bp) were used for genome sequence assembly. A hybrid genome assembly was performed using Unicycler v0.4.8 in conservative mode (8). Annotation was performed using the DDBJ Fast Annotation and Submission Tool (9). Default parameters were used for all software programs unless otherwise specified.

The *L. pneumophila* strain 80-045 genome consisted of a 3,438,257-bp circular chromosome with 38.2% G+C content; it had 2,994 predicted coding sequences, 9 rRNAs, 43 tRNAs, 2 CRISPR arrays, and genes for the Dot/Icm type-IVB secretion system. Based on phylogenetic analysis of the 16S rRNA (10) and *mip* genes (11), strain 80-045 belonged to *Legionella pneumophila*. Sequence-based typing indicated that the strain belonged to sequence type 118 (ST118), which has been observed only in Japan (12). Therefore, the genomic information uncovered in this study will be a useful reference for *L. pneumophila* serogroup 1 in Japan.

Data availability. The genomic sequence of the *L. pneumophila* isolate, 80-045, was deposited at DDBJ/ENA/GenBank under accession number AP024961. Additionally, the

short and long reads were deposited at the DDBJ/ENA/GenBank Sequence Read Archive under accession numbers DRR310440 and DRR310441, respectively.

REFERENCES

1. Berjeaud JM, Chevalier S, Schlusselhuber M, Portier E, Loiseau C, Aucher W, Lesouhaitier O, Verdon J. 2016. *Legionella pneumophila*: the paradox of a highly sensitive opportunistic waterborne pathogen able to persist in the environment. Front Microbiol 7:486. https://doi.org/10.3389/fmicb.2016.00486.
2. Fraser DW, Tsai TR, Orenstein W, Parkin WE, Beecham HJ, Sharrar RG, Harris J, Mallison GF, Martin SM, McDade JE, Shepard CC, Brachman PS. 1977. Legionnaires' disease: description of an epidemic of pneumonia. N Engl J Med 297:1189–1197. https://doi.org/10.1056/NEJM197712012972201.
3. Chien M, Morozova I, Shi S, Sheng H, Chen J, Gomez SM, Asamani G, Hill K, Nuara J, Feder M, Rineer J, Greenberg JJ, Steshenko V, Park SH, Zhao B, Teplitskaya E, Edwards JR, Pampou S, Georghiou A, Chou IC, Iannuccilli W, Ulz ME, Kim DH, Geringer-Sameth A, Goldsberry C, Morozov P, Fischer SG, Segal G, Qu X, Rzhetsky A, Zhang P, Cayanis E, De Jong PJ, Ju J, Kalachikov S, Shuman HA, Russo JJ. 2004. The genomic sequence of the accidental pathogen *Legionella pneumophila*. Science 305:1966–1968. https://doi.org/10.1126/science.1099776.
4. Saito A, Shimoda T, Nagasawa M, Tanaka H, Ito N, Shigeno Y, Yamaguchi K, Hirota M, Nakatomi M, Hara K. 1981. The first case of Legionnaires' disease in Japan. Kansenshogaku Zasshi 55:124–128. https://doi.org/10.11150/kansenshogakuzasshi1970.55.124.
5. Clarke J, Wu HC, Jayasinghe L, Patel A, Reid S, Bayley H. 2009. Continuous base identification for single-molecule Nanopore DNA sequencing. Nat Nanotechnol 4:265–270. https://doi.org/10.1038/nnano.2009.12.
6. Wick RR, Judd LM, Holt KE. 2019. Performance of neural network basecalling tools for Oxford Nanopore sequencing. Genome Biol 20:129. https://doi.org/10.1186/s13059-019-1727-y.
7. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Completing bacterial genome assemblies with multiplex MinION sequencing. Microb Genom 3:e000132. https://doi.org/10.1099/mgen.0.000132.
8. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
9. Tanizawa Y, Fujisawa T, Nakamura Y. 2018. DFAST: a flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics 34:1037–1039. https://doi.org/10.1093/bioinformatics/btx713.
10. Wilson DA, Reischl U, Hall GS, Procop GW. 2007. Use of partial 16S rRNA gene sequencing for identification of *Legionella pneumophila* and non-*pneumophila Legionella* spp. J Clin Microbiol 45:257–258. https://doi.org/10.1128/JCM.01552-06.
11. Ratcliff RM, Lanser JA, Manning PA, Heuzenroeder MW. 1998. Sequence-based classification scheme for the genus *Legionella* targeting the *mip* gene. J Clin Microbiol 36:1560–1567. https://doi.org/10.1128/JCM.36.6.1560-1567.1998.
12. Amemura-Maekawa J, Kura F, Chida K, Ohya H, Kanatani JI, Isobe J, Tanaka S, Nakajima H, Hiratsuka T, Yoshino S, Sakata M, Murai M, Ohnishi M, Working Group for Legionella in Japan. 2018. *Legionella pneumophila* and other *Legionella* species isolated from legionellosis patients in Japan between 2008 and 2016. Appl Environ Microbiol 84:e00721-18. https://doi.org/10.1128/AEM.00721-18.