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GENOME SEQUENCES

Draft Genome Sequences of Seven *Legionella pneumophila* Isolates from a Hot Water System of a Large Building

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ABSTRACT Public health data show that a significant fraction of the nation's waterborne disease outbreaks are attributable to premise plumbing. We report the draft genome sequences of seven *Legionella pneumophila* serogroup 1 isolates from hot water lines of a large building. Genomic analysis identified the isolates as belonging to sequence type 1.

Legionella pneumophila is a Gram-negative bacterium and is the major causative agent of Legionnaires' disease (1); serogroup 1 has been implicated in most cases associated with built environments (2). *Legionella* spp. colonize and persist in building water systems despite disinfection strategies that aim to mitigate their presence (3), due to the complexity of the pipe network (4). Regardless of the public health relevance of *Legionella* spp., very little information is available about their occurrence in large buildings (5).

Strains were isolated from drinking water obtained from branch lines serving a hot water system in a large occupational building. A 100-ml aliquot of each sample was concentrated by membrane filtration (0.2-μm pore size) and resuspended in 5 ml of Butterfield's phosphate buffer, and 100 μl was cultured on buffered charcoal-yeast extract (BCYE) selective agar (catalog number R110107; Remel, Lenexa, KS) for 5 days at 35°C. The agar is used for the selective recovery of *L. pneumophila* from potable water samples and contained vancomycin and anisomycin to suppress contaminating flora. A single colony was transferred to Remel BCYE agar (catalog number R01334), incubated for 2 days at 35°C, and screened with the *Legionella pneumophila* latex agglutination kit (Oxoid Ltd., Basingstoke, UK). Latex agglutination confirmed the identification of the seven isolates as *L. pneumophila* serogroup 1. DNA was extracted using the UltraClean DNA microbial isolation kit, following the manufacturer's instructions (Mo Bio Laboratories, Solana Beach, CA). Genomic libraries were prepared using the Nextera XT index kit v2 set A and sequenced on the HiSeq 4000 platform (Illumina, Inc., San Diego, CA) with a HiSeq 3000/4000 paired-end (PE) cluster kit (2 × 150 bp).

A total of 197,184,931 reads were generated. Prior to assembly, libraries were cleaned from adapters and phiX artifacts, error corrected, normalized (≤100×), and filtered to a minimum length of 100 nucleotides (nt) using the software package BBMap v37.90 (ktrim=r k = 23 mink = 11 hdist = 1 tbo tpe maxns = 0 trimq = 10 qtrim=r maq = 12 minlength = 100 ecco=t eccc=t ecct=t target = 100) (6). A reference-assisted *de novo* assembly approach were used to assemble the processed reads using Unicycler v0.4.4 (7). Average nucleotide identity (ANI) was calculated using the ANI calculator (8). ANI, an index of similarity between two genomes (9), was used to verify the taxonomic affiliation of the isolates (10). The *in silico* multilocus sequence type (MLST) based on seven alleles (11, 12) was obtained using MentaLiST v0.2.3 (13). Default

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TABLE 1 Summary statistics of whole-genome assemblies

Strain	Coverage (×)	Genetic element	No. of contigs	Assembly size (bp)	Contig N_{50} (bp)	G+C content (%)	Gene annotation data (no.)[a]				GenBank accession no.
							Genes	CDS	rRNAs	tRNAs	
L01C.1	193	Chromosome	10	3,518,411	2,171,451	38.33	3,361	3,308	9	43	QWDR00000000
		Plasmid 1	1	129,883	129,883	37.46					
		Plasmid 2	1	51,330	51,330	39.53					
L02C.1	193	Chromosome	4	3,527,736	2,108,061	38.32	3,171	3,117	9	44	QWDS00000000
L03B.1	190	Chromosome	10	3,523,457	2,176,402	38.34	3,362	3,309	9	43	QWDT00000000
		Plasmid 1	1	129,883	129,883	37.46					
		Plasmid 2	1	51,330	51,330	39.53					
L04A.1	190	Chromosome	9	3,501,953	2,172,681	38.32	3,150	3,097	9	43	QWDU00000000
L05B.1	192	Chromosome	10	3,527,329	2,180,468	38.36	3,310	3,257	9	43	QWDV00000000
		Plasmid 1	1	129,883	129,883	37.46					
L08A.1	194	Chromosome	9	3,512,166	2,171,040	38.32	3,303	3,250	9	43	QWDW00000000
		Plasmid 1	1	129,883	129,883	37.46					
L09A.1	197	Chromosome	9	3,510,538	2,181,930	38.36	3,294	3,241	9	43	QWDX00000000
		Plasmid 1	1	129,883	129,883	37.46					

[a] CDS, coding sequences.

parameters were used for all software unless otherwise specified. Genome quality and statistics were estimated with BBMap and annotated with Prokka v1.13.1 (14) (Table 1).

ANI calculations between isolates revealed an average genome similarity of 99.96%. The proposed cutoff for *Legionella* subspecies is 96% (15). The isolates share an average of 99.97% ANI with *L. pneumophila* subsp. *pneumophila* strain OLDA (16) and 91.87% to 90.55% ANI with *L. pneumophila* subsp. *fraseri* strain Lansing 3 and *L. pneumophila* subsp. *pascullei* strain U8W, respectively (15). *In silico* MLST identified the isolates as sequence type 1 (ST1) (11, 12), and gene annotation confirmed the presence of the *lpeAB* genes encoding a macrolide efflux pump, which confers reduced sensitivity to azithromycin (17, 18).

Data availability. The whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers listed in Table 1. The raw sequence reads have been submitted to the NCBI SRA under the accession numbers SRR8523645, SRR8523642, SRR8523643, SRR8523644, SRR8523646, SRR8523647, and SRR8523648. The versions described in this paper are the first versions.

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