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GENOME SEQUENCES

Complete Genome Sequence of a Suckermouth Catfish Outbreak Isolate, *Aeromonas hydrophila* Strain LP0103

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ABSTRACT *Aeromonas hydrophila* is the most common opportunistic pathogen that plagues freshwater and euryhaline fishponds. Here, we present the complete genome sequence of *A. hydrophila* strain LP0103, which was isolated from a bacterial septicemia outbreak among suckermouth catfish (*Pterygoplichthys pardalis*) at Lotus Pond in Kaohsiung City, Taiwan.

Aeromonas hydrophila is a Gram-negative, motile, facultatively anaerobic, rod-shaped bacterium linked to motile *Aeromonas* septicemia in various catfish species (1–6). The most cited case was the 2009 *A. hydrophila* ML09-119 epidemic among farmed channel catfish in Alabama, USA (7). That particular strain was also reported to have an Asian origin (8). In December 2021, over 500 suckermouth catfish per day on average were found dead/moribund at Lotus Pond, Kaohsiung City, Taiwan. We isolated *A. hydrophila* from the diseased catfish, named this strain LP0103, and sequenced its complete genome.

Wild moribund suckermouth catfish from Lotus Pond were collected, and a portion of each liver was streaked on premade blood agar (CMP0100311; Creative Life Science, Taiwan) and incubated at 27°C for 24 h following the veterinarian standard procedure (9, 10). The isolation showed a single colony type with hemolysis from at least five catfish screened. The Gentra Puregene yeast/bact. kit (Qiagen, Germany) was used to extract DNA from representative colonies isolated from each fish. Sanger sequencing of the 16S rRNA gene using the V1 to V9 region (i.e., with primers 27F [5′-AGAGTTTGATCMTGGCTCAG-3′] and 1492R [5′-TACGGYT ACCTTGTTACGACTT-3′]) was performed to confirm that the bacterial colonies were indistinguishable and identified as *A. hydrophila*.

A single colony of the isolated *A. hydrophila* was inoculated on brain heart infusion agar and incubated under the aforementioned conditions for subsequent genome sequencing. The same kit was used to extract 5.7 μg genomic DNA from roughly 10^{11} bacteria, and the DNA was subjected it to whole-genome sequencing. A g-TUBE (Covaris, USA) was used to shear 1 μg genomic DNA, which was purified using AMPure PB beads (Pacific Biosciences [PacBio], USA). Following the manufacturer's instructions, the sheared and purified DNA fragments were utilized as a template to prepare a 10-kb high-fidelity sequencing library using the SMRTbell template preparation kit v1.0 (PacBio). After damage and end repairs, the A-tailed inserts were ligated using barcoded overhang adapters, and small insert SMRTbell templates were removed using BluePippin size selection.

Genomics BioSci & Tech Co. (Taipei, Taiwan) performed single-molecule real-time (SMRT) sequencing with 100× coverage on a PacBio Sequel sequencer using a SMRT Cell 1M v3 tray with v3.0 chemistry. The Sequel system was used for the primary filtering analysis, and SMRT Link v9.0 (11) was used for the secondary analysis with default parameters. The pipeline includes consensus sequence determination, assembly, and quality evaluation.

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The sequence has 420,087 subreads and an average read length of 4,939 bp. The circular consensus reads were produced using Code Composer Studio v6 (PacBio), and the resulting reads provided base-level resolution with >99.9% accuracy. These reads were then used for assembling and polishing of the genome with hifiasm v0.15.3 (12) and GCpp v2.0.2 (https://github.com/PacificBiosciences/gcpp), respectively. Circlator v1.5.5 (13) was used to correct and circularize the genome, and QUAST v4.6.3 (14) evaluated the assembled genome quality. The *A. hydrophila* genome comprises 5,023,649 bp in one contig, with a GC content of 60.91%. It contains 4,606 predicted genes, of which 4,398 are protein-coding sequences. A total of 130 tRNAs and 31 rRNA operons were predicted using Prokka v1.12 (15). The final closed-circle version of the *A. hydrophila* LP0103 genome sequence was submitted to the PGAP v5.3 (16) for annotation, followed by submission to GenBank. Default parameters were used except where otherwise noted.

Further studies of the genome of *Aeromonas hydrophila* LP0103 and *in vivo* challenge experiments are of interest and may reveal more information on pathogenicity and host specificity for the suckermouth catfish.

Data availability. The complete genome sequence of *A. hydrophila* LP0103 was deposited in GenBank under the accession number CP092906. The raw sequencing reads were deposited in the NCBI Sequence Read Archive (SRA) under accession number SRR18355516.

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