

# Draft Genome Sequences of Three *Flavobacterium psychrophilum* Strains Isolated from Coldwater Disease Outbreaks at Three Production Hatcheries

Regg Neiger,<sup>a</sup> Milton Thomas,<sup>a</sup> Seema Das,<sup>a</sup> Michael Barnes,<sup>b</sup> Brian Fletcher,<sup>b</sup> Kevin Snekvik,<sup>c</sup> Jim Thompson,<sup>c</sup> Joy Scaria<sup>a</sup>

Department of Veterinary and Biomedical Sciences, South Dakota State University, Brookings, South Dakota, USA<sup>a</sup>; Department of Game, Fish and Parks, Pierre, South Dakota, USA<sup>b</sup>; Washington Animal Disease Diagnostic Laboratory, Pullman, Washington, USA<sup>c</sup>

**We report here the genome sequences of three *Flavobacterium psychrophilum* strains causing a bacterial coldwater disease (BCWD) outbreak, isolated from infected rainbow trout from hatcheries in Montana and South Dakota. The availability of these virulent outbreak-causing strain genome sequences will help further understand the pathogenesis of BCWD.**

Received 19 January 2016 Accepted 28 January 2016 Published 10 March 2016

**Citation** Neiger R, Thomas M, Das S, Barnes M, Fletcher B, Snekvik K, Thompson J, Scaria J. 2016. Draft genome sequences of three *Flavobacterium psychrophilum* strains isolated from coldwater disease outbreaks at three production hatcheries. *Genome Announc* 4(2):e00035-16. doi:10.1128/genomeA.00035-16.

**Copyright** © 2016 Neiger et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Joy Scaria, joy.scaria@sdsu.edu.

Bacterial coldwater disease (BCWD) is a disease that affects a number of free-ranging and cultured salmonid and a variety of nonsalmonid fish species (1–4). BCWD typically occurs in water temperatures just above freezing to 30°C, and it is most prevalent and serious at ≤10°C. BCWD has been reported in Australia, Belgium, Canada, Chile, Denmark, France, Finland, Germany, Italy, Japan, South Korea, Spain, the United Kingdom, and the United States (2). The causative agent of BCWD is the yellow-pigment-producing bacterium *Flavobacterium psychrophilum*. The most commonly recognized symptoms of BCWD are tailrot or peduncle disease, necrotic myositis, and cephalic osteochondritis (2). BCWD has a significant economic impact on aquaculture operations in the United States and Canada. The genome sequences of the *F. psychrophilum* vaccine strain (5), type strain (6), and a European isolate are available (7). Here, we report the sequencing of three BCWD outbreak-associated *F. psychrophilum* strains isolated from rainbow trout (*Oncorhynchus mykiss*) from three different hatcheries in the United States.

For isolating genomic DNA, strains were grown in tryptone yeast extract medium for 5 days at 20°C. DNA from each strain was isolated from 1.0 ml of grown cultures using the E.Z.N.A. bacterial DNA kit (Omega Bio-tek, Norcross, GA). In accordance with the manufacturer's protocol, sequencing libraries were prepared using 1.0 ng of genomic DNA using the Nextera XT kit (Illumina, San Diego, CA). The genomes were sequenced on an Illumina MiSeq platform using V2 paired-end chemistry (2 × 250 bp). The sequencing reads were assembled

into contigs using the SPAdes genome assembler version 3.5.0. The genomes were then annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP) ([http://www.ncbi.nlm.nih.gov/genome/annotation\\_prok](http://www.ncbi.nlm.nih.gov/genome/annotation_prok)).

The general properties of the sequenced *F. psychrophilum* strains are shown in Table 1. The genome size and G+C content were similar to those of the previously sequenced type and reference strains of *F. psychrophilum*. Analysis revealed the presence of efflux and response elements conferring resistance to copper, lead, cadmium, and zinc in all three genomes. Since the exposure of fish to copper sulfate solution is one of the treatments used against *F. psychrophilum* infection (8), the presence of these resistance elements in these outbreak-causing strains might be conferring resistance. Genes conferring resistance to fluoroquinolones and beta-lactam antibiotics were also detected in all three genomes. The addition of antibiotics to diluents and water-hardening solutions in hatcheries is a suggested treatment for preventing the spread of BCWD (9). The presence of multiple antibiotic resistance genes in the outbreak-associated strains might have aided in the survival of these strains. The availability of these genomes of outbreak-causing strains, along with other genomes of strains published previously, could be helpful in further understanding the mechanisms behind the pathogenesis of *F. psychrophilum*.

**Nucleotide sequence accession numbers.** The accession numbers of the three *F. psychrophilum* genome sequences are listed in Table 1.

**TABLE 1** Metadata for *F. psychrophilum* strains isolated from coldwater disease outbreaks in U.S. hatcheries

Strain name	GenBank accession no.	Source tissue	Source state	Yr	Genome size (bp)
11754	<a href="https://genbank.ncbi.nlm.nih.gov/GenBank/FASTA/seqview.fcgi?acc=LNTP00000000">LNTP00000000</a>	Whole tissue homogenate	South Dakota	2011	2,945,333
17830	<a href="https://genbank.ncbi.nlm.nih.gov/GenBank/FASTA/seqview.fcgi?acc=LNTQ00000000">LNTQ00000000</a>	Swab from sagittal section of head	South Dakota	2011	2,822,685
Pullman	<a href="https://genbank.ncbi.nlm.nih.gov/GenBank/FASTA/seqview.fcgi?acc=LNNF00000000">LNNF00000000</a>	Spleen	Montana	2010	2,713,260

## ACKNOWLEDGMENTS

This work was supported in part by the USDA National Institute of Food and Agriculture, Hatch projects SD00H532-14 and SD00R540-15, awarded to J.S. The funders had no role in the study design, data collection and interpretation, or the decision to submit the work for publication.

## FUNDING INFORMATION

This work, including the efforts of Joy Scaria, was funded by U.S. Department of Agriculture (USDA) (SD00R540-15 and SD00H532-14).

## REFERENCES

1. Nematollahi A, Decostere A, Pasmans F, Haesebrouck F. 2003. *Flavobacterium psychrophilum* infections in salmonid fish. *J Fish Dis* 26:563–574. <http://dx.doi.org/10.1046/j.1365-2761.2003.00488.x>.
2. Hesami S, Allen KJ, Metcalf D, Ostland VE, MacInnes JI, Lumsden JS. 2008. Phenotypic and genotypic analysis of *Flavobacterium psychrophilum* isolates from Ontario salmonids with bacterial coldwater disease. *Can J Microbiol* 54:619–629. <http://dx.doi.org/10.1139/w08-058>.
3. Hesami S, Metcalf DS, Lumsden JS, Macinnes JI. 2011. Identification of cold-temperature-regulated genes in *Flavobacterium psychrophilum*. *Appl Environ Microbiol* 77:1593–1600. <http://dx.doi.org/10.1128/AEM.01717-10>.
4. Chen YC, Davis MA, Lapatra SE, Cain KD, Snekvik KR, Call DR. 2008. Genetic diversity of *Flavobacterium psychrophilum* recovered from commercially raised rainbow trout, *Oncorhynchus mykiss* (Walbaum), and spawning coho salmon, *O. kisutch* (Walbaum). *J Fish Dis* 31:765–773. <http://dx.doi.org/10.1111/j.1365-2761.2008.00950.x>.
5. Wiens GD, LaPatra SE, Welch TJ, Rexroad C III, Call DR, Cain KD, LaFrentz BR, Vaisvil B, Schmitt DP, Kapatral V. 2014. Complete genome sequence of *Flavobacterium psychrophilum* strain CSF259-93, used to select rainbow trout for increased genetic resistance against bacterial cold water disease. *Genome Announc* 2(5):e00889-14. <http://dx.doi.org/10.1128/genomeA.00889-14>.
6. Wu AK, Kropinski AM, Lumsden JS, Dixon B, MacInnes JI. 2015. Complete genome sequence of the fish pathogen *Flavobacterium psychrophilum* ATCC 49418<sup>T</sup>. *Stand Genomic Sci* 10:3.
7. Duchaud E, Boussaha M, Loux V, Bernardet JF, Michel C, Kerouault B, Mondot S, Nicolas P, Bossy R, Caron C, Bessières P, Gibrat JF, Claverol S, Dumetz F, Le Hénaff M, Benmansour A. 2007. Complete genome sequence of the fish pathogen *Flavobacterium psychrophilum*. *Nat Biotechnol* 25:763–769. <http://dx.doi.org/10.1038/nbt1313>.
8. Darwish AM, Bebak JA, Schrader KK. 2012. Assessment of Aquaflor, copper sulphate and potassium permanganate for control of *Aeromonas hydrophila* and *Flavobacterium columnare* infection in sunshine bass, *Morone chrysops* female x *Morone saxatilis* male. *J Fish Dis* 35:637–647. <http://dx.doi.org/10.1111/j.1365-2761.2012.01393.x>.
9. Oplinger RW, Wagner EJ, Cavender W. 2015. Use of penicillin and streptomycin to reduce spread of bacterial coldwater disease II: efficacy of using antibiotics in diluents and during water hardening. *J Aquat Anim Health* 27:32–37. <http://dx.doi.org/10.1080/08997659.2014.945049>.