



# Draft Genome Sequences of Type VI Secretion System-Encoding *Vibrio fischeri* Strains FQ-A001 and ES401

Katherine M. Bultman,<sup>a</sup> Andrew G. Cecere,<sup>b</sup> Tim Miyashiro,<sup>b</sup> Alecia N. Septer,<sup>c</sup>  Mark J. Mandel<sup>a</sup>

<sup>a</sup>Department of Medical Microbiology and Immunology, University of Wisconsin–Madison, Madison, Wisconsin, USA

<sup>b</sup>Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, Pennsylvania, USA

<sup>c</sup>Department of Marine Sciences, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina, USA

**ABSTRACT** The type VI secretion system (T6SS) facilitates lethal competition between bacteria through direct contact. Comparative genomics has facilitated the study of these systems in *Vibrio fischeri*, which colonizes the squid host *Euprymna scolopes*. Here, we report the draft genome sequences of two lethal *V. fischeri* strains that encode the T6SS, FQ-A001 and ES401.

The bacterially encoded type VI secretion system (T6SS) is a membrane-embedded syringe-like structure that delivers effectors to bacterial and, in some cases, eukaryotic cells (1). A role for the T6SS has been demonstrated in mediating strain separation of incompatible *Vibrio fischeri* strains during colonization of *Euprymna scolopes* juvenile squid (2). *V. fischeri* FQ-A001 (3) and ES401 (4) are strains that were isolated decades apart, yet both exhibit the lethal phenotype, defined as the ability to kill *V. fischeri* ES114 in direct competition (2). Experimental analysis of FQ-A001 has demonstrated that a specific T6SS locus (T6SS2) is responsible for the observed lethal phenotype (2). Draft genomes for FQ-A001 and ES401, both of which have an intact T6SS2 locus, were obtained as follows.

Strain FQ-A001 was isolated from an *E. scolopes* adult female (21-mm mantle length) from Kaneohe Bay, Oahu, HI, in 2015, and was cultivated in the laboratory on LB salt (LBS) medium (3). For Illumina MiSeq sequencing (2 × 250 bp; Penn State Genomics Core Facility), genomic DNA was isolated using the MasterPure DNA purification kit (Epicentre, Madison, WI), and the library was constructed using the TruSeq DNA PCR-free kit (Illumina, San Diego, CA). For PacBio RS II single-molecule real-time (SMRT) sequencing (UNC Chapel Hill High-Throughput Sequencing Facility), genomic DNA was isolated by phenol-chloroform extraction, and the sequencing library was prepared using the SMRTbell library prep kit (10-kb size selection). Approximately 9.4 × 10<sup>8</sup> bp (MiSeq) and 1.1 × 10<sup>9</sup> bp (RS II) of sequence data were obtained, yielding approximately 470-fold coverage of the FQ-A001 genome. *De novo* assembly of the FQ-A001 genome was conducted using SPAdes version 3.13.0 (with the parameters “-careful -k 127”) (5), yielding four contigs of >1,000 bp. Contigs were reordered using Mauve Contig Mover, with the ES114 genome as a reference (6–8). For exploratory analysis, the genome was annotated with Prokka 1.13.3 (9), and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP; accessed 4 March 2019) was used to annotate the contigs for deposition to GenBank (10).

Strain ES401 was isolated from an *E. scolopes* juvenile (3.5-mm mantle length) from Maunalua Bay, Oahu, HI, in 1990 and can be cultivated in the laboratory on LBS medium (4). For Illumina MiSeq sequencing (2 × 300 bp; University of Wisconsin Biotechnology Center), genomic DNA was isolated using the Gram-negative bacterial protocol in the DNeasy blood and tissue kit (Qiagen USA, Germantown, MD), and the library was constructed using the TruSeq Nano kit (Illumina, San Diego, CA). PacBio sequencing

**Citation** Bultman KM, Cecere AG, Miyashiro T, Septer AN, Mandel MJ. 2019. Draft genome sequences of type VI secretion system-encoding *Vibrio fischeri* strains FQ-A001 and ES401. *Microbiol Resour Announc* 8:e00385-19. <https://doi.org/10.1128/MRA.00385-19>.

**Editor** Julie C. Dunning Hotopp, University of Maryland School of Medicine

**Copyright** © 2019 Bultman 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 Mark J. Mandel, [mmandel@wisc.edu](mailto:mmandel@wisc.edu).

**Received** 9 April 2019

**Accepted** 25 April 2019

**Published** 16 May 2019

**TABLE 1** *Vibrio fischeri* genomes described in this report

Strain	Yr isolated	Genome size (bp)	No. of contigs	$N_{50}$	Total no. of genes	G+C content (%)	GenBank accession no.	Genes corresponding to MJ11 T6SS2
FQ-A001	2015	4,286,900	4	2,417,280	4,015	38.3	SJSX00000000	VFFQA001_15465 to VFFQA001_15635
ES401	1990	4,282,653	6	1,351,222	4,011	38.3	SRJG00000000	VFES401_15680 to VFES401_15865

was conducted as described above for FQ-A001. Approximately  $2.7 \times 10^8$  bp (MiSeq) and  $1.8 \times 10^9$  bp (RS II) of sequence data were obtained, yielding approximately 450-fold coverage of the ES401 genome. Assembly, annotation, and GenBank deposition of the resulting 6 contigs of >1,000 bp were conducted as described above for FQ-A001 but with the SPAdes parameters “-careful -k 125” and with PGAP (accessed 2 April 2019).

For quality control, the original Illumina and PacBio base calling resulted in 0 sequences flagged as poor quality by FastQC (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc>). Upon submission to the PGAP, an additional fifth contig in the FQ-A001 sequence contained only the PhiX phage sequence and was excluded from the assembly and further analysis. PGAP additionally identified an Illumina adapter sequence at one location in the ES401 genome; we conducted Sanger sequencing of this region, which clarified the correct sequence. The correct sequence was resubmitted to PGAP, and the surrounding genomic context was not affected.

Analysis using progressiveMauve (snapshot 2015\_02\_25, macOS) revealed genomes that are colinear with MJ11 and the presence of the lethal strain-specific T6SS on chromosome II in both FQ-A001 and ES401 (Table 1) (2, 11, 12). Default parameters for software were used except where additional parameters are noted above.

**Data availability.** The data for this paper are available at the NCBI as FQ-A001 genome accession number [SJSX00000000](https://ncbi.nlm.nih.gov/assembly/SJSX00000000) (the version reported here is the first version, SJSX01000000), FQ-A001 read accession numbers [SRR8647323](https://ncbi.nlm.nih.gov/sra/SRR8647323) and [SRR8647324](https://ncbi.nlm.nih.gov/sra/SRR8647324), ES401 genome accession number [SRJG00000000](https://ncbi.nlm.nih.gov/assembly/SRJG00000000) (first version, SRJG01000000), and ES401 read accession numbers [SRR8708068](https://ncbi.nlm.nih.gov/sra/SRR8708068) and [SRR8708069](https://ncbi.nlm.nih.gov/sra/SRR8708069).

## ACKNOWLEDGMENTS

We gratefully acknowledge Ned Ruby for providing strain ES401 and Ella Rotman for assistance with sample preparation.

This work was funded by NIH grants R35GM119627 (to M.J.M.), R01GM129133 (to T.M.), and R21AI117262 (to M.J.M.) and NSF grant IOS-1757297 (to M.J.M.). Trainee support was provided on NIH grant T32GM008349 (to K.M.B.). This work used the Jetstream service via the Extreme Science and Engineering Discovery Environment (XSEDE) through allocation MCB180165 (13, 14).

## REFERENCES

- Coulthurst S. 2019. The type VI secretion system: a versatile bacterial weapon. *Microbiology* 165:503–515. <https://doi.org/10.1099/mic.0.000789>.
- Speare L, Cecere AG, Guckes KR, Smith S, Wollenberg MS, Mandel MJ, Miyashiro T, Septer AN. 2018. Bacterial symbionts use a type VI secretion system to eliminate competitors in their natural host. *Proc Natl Acad Sci U S A* 115:E8528–E8537. <https://doi.org/10.1073/pnas.1808302115>.
- Sun Y, LaSota ED, Cecere AG, LaPenna KB, Larios-Valencia J, Wollenberg MS, Miyashiro T. 2016. Intraspecific competition impacts *Vibrio fischeri* strain diversity during initial colonization of the squid light organ. *Appl Environ Microbiol* 82:3082–3091. <https://doi.org/10.1128/AEM.04143-15>.
- Ruby EG, Lee KH. 1998. The *Vibrio fischeri*-*Euprymna scolopes* light organ association: current ecological paradigms. *Appl Environ Microbiol* 64:805–812.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Rissman AI, Mau B, Biehl BS, Darling AE, Glasner JD, Perna NT. 2009. Reordering contigs of draft genomes using the Mauve aligner. *Bioinformatics* 25:2071–2073. <https://doi.org/10.1093/bioinformatics/btp356>.
- Ruby EG, Urbanowski M, Campbell J, Dunn A, Faini M, Gunsalus R, Lostroh P, Lupp C, McCann J, Millikan D, Schaefer A, Stabb E, Stevens A, Visick K, Whistler C, Greenberg EP. 2005. Complete genome sequence of *Vibrio fischeri*: a symbiotic bacterium with pathogenic congeners. *Proc Natl Acad Sci U S A* 102:3004–3009. <https://doi.org/10.1073/pnas.0409900102>.
- Mandel MJ, Stabb EV, Ruby EG. 2008. Comparative genomics-based investigation of resequencing targets in *Vibrio fischeri*: focus on point miscalls and artefactual expansions. *BMC Genomics* 9:138. <https://doi.org/10.1186/1471-2164-9-138>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. *Nucleic Acids Res* 44:6614–6624. <https://doi.org/10.1093/nar/gkw569>.

11. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 5:e111147. <https://doi.org/10.1371/journal.pone.0011147>.
12. Mandel MJ, Wollenberg MS, Stabb EV, Visick KL, Ruby EG. 2009. A single regulatory gene is sufficient to alter bacterial host range. *Nature* 458: 215–218. <https://doi.org/10.1038/nature07660>.
13. Stewart CA, Turner G, Vaughn M, Gaffney NI, Cockerill TM, Foster I, Hancock D, Merchant N, Skidmore E, Stanzione D, Taylor J, Tuecke S. 2015. Jetstream: a self-provisioned, scalable science and engineering cloud environment, p 1–8. *In* Proceedings of the 2015 XSEDE Conference on Scientific Advancements Enabled by Enhanced Cyberinfrastructure–XSEDE '15. ACM Press, New York, NY.
14. Towns J, Cockerill T, Dahan M, Foster I, Gauthier K, Grimshaw A, Hazelwood V, Lathrop S, Lifka D, Peterson GD, Roskies R, Scott JR, Wilkins-Diehr N. 2014. XSEDE: accelerating scientific discovery. *Comput Sci Eng* 16:62–74. <https://doi.org/10.1109/MCSE.2014.80>.