Draft Genome Sequences of *Legionella* Presumptive Novel Species Isolated during Environmental Surveillance in Artificial Water Systems

Luna Girolamini,a Silvano Salaris,a Massimiliano Orsini,b Maria Rosaria Pascale,a Marta Mazzotta,a Antonella Grottola,c Sandra Cristinoa

aDepartment of Biological, Geological, and Environmental Sciences, University of Bologna, Bologna, Italy
bLaboratory of Microbial Ecology and Genomics of Microorganisms, Istituto Zooprofilattico Sperimentale delle Venezie, Legnaro, Italy
cRegional Reference Laboratory for Clinical Diagnosis of Legionellosis, Unit of Microbiology and Virology, Modena University Hospital, Modena, Italy

Luna Girolamini and Silvano Salaris contributed equally to this work. Author order was determined by drawing straws.

**ABSTRACT** We present the draft genome sequences of three *Legionella* strains that were isolated from a hotel water distribution system. *Legionella* species identification was performed by macrophage infectivity potentiator (*mip*) and RNA polymerase β subunit (*rpoB*) gene sequencing. Whole-genome sequencing and average nucleotide identity results supported the hypothesis of new *Legionella* species isolation.

The *Legionella* genus contains pathogenic Gram-negative bacteria that are ubiquitous in soil and water environments. It consists of more than 60 species, all of them potentially able to cause Legionnaires’ disease, a severe form of pneumonia (1).

The *Legionella* sp. strains 27fs60 (S60), 30fs61 (S61), and 30cs62 (S62) were isolated from three different samples from a hotel’s hot water distribution system in the Emilia-Romagna region (Italy) during a routine *Legionella* surveillance program. Water sampling and *Legionella* isolation were performed according to ISO 19458:2006 and ISO 11731:2017, respectively (2, 3). Samples were seeded onto selective medium with glycine-vancomycin-polymyxin B-cycloheximide (GVPC) and were incubated for 15 days at 35°C ± 2°C in 2.5% CO₂. Suspected colonies were subcultured on buffered charcoal yeast extract (BCYE) without L-cysteine (Thermo Fisher Scientific, Basingstoke, UK).

The DNA was extracted with InstaGene matrix (Bio-Rad, Hercules, CA, USA), and identification of isolates was performed by macrophage infectivity potentiator (*mip*) and RNA polymerase β subunit (*rpoB*) gene sequencing (4, 5). Amplicons were sequenced using BigDye chemistry and analyzed on an ABI Prism 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA). The *mip* sequences were compared with the European Working Group for *Legionella* Infections (EWGLI) database. A BLAST search of the NCBI database was carried out for both *mip* and *rpoB* gene sequences. The best match returned was *Legionella quateirensis* reference strain ATCC 49507 (GenBank accession number GCA_001467955.1), with similarities of 98.45% and 94.8% for *mip* and *rpoB*, respectively.

One hundred nanograms of genomic DNA was used for next-generation sequencing (NGS) library preparation using the Illumina Nextera XT DNA library preparation kit (New England Biolabs, Ipswich, MA, USA). Sequencing was performed on the Illumina NextSeq 500 platform (2 × 150-bp paired-end reads). Raw reads were used as input data for TORMES v.1.2.0 (6), an automated pipeline for analysis of whole bacterial genomes. TORMES includes sequence quality filtering (PRINSEQ v.0.20.4) (7) and *de novo* genome assembly (SPAdes v.13.4.1) (8), as well as other downstream analyses not used for our purpose. Scaffolding was performed using TORMES contigs as input for CSAR v.1.1.1 (9) with an evolutionarily related reference genome, i.e., *Legionella fallonii* (GenBank accession

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**Address correspondence to Sandra Cristino, sandra.cristino@unibo.it.**

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The final assemblies were further improved using Geneious Prime v.2020.2.4 software (10) and were submitted to GenBank with annotation by the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v.4.3 (11). Default parameters were used for all software tools unless otherwise noted. Table 1 summarizes results from assembly and annotation by the PGAP and the completeness of genome assembly determined by Benchmarking Universal Single-Copy Orthologs (BUSCO) v.5.0.0 (12). The FastANI tool (13) was used to compare the average nucleotide identity (ANI) of the three strains against 1,009 Legionella sequences that had been downloaded from the NCBI database using the ncbi-genome-download tool (https://github.com/kblin/ncbi-genome-download). FastANI identified the closest relative of strain S60 to be L. quateirensis NCTC 12376 (GenBank accession number GCA_900452695.1) (91.31%) and the closest relative of strains S61 and S62 to be L. quateirensis ATCC 49507 (91.45% and 91.44%, respectively). Since the assumption is that two strains showing pairwise ANI values below a given threshold (95% or 96 %) belong to different species (14), our results led us to consider these strains new species.

Studying the whole genome allows investigators to better identify already known species and to discover new ones, improving the knowledge of the ecological, virulence, and resistance characteristics of Legionella.

Data availability. The draft genome assemblies are available in the GenBank database and can be accessed with SRA and assembly accession numbers SRP292355 and JADOBG000000000 (S60), SRP295125 and JADWVM000000000 (S61), and SRP295130 and JADWVN000000000 (S62).

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L.G., S.S., and S.C. conceived and designed the experiments and wrote the paper. M.R.P. and M.M. performed sample collection and culture experiments. M.O. performed the whole-genome sequencing. A.G. performed gene sequencing. L.G., S.S., and M.O. performed the bioinformatics analysis.

REFERENCES

1. Jomehzadeh N, Moosavian M, Saki M, Rashno M. 2019. Legionella and Legionnaires’ disease: an overview. J Acute Dis 8:221–232.
2. International Organization for Standardization. 2006. ISO 19458:2006: water quality: sampling for microbiological analysis. International Organization for Standardization, Geneva, Switzerland.
3. International Organization for Standardization. 2017. ISO 11731:2017:

### TABLE 1 Genome statistics from NCBI and BUSCO quality analyses

| Attribute                        | Data for strain:       |
|----------------------------------|------------------------|
| No. of reads                     | 27fs60 (S60)           |
| Avg read length (bp)             | 149                    |
| Coverage (%)                     | 142                    |
| Total length (bp)                | 4,211,919              |
| No. of contigs                   | 23                     |
| GC content (%)                   | 39.00                  |
| N50 (bp)                         | 312,097                |
| No. of coding sequences          | 3,542                  |
| No. of rRNAs                     | 3                      |
| BUSCO results (% [no. of genes]) |                        |
| Complete                         | 99.2 (123)             |
| Single-copy complete             | 99.2 (123)             |
| Duplicated complete              | 0.0 (0)                |
| Fragmented                       | 0.8 (1)                |
| Missing                           | 0 (0)                  |
| Total no. of BUSCO genes         | 124                    |
water quality: enumeration of Legionella. International Organization for Standardization, Geneva, Switzerland.

4. 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.

5. Ko KS, Lee HK, Park MY, Lee KH, Yun YJ, Woo SY, Miyamoto H, Kook YH. 2002. Application of RNA polymerase β-subunit gene (rpoB) sequences for the molecular differentiation of Legionella species. J Clin Microbiol 40:2653–2658. https://doi.org/10.1128/JCM.40.7.2653-2658.2002.

6. Quijada NM, Rodríguez-Lázaro D, Eiros JM, Hernández M. 2019. TORMES: an automated pipeline for whole bacterial genome analysis. Bioinformatics 35:4207–4212. https://doi.org/10.1093/bioinformatics/btz220.

7. Schmieder R, Edwards R. 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27:863–864. https://doi.org/10.1093/bioinformatics/btr026.

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. Chen KT, Liu CL, Huang SH, Shen HT, Shieh YK, Chiu HT, Lu CL. 2018. CSAR: a contig scaffolding tool using algebraic rearrangements. Bioinformatics 34:109–111. https://doi.org/10.1093/bioinformatics/btx543.

10. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199.

11. 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.

12. Seppey M, Manni M, Zdobnov EM. 2019. BUSCO: assessing genome assembly and annotation completeness. Methods Mol Biol 1962:227–245. https://doi.org/10.1007/978-1-4939-9173-0_14.

13. Jain C, Rodriguez-R LM, Phillippy AM, Konstantinidis KT, Aluru S. 2018. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun 9:5114. https://doi.org/10.1038/s41467-018-07641-9.

14. Kim M, Oh HS, Park SC, Chun J. 2014. Towards a taxonomic coherence between average nucleotide identity and 16S rRNA gene sequence similarity for species demarcation of prokaryotes. Int J Syst Evol Microbiol 64:346–351. https://doi.org/10.1099/ijs.0.059774-0.