

Complete Genome Sequence of *Flavobacterium psychrophilum* Strain CSF259-93, Used To Select Rainbow Trout for Increased Genetic Resistance against Bacterial Cold Water Disease

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The genome sequence of *Flavobacterium psychrophilum* strain CSF259-93, isolated from rainbow trout (*Oncorhynchus mykiss*), consists of a single circular genome of 2,900,735 bp and 2,701 predicted open reading frames (ORFs). Strain CSF259-93 has been used to select a line of rainbow trout with increased genetic resistance against bacterial cold water disease.

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Infectious disease causes significant losses in aquaculture, and bacterial cold water disease (BCWD) is a common cause of rainbow trout loss (1, 2). Disease resistance can be improved through selective breeding (3, 4), and recent farm trials of a selectively bred line (ARS-Fp-R) demonstrated significantly higher survival at locations where BCWD is endemic (5, 6). *Flavobacterium psychrophilum* is the causative agent of BCWD and a reference genome sequence is available (7). Herein we report the complete genome sequence of *F. psychrophilum* strain CSF259-93 utilized in the National Center for Cool and Cold Water Aquaculture (NCCCWA) rainbow trout selective breeding program. The CSF259-93 strain has been used to derive a live attenuated vaccine (8, 9) and has been characterized biochemically and immunologically (10–16). The strain belongs to multilocus sequence type 10 (17).

A single colony of strain CSF259-93 was subcultured and grown in tryptone yeast extract salt (TYES) broth, and a large stock was stored at -80°C for challenge and DNA isolation (5). Isolated DNA was sheared and a random library prepared and sequenced using the Sanger di-deoxy method to $8\times$ coverage. Sequences were assembled using larger contigs with *de novo* assembly with the Phred-Phrap-Consed package. The contig scaffolds were closed using a 40-kb fosmid library and end sequencing by use of the Sanger di-deoxy method followed by gap closure using PCR. The genome was closed to a single chromosome and assembly validated by optical mapping using NcoI. The genome of strain CSF259-93 is 2,900,735 bp, with an average G+C content of 32% and 2701 open reading frames (ORFs) including 49 tRNAs species and 6 rRNA operons. The genome was analyzed with the ERGO genome application platform (18), using previously described annotation methods (19), and 63% of the ORFs were assigned functions.

Comparative sequence analysis identified 2,481,839 bp common between CSF259-93 and strain JIP02/86 (7), with 1,471 single nucleotide polymorphisms (SNPs) present within protein-coding ORFs (617 nonsynonymous SNPs), 4 SNPs within RNA ORFs, and 119 SNPs in noncoding DNA. Five notable regions of difference were identified between strains. There is an expansion of 19 tandem leucine-rich repeat genes in CSF259-93 (nucleotides [nt] 215,399 to 235,946) compared to 15 tandem genes in the JIP02/86 genome. The number of repeats within each gene varies from 3 to 11, with each repeat encoding ~ 23 amino acids. This locus exhibits rearrangement in gene synteny and contains an overabundance of nonsynonymous SNPs ($n = 64$ out of 99 putative SNPs). There is an ~ 1.9 -Mb chromosomal inversion between the putative adhesins, FPSM_00355 and FPSM_02084. There is a large genomic island of $\sim 146,000$ kb, a segment (597,708 to 743,794) present in the CSF259-93 genome containing 26 transposase ORFs, type II and III restriction modification systems, two putative tetracycline resistance genes (FPSM_00635 and FPSM_00640) (20, 21), and an integrase (FPSM_00578) absent from the JIP02/86 genome. There is a substitution of several lipopolysaccharide (LPS) biosynthesis genes (FPSM_02190 through FPSM_02194 and FPSM_02202). Finally, strain CSF259-93 lacks an integrated prophage that is found in the JIP02/86 genome (22).

The availability of complete and draft genome sequences combined with laboratory challenge data will facilitate definition of host specificity and mechanisms of bacterial cold water disease resistance.

Nucleotide sequence accession number. The genome sequence for *Flavobacterium psychrophilum* strain CSF259-93 has been deposited in GenBank under the accession number CP007627.

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We declare that we have no conflicts of interest.

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