AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology Resource Announcements

| Environmental Microbiology | Announcement

Draft genome sequence of *Vibrio pectenicida* strain FHCF-3, a causative agent of sea star wasting disease in the sunflower sea star (*Pycnopodia helianthoides*), reveals the genetic potential to produce aerolysin-like toxins

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ABSTRACT Here, we report the genomic sequence of *Vibrio pectenicida* strain FHCF-3, isolated from the coelomic fluid of a sunflower sea star (*Pycnopodia helianthoides*) showing signs of wasting disease. The draft genome is 4,368,354 bp with 3,903 coding sequences, including 3 with significant sequence similarity to aerolysin-like toxins.

KEYWORDS sea star wasting disease, genomes, whole-genome sequencing (WGS), *Vibrio pectenicida*, aerolysin, sunflower sea star (*Pycnopodia helianthoides)*

Sunflower sea stars (*Pycnopodia helianthoides*) along the Pacific coast have been devastated by sea star wasting disease (SSWD) (1). Here, we report the draft genome of *Vibrio pectenicida* strain FHCF-3, isolated from the coelomic fluid of a sunflower sea star exhibiting signs of SSWD, collected near Friday Harbor, Washington, USA, in February 2024.

Coelomic fluid samples were withdrawn from the sea star coelomic cavity using a syringe fitted with a 26G needle. Samples were transported on ice to The University of British Columbia and spread onto MLB (2, 3) plates (0.05% each of casamino acids, peptone, and yeast extract, 0.3% glycerol, 1.2% agar, in 24 practical salinity units [PSU] seawater) within 8 hours of collection. After incubation at room temperature (ca. 21°C) for 5–7 days, individual colonies were picked and re-streaked repeatedly to achieve axenic clonal cultures of the bacterium. We used whole-genome sequencing (WGS) and phylogenetic analysis to identify FHCF-3 as a new strain of *Vibrio pectenicida*.

To prepare samples for WGS, marine broth (0.5% peptone, 0.1% yeast extract, and 24 PSU seawater) was seeded with clonal colonies and incubated at 18°C for 3 days. Cells were harvested by centrifugation at 11,000 × *g* for 5 minutes and sent to Seq-center (Pittsburgh, PA) for genomic DNA extraction and hybrid assembly sequencing (Nanopore/Illumina Combo), following Materials and Methods as described previously (4). Briefly, Illumina libraries were prepared with the Illumina DNA Library Prep Kit (Illumina Inc., San Diego, CA) and sequenced on a NextSeq2000 (151 bp paired end), yielding 22,547,344 short reads (Table 1). Nanopore libraries were prepared with the Oxford Nanopore Technologies (ONT, UK) Ligation-Sequencing Kit (no size selection for genomic DNA), sequenced on a MinION (R9.4.1 flow cell), and base called using Guppy v4.2.2, yielding 515,036 long reads (N_{50} = 6,342 bp). Adapters and low-quality reads were trimmed using bcl2fastq v2.19.0 (5) and Porechop v0.2.4 (6) for Illumina and ONT sequences, respectively. Unicycler v0.5.0 (7) was used to perform hybrid assembly of both read types. The draft genome of FHCF-3 is 4,368,354 bp in size, consisting of five contigs (N_{50} = 1,415,317 bp), with an average GC content of 41.5% (Table 1). Analysis

Editor Elinne Becket, California State University San Marcos, San Marcos, California, USA

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The authors declare no conflict of interest.

See the funding table on p. 4.

Received 1 April 2025
Accepted 24 May 2025
Published 14 August 2025

TABLE 1 Summary of the draft genome sequences of *Vibrio pectenicida* strain FHCF-3

Variable	Data
Species	*Vibrio pectenicida*
Strain	FHCF-3
NCBI accession no.	JBLZMR000000000
Isolation source	Sunflower sea star (*Pycnopodia helianthoides*) coelomic fluid
Source location	University of Washington Friday Harbor Laboratories, Friday Harbor, Washington, USA (48.5479° N, 123.0142° W)
Genome size (bp)	4,368,354
G + C content (%)	41.5
Mean coverage (×)	797
No. of raw reads (Illumina)	22,547,344
No. of raw reads (Nanopore)	515,036
No. of contigs	5 (Contigs 1–5)
N50 (bp)	1,415,317
No. of genes	4,024
No. of coding sequences (total)	3,903
No. of coding sequences (with protein)	3,735
No. of rRNAs	8, 9, 8 (5S, 16S, 23S)
No. of tRNAs	92
No. of ncRNAs	4
Locus positions of genes annotated to encode aerolysin-like toxins (e.g., aerolysin family beta-barrel pore-forming toxin)	195,744–197,188 In contig 1 420,801–422,249 In contig 3 1,428,404–1,429,882 In contig 3

using CheckM v1.0.18 (8) revealed that the FHCF-3 genome was 100% complete and had 0.45% contamination compared to other reference genomes in the database.

Annotation of the genome using the NCBI Prokaryotic Genome Annotation Pipeline v6.9 (9) predicted 3,903 coding sequences, and 25 rRNA, 4 ncRNA, and 92 tRNA genes, including 3 identified to encode proteins with sequence similarity to aerolysin (e.g., aerolysin family beta-barrel pore-forming toxin) (Table 1); a well-known pore-forming toxin that can disrupt cellular membranes and is commonly associated with virulence (10–13). Given FHCF-3 is the causative agent of SSWD (14), these aerolysin-like toxins may play a role in the disease process.

To determine the evolutionary relationship of strain FHCF-3 to other bacteria, a whole-genome phylogeny was constructed using GTDBtk v.2.3.2 (15). Strain FHCF-3 clustered in a well-supported clade (bootstrap support, 99.8/100) comprising members of *Vibrio pectenicida* (Fig. 1).

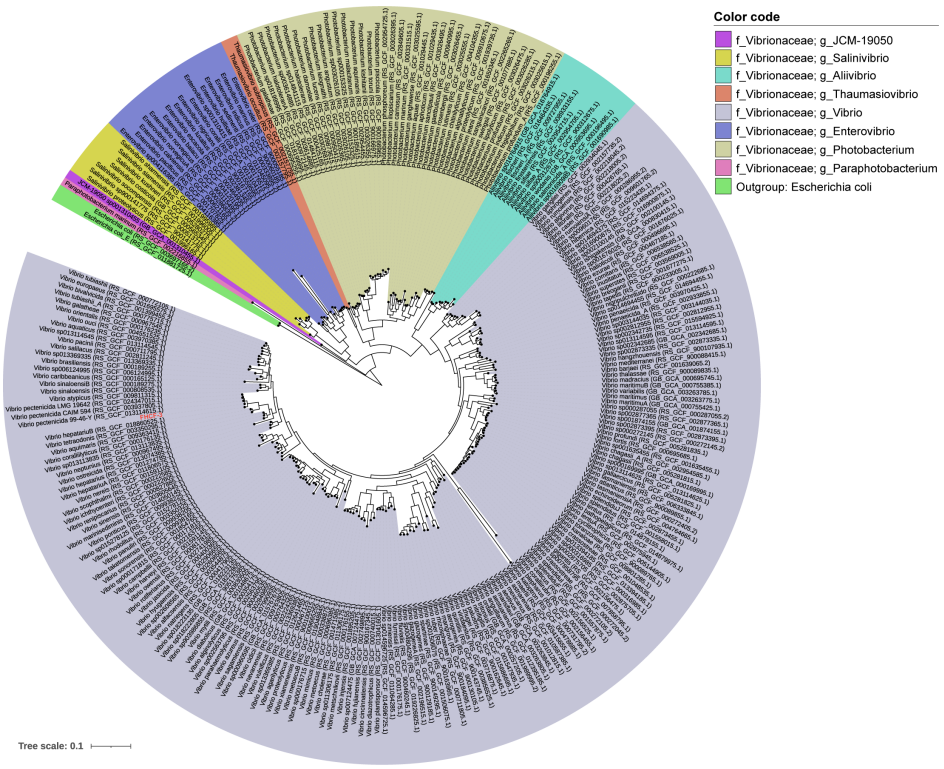

FIG 1 Phylogenomic tree of bacteria in the family Vibrionaceae. This phylogenomic tree illustrates the placement of strain FHCF-3 within the family Vibrionaceae, using genome data extracted from the Genome Taxonomy Database (GTDB, version 217) and NCBI Reference Sequence Database (as of 14 November 2024). The tree was constructed from genome sequences using GTDBtk v.2.3.2 (15) and rooted using *Escherichia coli* (GTDBtk accession numbers: RS_GCF_011881725.1 and RS_GCF_003697165.2) as an outgroup. Strain FHCF-3 is highlighted in red text.

Default parameters were used for all software.

ACKNOWLEDGMENTS

We thank Jason Hodin for providing access to sunflower sea stars for this study.

This work was supported by funds provided to C.A.S. from the Natural Sciences and Engineering Research Council of Canada (Discovery Grant RPGIN-2020-06515), The University of British Columbia, and the Canada Foundation for Innovation and British Columbia Knowledge Development Fund (Project #25412); a grant from the Tula Foundation to A.-L.M.G. and C.A.S., and a grant from The Nature Conservancy of California to D.H. Additional support was provided by an NSERC Undergraduate Student Research Award and a Biodiversity Research Centre Undergraduate Diversity Research Grant from the University of British Columbia to Y.G.

A.-L.M.G. and M.B.P. collected the coelomic fluid, A.M.C. isolated, cultured, and characterized the bacterium *V. pectenicida* strain FHCF-3. K.X.Z. led and conducted the bioinformatic analysis, Y.G. assisted. K.X.Z. and A.M.C. wrote the paper with input from all co-authors.

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FUNDING

Funder	Grant(s)	Author(s)
Natural Sciences and Engineering Research Council of Canada	Discovery Grant RPGIN-2020-06515	Curtis A. Suttle
The University of British Columbia	Biodiversity Research Centre Undergraduate Diversity Research Grant	Kevin Xu Zhong Yasmine Gouin Curtis A. Suttle
Canada Foundation for Innovation		Curtis A. Suttle
British Columbia Knowledge Development Fund	Project #25412	Curtis A. Suttle
Tula Foundation		Alyssa-Lois M. Gehman Curtis A. Suttle
Nature Conservancy of California		Drew Harvell
Natural Sciences and Engineering Research Council of Canada	Undergraduate Student Research Award	Yasmine Gouin Curtis A. Suttle

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Kevin Xu Zhong, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft, Writing – review and editing | Amy M. Chan, Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review and editing | Melanie B. Prentice, Resources, Writing – review and editing | Yasmine Gouin, Formal analysis, Investigation, Software, Writing – review and editing | Drew Harvell, Funding acquisition, Resources, Writing – review and editing | Alyssa-Lois M. Gehman, Funding acquisition, Resources, Writing – review and editing | Curtis A. Suttle, Conceptualization, Funding acquisition, Investigation, Project administration, Resources, Supervision, Validation, Writing – review and editing

DATA AVAILABILITY

The genome sequence of *V. pectenicida* strain FHCF-3 was deposited in GenBank under accession number JBLZMR000000000. Raw Nanopore and Illumina sequencing reads were deposited in NCBI SRA database under accession numbers SRR32620450 and

SRR32620451, respectively. Strain FHCF-3 is available upon request from the corresponding authors.

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