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GENOME SEQUENCES

Draft Genome Sequences of *Flavobacterium covae* Strains LSU-066-04 and LV-359-01

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ABSTRACT *Flavobacterium covae* is one of four *Flavobacterium* spp. that cause columnaris disease in teleost fish. Here, we report the draft genomes of two isolates, LSU-066-04 and LV-359-01, and their predicted virulence factors.

The fish pathogen *Flavobacterium columnare* was previously organized into four genetic groups, but recent phylogenetic analysis has shown that *F. columnare* encompasses four separate species, all of which are etiologic agents of columnaris disease (1–3). In 2004, the bacterial isolate LSU-066-04 was cultured from a largemouth bass showing symptoms of columnaris disease from the Louisiana Fish and Game Hatchery in Forest Hill, Louisiana (4). The isolate LV-359-01 was cultured from a channel catfish (*Ictalurus punctatus*) production farm experiencing a columnaris outbreak in Lake Village, Arkansas in 2001. Both isolates were initially described as *F. columnare* genomovar II using methods from Arias et al. and were shown to be virulent in channel catfish disease models (4, 5).

From cryogenic stocks, cultures of both isolates were grown overnight in 10 mL of modified Shieh medium at 28°C with shaking (6). Genomic DNA was isolated using an E.Z.N.A. bacterial DNA isolation kit (Omega Bio-tek, Norcross, GA, USA) following the manufacturer's protocol. Genomic DNA was quantified using AccuGreen fluorescence on the Qubit 3.0 fluorometer using the double-stranded DNA (dsDNA) high sensitivity program (Biotium, Fremont, CA, USA). Paired-end reads of 250 bp were generated using Illumina Nextera with indexed fragment libraries on an Illumina NovaSeq 6000 sequencer (Novogene, Durham, NC, USA). End trimming, error correction, and contig assembly were conducted using CLC Genomics Workbench 20.0 (Qiagen, Aarhus, Denmark). Contig sequences of >1 kb were uploaded to KBase, and CheckM was used to assess genome purity. Genome annotation was conducted using Rapid Annotation using Subsystem Technology (RAST) v. 2.0 (7, 8). Using the published genomes, average nucleotide identities (ANI) and DNA-DNA hybridization estimate comparisons for each type strain of *F. columnare* (accession number PCMX01000000), *F. covae* (accession number CP067379), *F. davisii* (accession number CP067378), and *F. oreochromis* (accession number CP067377) were calculated using OrthoANI algorithm in the Orthologous Average Nucleotide Identity Tool (OAT) and Genome-to-Genome Distance Calculator (GGDC) 3.0 (Leibniz Institute DSMZ, Braunschweig, Germany) (9, 10). LSU-066-04 had a genome size of 3.07 Mb with 100 contigs with an N_{50} of 75,071, G+C% content of 30.4%, 2,724 coding sequences, and 64 total RNAs. LV-359-01 had a genome size of 3.14 Mb with 95 contigs with an N_{50} of 70,015, G+C% content of 30.5%, 2,832 coding sequences, and 62 total RNAs. Both isolates had ANI values of >99% to *Flavobacterium covae* AL-02-36^{T}and DNA-DNA hybridization estimates of >95%. The ANI and GGDC values to *F. columnare* ATCC 23463^T were 92 to 93% and 4 to 11%, indicating that both strains belong to the species *F. covae* (1, 11, 12).

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TABLE 1 Virulence factors predicted to be produced by *F. covae* LSU-066-04 and *F. covae* LV-359-01 based on previous studies; percent similarities are to *F. covae* AL-02-36^T

Gene	Reference	Strain(s) used	*Flavobacterium* species	LSU-066-04 percent similarity; E value; query cover	LV-359-01 percent similarity; E value; query cover
porV	13, 14	C#2 and MS-FC-4	*F. covae* and F. *columnare*	99.29%; 0.0; 100%	99.29%; 0.0; 100%
gldN	13, 14	C#2, IA-S-4, and MS-FC-4	*F. covae*, *F. columnare*, and *F. columnare*	98.67%; 0.0; 100%	98.67%; 0.0; 100%
gldK	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	99.78%; 0.0; 100%	100.00%; 0.0; 100%
gldL	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	100.00%; 4e−116; 100%	100.00%; 4e−116; 100%
gldM	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	99.50%; 0.0; 100%	99.50%; 0.0; 100%
sprA	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	99.36%; 0.0; 100%	99.84%; 0.0; 100%
sprE	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	97.44%; 0.0; 100%	97.18%; 0.0; 100%
sprT	13	C#2 and IA-S-4	*F. covae* and *F. columnare*	99.15%; 6e−163; 100%	99.15%; 6e−163; 100%
nirS	15	94-801	*F. covae*	100.00%; 0.0; 100%	100.00%; 0.0; 100%
cylA	15	MS-FC-4	*F. columnare*	100.00%; 0.0; 100%	100.00%; 0.0; 100%
C6N29_11540	14	MS-FC-4	*F. columnare*	100.00%; 0.0; 100%	100.00%; 0.0; 100%
C6N29_11545	14	MS-FC-4	*F. columnare*	99.44%; 0.0; 100%	99.37%; 0.0; 100%

To predict the presence of virulence factors in *F. covae* LSU-066-04 and *F. covae* LV-359-01, a tBLASTx analysis of each genome was conducted with previously described virulence factors associated with columnaris disease. Interestingly, both *F. covae* isolates were predicted to carry all of the genes previously determined to have a role in columnaris disease, with high percent similarities to each gene (Table 1). These predicted virulence factors included genes associated with T9SS, denitrification, and anaerobic growth. It is likely that there are many other virulence factors expressed by these *F. covae* strains, but their functional roles in virulence have not yet been determined. The genome sequences for these two virulent strains LSU-066-04 and LV-359-01 will provide a resource to explore the specific factors that contribute to *F. covae* virulence and contrast this with virulence factors expressed by the other *Flavobacterium* spp. that cause columnaris disease.

Data availability. This Whole Genome Shotgun project for both LSU-066-04 and LV-359-01 has been deposited at GenBank under the accession numbers JALDSR000000000 and JALDSS000000000, respectively. The versions described in this paper are versions JALDSR010000000 and JALDSS010000000, respectively. LSU-066-04 is under the BioSample accession number SAMN26856626, and LV-359-01 is under the BioSample accession number SAMN26856627. Both strains are under BioProject accession number PRJNA818358 with the SRA accession numbers SRX15461022 and SRX1461023.

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