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Draft Genome Sequence of the Fish Pathogen *Flavobacterium columnare* Strain MS-FC-4

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ABSTRACT *Flavobacterium columnare* MS-FC-4 is a highly virulent genetic group 1 (formerly genomovar I) strain isolated from rainbow trout (*Oncorhynchus mykiss*). The draft genome consists of three contigs totaling 3,449,277 bp with 2,811 predicted open reading frames. *F. columnare* MS-FC-4 is a model strain for functional genomic analyses.

Flavobacterium columnare causes columnaris disease in wild and cultured fish (1, 2). It is a common pathogen of farmed freshwater fish and causes major economic losses worldwide. *F. columnare* isolates are genetically diverse and were classified historically into multiple genomovars (3, 4). Recently, the genomovars were described as four distinct genetic groups (5). Almost all isolates recovered from salmonids belong to genetic group 1 (formerly genomovar 1). *F. columnare* MS-FC-4 is a highly virulent strain that was isolated from rainbow trout (*Oncorhynchus mykiss*) (6). It is amenable to genetic manipulation, making it attractive for functional genomic studies of virulence.

F. columnare MS-FC-4 was cultured for 24 h in tryptone yeast extract salt medium at 30°C and 150 rpm. Genomic DNA (gDNA) was isolated using a cetyltrimethylammonium bromide (CTAB)/phenol-chloroform/isoamyl alcohol protocol (7) optimized for *F. columnare*.

For long-read sequencing, a gDNA library was prepared according to the standard Pacific Biosciences RS II large-insert library protocol. Two flow cells (C4 chemistry) were each loaded with 0.06 nM of a BluePippin (Sage Science, Beverly, MA, USA) size-selected (>10-kb) SMRTbell gDNA library. One microgram of MS-FC-4 gDNA was also sequenced on an Illumina HiSeq 2000 platform with 100-bp paired-end reads. PacBio reads were assembled using Canu version 1.6 with the following options: genome size 3.3 Mb, corrected error rate 0.035, and corMaxEvidenceErate 0.15 (8). Subread coverage of the genome was ~865×. The Illumina HiSeq paired-end reads were mapped to the PacBio assembly using Bowtie 2 version 2.3.4 (9) with the no-unaligned parameter to discard unaligned reads. HiSeq coverage of the contigs was ~360×. The output SAM file was converted to a BAM file using SAMtools version 1.4 (10). Using the Canu assembly and the BAM file as input, Pilon version 1.22 (11) corrected two insertions, seven deletions, and one substitution. The Pilon corrections did not close the gaps in the Canu PacBio assembly, resulting in three contigs of 2,709,164, 716,735, and 23,378 bp. The corrected MS-FC-4 genome assembly was annotated using the NCBI Prokaryotic Genome Annotation Pipeline (12).

The draft *F. columnare* MS-FC-4 genome has a cumulative total size of 3,449,277 bp with a G+C content of 31.9%. The annotated genome had 2,811 coding genes, 19 complete rRNA operons, 95 tRNAs, and 2 clustered regularly interspaced short palin-

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dromic repeat (CRISPR) arrays. Two incomplete prophage regions of 12.4 and 25.9 kb were identified using PHASTER (13). The two-way average nucleotide identities (ANIs) (14) between the MS-FC-4 genome sequence and those of strains ATCC 49512 (15), Pf1 (16), and CSF 298-10 (17) were >99%, confirming the *F. columnare* genetic group 1 (genomovar I) classification (5, 6). The MS-FC-4 genome contains the core genes of the *Bacteroidetes*-specific type IX secretion system linked to gliding motility and virulence (18, 19). The availability of the MS-FC-4 genome sequence should facilitate construction of targeted gene deletions to identify critical *F. columnare* virulence factors.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession number PVLU00000000. The version described in this paper is the first version, PVLU01000000.

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