AMERICAN SOCIETY FOR MICROBIOLOGY | genomeAnnouncements™

PROKARYOTES

Complete Genome Sequence of *Flavobacterium psychrophilum* Strain OSU THCO2-90, Used for Functional Genetic Analysis

Tatiana Rochat,[a] Paul Barbier,[a]* Pierre Nicolas,[b] Valentin Loux,[b] David Pérez-Pascual,[a] José A. Guijarro,[c] Jean-François Bernardet,[a] Eric Duchaud[a]

VIM, INRA, Université Paris-Saclay, Jouy-en-Josas, France[a]; Malage, INRA, Université Paris-Saclay, Jouy-en-Josas, France[b]; Área de Microbiología, Departamento de Biología Funcional, Facultad de Medicina, Instituto de Biotecnología de Asturias, Universidad de Oviedo, Oviedo, Spain[c]

ABSTRACT We report here the complete annotated genome sequence of *Flavobacterium psychrophilum* OSU THCO2-90, isolated from Coho salmon (*Oncorhynchus kisutch*) in Oregon. The genome consists of a circular chromosome with 2,343 predicted open reading frames. This strain has proved to be a valuable tool for functional genomics.

Received 9 December 2016 **Accepted** 16 December 2016 **Published** 23 February 2017

Citation Rochat T, Barbier P, Nicolas P, Loux V, Pérez-Pascual D, Guijarro JA, Bernardet J-F, Duchaud E. 2017. Complete genome sequence of *Flavobacterium psychrophilum* strain OSU THCO2-90, used for functional genetic analysis. Genome Announc 5:e01665-16. https://doi.org/10.1128/genomeA.01665-16.

Address correspondence to Eric Duchaud, eric.duchaud@inra.fr.

* Present address: Paul Barbier, Department of Biological Sciences, University of Wisconsin–Milwaukee, Milwaukee, Wisconsin, USA.

Flavobacterium psychrophilum is a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. This bacterium, initially reported from North America, is now documented worldwide (1–5) and is currently one of the most devastating bacterial pathogens of farmed salmonids reared in freshwater (6). The two main clinical forms are rainbow trout fry syndrome and bacterial coldwater disease (7). As a complement to the *F. psychrophilum* genomes already available (8–10), we report here the complete genome sequence of strain OSU THCO2-90 (11). This strain was isolated by R. A. Holt (Oregon State University) from the kidney of a Coho salmon (*Oncorhynchus kisutch*) in Oregon in 1990. Its sequence type is ST9 (3), and it is moderately virulent in a rainbow trout experimental infection model using injection (12) or bath challenge (unpublished). Importantly, it is so far the only *F. psychrophilum* strain that can be successfully genetically manipulated (13, 14). Indeed, this strain has proved to be a valuable tool for functional genomics and has already contributed to the characterization of several genes involved in pathogenicity (12, 15–17).

Sequencing used a combination of Sanger (ABI3730, Applied Biosystems), 454 (GS-FLX, Roche), and Solexa (GAIIx) sequencing with 2.5-fold, 17-fold, and 75-fold coverage, respectively. The 454 reads were assembled into 186 contigs (>500 bp) and 30 scaffolds using Newbler (Roche). These contigs and the Sanger reads were assembled using Phrap (18). Gaps were closed using primer walking on pCNS clones (10-kb fragments on average) used for Sanger sequencing or by PCR sequencing; Solexa reads were used to correct residual sequencing errors. The genome was closed to a single chromosome and the assembly was validated by optical mapping using *Nco*I (19). Genome annotation was performed using the AGMIAL annotation platform and then manually validated and enriched (20).

The genome of *F. psychrophilum* OSU THCO2-90 consists of a circular chromosome of 2,783,852 bp with an overall G+C content of 32.61%. The genome is predicted to encode 2,343 protein-coding genes, 49 tRNA genes, and six rRNA operons.

F. psychrophilum OSU THCO2-90 belongs to the same clonal complex as the type strain ATCC 49418, which was also isolated from Coho salmon but differs from isolates retrieved from rainbow trout, thus demonstrating host specificity (3). Strains OSU THCO2-90 and ATCC 49418^T share 2,259 protein-coding genes (≥80% protein identity; 80% protein overlap). The most striking differences between these two strains are (i) a bona fide CRISPR locus encompassing 42 direct repeats in strain OSU THCO2-90,

whereas strain ATCC 49418^T contains only a remnant CRISPR system; (ii) a genomic island in strain OSU THCO2-90 (genomic coordinates: 1,323,198 to 1,398,091); and (iii) the absence of a 2.7-kb plasmid in strain OSU THCO2-90 (19), which probably makes it more amenable to genetic manipulations (13).

The availability of this complete genome may help in understanding host specificity and genome evolution and will facilitate functional genomics studies. Using Tn*4351*-mediated random mutagenesis, we already obtained about 1,000 mutants whose precise sites of transposon integration into the chromosome can easily be determined by inverse PCR (15).

Accession number(s). This genome has been deposited in ENA under accession number LT670843.

ACKNOWLEDGMENTS

This work has benefited from the platforms and expertise of the High-Throughput Sequencing Platform of CEA/Genoscope, supported by the France Génomique National infrastructure, funded as part of an "Investissement d'avenir" program managed by Agence Nationale pour la Recherche (contrat ANR-10-INBS-09).

We are indebted to M. Whipple (OSU) for kindly providing the strain, to J. Poulain and V. Barbe (CEA/Genoscope) for sequencing and optical mapping, and to the INRA MIGALE bioinformatics platform (http://migale.jouy.inra.fr) for providing computational resources, help, and support.

REFERENCES

1. Avendaño-Herrera R, Houel A, Irgang R, Bernardet JF, Godoy M, Nicolas P, Duchaud E. 2014. Introduction, expansion and coexistence of epidemic *Flavobacterium psychrophilum* lineages in Chilean fish farms. Vet Microbiol 170:298–306. https://doi.org/10.1016/j.vetmic.2014.02.009.
2. Fujiwara-Nagata E, Chantry-Darmon C, Bernardet JF, Eguchi M, Duchaud E, Nicolas P. 2013. Population structure of the fish pathogen *Flavobacterium psychrophilum* at whole-country and model river levels in Japan. Vet Res 44:34. https://doi.org/10.1186/1297-9716-44-34.
3. Nicolas P, Mondot S, Achaz G, Bouchenot C, Bernardet JF, Duchaud E. 2008. Population structure of the fish-pathogenic bacterium *Flavobacterium psychrophilum*. Appl Environ Microbiol 74:3702–3709. https://doi.org/10.1128/AEM.00244-08.
4. Nilsen H, Sundell K, Duchaud E, Nicolas P, Dalsgaard I, Madsen L, Aspán A, Jansson E, Colquhoun DJ, Wiklund T. 2014. Multilocus sequence typing identifies epidemic clones of *Flavobacterium psychrophilum* in Nordic countries. Appl Environ Microbiol 80:2728–2736. https://doi.org/10.1128/AEM.04233-13.
5. Van Vliet D, Wiens GD, Loch TP, Nicolas P, Faisal M. 2016. Genetic diversity of *Flavobacterium psychrophilum* isolates from three *Oncorhynchus* spp. in the United States, as revealed by multilocus sequence typing. Appl Environ Microbiol 82:3246–3255. https://doi.org/10.1128/AEM.00411-16.
6. Nematollahi A, Decostere A, Pasmans F, Haesebrouck F. 2003. *Flavobacterium psychrophilum* infections in salmonid fish. J Fish Dis 26:563–574. https://doi.org/10.1046/j.1365-2761.2003.00488.x.
7. Cipriano RC, Holt RA. 2005. *Flavobacterium psychrophilum*, cause of bacterial cold-water disease and rainbow trout fry syndrome. Fish Dis Leaflet 86:1–44.
8. 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. https://doi.org/10.1038/nbt1313.
9. 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. https://doi.org/10.1128/genomeA.00889-14.
10. Wu AK, Kropinski AM, Lumsden JS, Dixon B, MacInnes JI. 2015. Complete genome sequence of the fish pathogen *Flavobacterium psychrophilum* ATCC 49418^T. Stand Genomic Sci 10:3. https://doi.org/10.1186/1944-3277-10-3.
11. Bertolini JM, Wakabayashi H, Watral VG, Whipple MJ, Rohovec JS. 1994. Electrophoretic detection of proteases from selected strains of *Flexibacter psychrophilus* and assessment of their variability. J Aquat Anim Health 6:224–233. https://doi.org/10.1577/1548-8667(1994)006<0224:EDOPFS>2.3.CO;2.
12. Alvarez B, Alvarez J, Menéndez A, Guijarro JA. 2008. A mutant in one of two *exbD* loci of a TonB system in *Flavobacterium psychrophilum* shows attenuated virulence and confers protection against cold water disease. Microbiology 154:1144–1151. https://doi.org/10.1099/mic.0.2007/010900-0.
13. Alvarez B, Secades P, McBride MJ, Guijarro JA. 2004. Development of genetic techniques for the psychrotrophic fish pathogen *Flavobacterium psychrophilum*. Appl Environ Microbiol 70:581–587. https://doi.org/10.1128/AEM.70.1.581-587.2004.
14. Gómez E, Álvarez B, Duchaud E, Guijarro JA. 2015. Development of a markerless deletion system for the fish-pathogenic bacterium *Flavobacterium psychrophilum*. PLoS One 10:e0117969. https://doi.org/10.1371/journal.pone.0117969.
15. Alvarez B, Secades P, Prieto M, McBride MJ, Guijarro JA. 2006. A mutation in *Flavobacterium psychrophilum tlpB* inhibits gliding motility and induces biofilm formation. Appl Environ Microbiol 72:4044–4053. https://doi.org/10.1128/AEM.00128-06.
16. Pérez-Pascual D, Gómez E, Álvarez B, Méndez J, Reimundo P, Navais R, Duchaud E, Guijarro JA. 2011. Comparative analysis and mutation effects of *fpp2–fpp1* tandem genes encoding proteolytic extracellular enzymes of *Flavobacterium psychrophilum*. Microbiology 157:1196–1204. https://doi.org/10.1099/mic.0.046938-0.
17. Pérez-Pascual D, Gómez E, Guijarro JA. 2015. Lack of a type-2 glycosyltransferase in the fish pathogen *Flavobacterium psychrophilum* determines pleiotropic changes and loss of virulence. Vet Res 46:1. https://doi.org/10.1186/s13567-014-0124-5.
18. Ewing B, Green P. 1998. Base-calling of automated sequencer traces using *Phred*. II. Error probabilities. Genome Res 8:186–194. https://doi.org/10.1101/gr.8.3.186.
19. Latreille P, Norton S, Goldman BS, Henkhaus J, Miller N, Barbazuk B, Bode HB, Darby C, Du Z, Forst S, Gaudriault S, Goodner B, Goodrich-Blair H, Slater S. 2007. Optical mapping as a routine tool for bacterial genome sequence finishing. BMC Genomics 8:321. https://doi.org/10.1186/1471-2164-8-321.
20. Bryson K, Loux V, Bossy R, Nicolas P, Chaillou S, van de Guchte M, Penaud S, Maguin E, Hoebeke M, Bessières P, Gibrat JF. 2006. AGMIAL: implementing an annotation strategy for prokaryote genomes as a distributed system. Nucleic Acids Res 34:3533–3545. https://doi.org/10.1093/nar/gkl471.