

Genome Sequence of the Fish Pathogen *Flavobacterium columnare* ATCC 49512

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Flavobacterium columnare is a Gram-negative, rod-shaped, motile, and highly prevalent fish pathogen causing columnaris disease in freshwater fish worldwide. Here, we present the complete genome sequence of *F. columnare* strain ATCC 49512.

lavobacterium columnare is considered ubiquitous in the warm water environment, and it is the etiological agent of columnaris disease, which affects cultured, wild, and ornamental fish throughout the world (2, 7, 11). F. columnare is a yellow-pigmented, motile, Gram-negative rod in the family Flavobacteriaceae, one of the main phyletic lines within the Bacteroidetes group (5). F. columnare shows wide genetic heterogeneity and colony morphology as well as significant differences in virulence within the species. F. columnare strains are divided into three genomovars that demonstrate various levels of virulence for different fish species (1, 6, 12-14). F. columnare strain ATCC 49512 (CIP 103533 [TG 44/87]), isolated in 1987 from a skin lesion of a brown trout fry in France (3), belongs to genomovar I (10) and is avirulent in channel catfish (13). The average reported genome size of bacterial species in the genus Flavobacterium and estimated average G+C content of the F. columnare genome have been reported as 4.1 ± 1 Mb and 32.5%, respectively (4, 8).

To obtain a complete sequence of the F. columnare genome, small and medium insert libraries (average insert sizes, 3 to 6 kb, 6 to 10 kb, and 10 to 12 kb) were constructed at Mississippi State University (9). Shotgun paired-end sequencing was conducted to 8-fold coverage at The Laboratory for Genomics and Bioinformatics at the University of Oklahoma Health Sciences Center. Sequences were assembled using Phred/Phrap. In addition to Sanger sequencing, a sequencing run was conducted using a GS FLX system from 454 Life Sciences, in which F. columnare genomic DNA was mixed with amplified gaps from the Sanger assembly and used as the template (269,256 reads with 269.4-bp average read length; Roche). Both Sanger and 454 sequencing generated an assembly with approximately 30-fold coverage. Quality-filtered 454 sequences were assembled using the Newbler assembler, and 454 contigs were added to the Sanger assembly. This hybrid assembly resulted in about 6 large contigs (>2 kb), and gaps were closed by primer walking using small or large insert plasmids or PCR amplicons as templates. The DNA sequence was submitted to the NCBI's Prokaryotic Genomes Automatic Annotation Pipeline (PGAAP) for annotation, and then the annotated genome was submitted to GenBank.

The complete genome of *F.columnare* consists of a 3,162,432-bp chromosome with an average G+C content of 31.5%. The genome is 85.2% coding and has 2,731 predicted genes consisting of 2,642 protein coding, 15 rRNA, and 74 tRNA genes. For the protein coding genes, the average length is 1,021 bp and 1,625 genes (61.5%) have assigned functions. The *F. columnare* genome contains 5 rRNA oper-

ons, two of which are tandemly arranged. Analysis of the *F. columnare* genome sequence reveals many features common to other sequenced *Flavobacterium* species. We identified chondroitin AC lyase, proteases, and collagenases as well as genes involved in biofilm formation, secretion systems, and iron acquisition. Thirteen gliding motility genes were also identified.

Nucleotide sequence accession number. The genome sequence was deposited in GenBank under accession number CP003222, version CP003222.2, GI:372863588.

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