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GENOME SEQUENCES

Closed Genome and Plasmid Sequences of *Legionella pneumophila* AW-13-4, Isolated from a Hot Water Loop System of a Large Occupational Building

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ABSTRACT Unused water in unoccupied buildings can become stagnant, with reductions in temperature and levels of disinfectant resulting in increased microbial growth. We report the closed and complete genome and plasmid of *Legionella pneumophila* strain AW-13-4 (serogroup 1), which was isolated from a hot water loop system of a large building.

In March 2020, state and local government officials in the United States issued shelter-in-place recommendations ("social distancing") and ordered the closing of high-occupancy venues (e.g., nonessential buildings) to stop the global pandemic caused by novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]) disease 2019 (COVID-19) (1–3). During this time, unoccupied and low-occupancy buildings might have experienced extended periods of low water demand without proper water management plans (i.e., mitigation) (4). As conditions allow, the workforce will steadily return to occupational buildings with their safety being a key priority for facility managers. It is important to understand the impact of the lockdown and mitigation efforts in the building water distribution system. Here, we report the assembled genome and plasmid of *Legionella pneumophila* strain AW-13-4, a Gram-negative bacterium and major causative agent of Legionnaires' disease (5, 6).

Strain AW-13-4 was isolated in April 2019 from drinking water obtained from a hot water recirculating loop pipe in a large occupational building. At the time of publication, monthly sampling events confirmed the presence of *L. pneumophila* in the drinking water system. A 100-ml water sample was concentrated by membrane filtration (0.2 μm), and the subsequent selective recovery of *L. pneumophila* was performed as described by Gomez-Alvarez et al. (7). Briefly, the concentrate was resuspended in 5 ml of Butterfield's phosphate buffer, and 100 μl was cultured on buffered CYE selective agar (catalogue 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 contains vancomycin and anisomycin to suppress contaminating flora. A single colony was transferred to BCYE agar (catalogue number R01334; Remel) and incubated for 2 days at 35°C. The isolate was further characterized as serogroup 1 by latex agglutination (Oxoid Ltd., Basingstoke, UK). High-quality DNA was extracted using the UltraClean DNA microbial isolation kit (MO BIO Laboratories, Solana Beach, CA) following the manufacturer's instructions. A genomic library was prepared using the Nextera XT index kit (Illumina, Inc., San Diego, CA) and sequenced on the Illumina HiSeq 4000 platform (paired-end 150-nucleotide [nt] reads). Read processing was performed as described previously (7); short-read libraries were cleaned of adapters and phiX artifacts, error corrected, normalized (≤100×), and filtered to a minimum length of 100 nt (BBMap v38.67; 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). A long-read library was prepared

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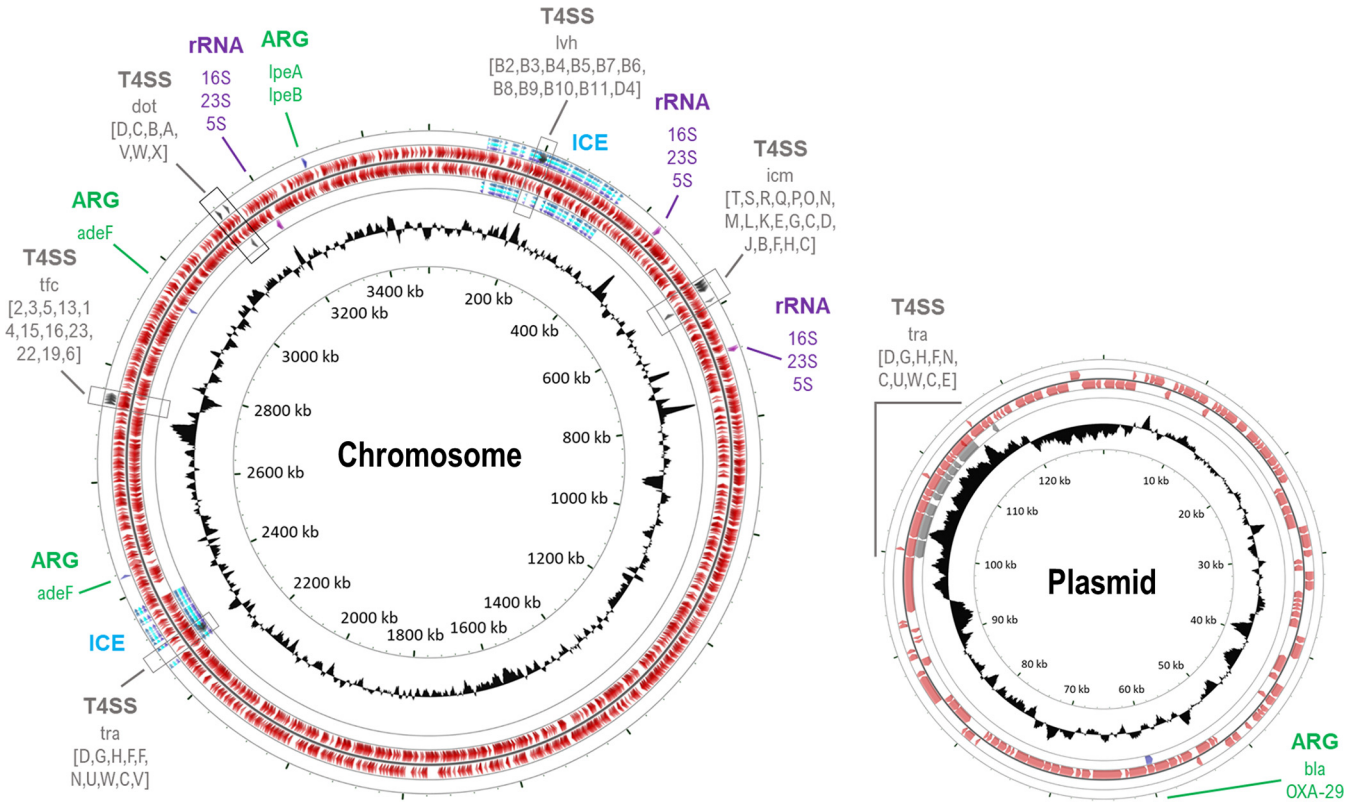

FIG 1 Circular maps of the AW-13-4 genome and plasmid generated with the CGView server (23). The rings, from outside to inside, denote AMR genes (ARG), secretion systems (T4SS), and rRNA coding genes on both the forward and reverse strands (rings 1 and 4), protein coding genes on both the forward and reverse strands (rings 2 and 3), and GC content (ring 5). Blue regions represent *in silico* identification of ICEs or mobile genetic elements (21) using the VRprofile server (20).

using the ligation sequencing kit (1D SQK-LSK109) and the native barcoding kit (EXP-NBD104) on a MinION device (Oxford Nanopore Technologies [ONT], Inc., Oxford, UK). Fast5 reads were base called using Guppy v3.1.5 (ONT), trimmed with Porechop v0.2.3 (8) using a 90% identity threshold, and then filtered to a minimum length of 25,000 nt. Short- and long-read sequencing produced 45,606,672 reads (average length, 132 nt [range, 100 to 150 nt]; coverage, 1,599×) and 10,313 reads (average length, 30,056 nt [range, 25,001 to 68,983 nt]; coverage, 142,495×), respectively. A hybrid assembly approach was used to assemble the processed reads using Unicycler v0.4.8 (9). Default parameters were used for all software unless otherwise specified.

The final assembly contains a single chromosome spanning the complete 3.512-Mbp length of the genome (GC content of 38%) and a single 1.299-kbp plasmid (Fig. 1). Gene annotation with Prokka v1.14.6 (10) identified 3,100 coding sequences, 9 rRNAs, and 43 tRNAs. The average nucleotide identity (ANI) was calculated using FastANI v1.31 (11), which indicated 99.99% ANI with *L. pneumophila* strain OLDA (12). The *in silico* multilocus sequence typing (MLST) sequence type (13, 14) was determined using MentaLiST v0.2.3 (15), and the presence of antimicrobial resistance (AMR) genes was determined with the RGI CARD Web-based tool (16). AW-13-4 was identified as sequence type 1, and gene annotation confirmed the presence of *lpeAB* genes (Fig. 1). Previous studies demonstrated the involvement of LpeAB in an efflux pump responsible for reduced sensitivity to azithromycin in *L. pneumophila* strains (17, 18). The plasmid contained a class D β-lactamase ($bla_{\text{OXA-29}}$) (19). The VRprofile server (20) detected the presence of integrative and conjugative elements (ICEs) (21) (Fig. 1), which are often associated with transfer/conjugative elements such as the type IV secretion system (T4SS) (22).

Data availability. This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under BioProject number PRJNA487286 with the accession numbers CP061840 (chromosome) and CP061841 (plasmid). The raw sequence reads have

been submitted to the NCBI SRA under the accession numbers SRR13076822 and SRR13076823. The versions described in this paper are the first versions.

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