AMERICAN SOCIETY FOR MICROBIOLOGY
genomeAnnouncements

Draft Genome Sequence of *Piscirickettsia litoralis*, Isolated from Seawater

Xuehua Wan,[a] Alex J. Lee,[b] Shaobin Hou,[a] Blake Ushijima,[b] Yen P. Nguyen,[b] Jessica A. Thawley,[b] Stuart P. Donachie[a,b]

Advanced Studies in Genomics, Proteomics and Bioinformatics, University of Hawai'i at Mānoa, Honolulu, Hawai'i, USA[a]; Department of Microbiology, University of Hawai'i at Mānoa, Honolulu, Hawai'i, USA[b]

One species of *Piscirickettsia*, a pathogen of salmonid fish, has been described. The genome sequence of a putative second and free-living species may provide insights into the evolution of pathogenicity in the genus.

Received 12 September 2016 **Accepted** 12 September 2016 **Published** 3 November 2016

Citation Wan X, Lee AJ, Hou S, Ushijima B, Nguyen YP, Thawley JA, Donachie SP. 2016. Draft genome sequence of *Piscirickettsia litoralis*, isolated from seawater. Genome Announc 4(6):e01252-16. doi:10.1128/genomeA.01252-16.

Address correspondence to Stuart P. Donachie, donachie@hawaii.edu.

Strain Y2 was cultivated from seawater at Waimanalo Beach Park, O'ahu, Hawai'i. The nearest neighbor based on 16S rRNA gene nucleotide identity is *Piscirickettsia salmonis* LF-89^T (97.3%), an economically important obligate intracellular pathogen of salmonids (1, 2). We sequenced the genome of Y2 to investigate the evolutionary relationship between free-living Y2 and the pathogenic *P. salmonis* LF89^T and to provide insights into their lifestyles.

Genomic DNA was isolated from Y2 with the Wizard genomic DNA purification kit (Promega, USA), additional cetyltrimethylammonium bromide (CTAB) and phenol extraction steps, and a 30% ethanol wash to remove excess exopolysaccharides. Roche 454 GS FLX+ pyrosequencing generated 66.6 Mb of shotgun sequences and 29.1 Mb of 8-kb paired-end sequences. Newbler 2.8 built 16 scaffolds spanning 3,897,429 bp (scaffold N_{50}, 3,047,478 bp), with 263 contigs oriented in the scaffolds. The G+C content is 39.9%.

The Y2 genome was annotated in the NCBI's Prokaryotic Genome Annotation Pipeline (PGAP), Rapid Annotation using Subsystem Technology (RAST), and Prokka 1.11 (3–6). PGAP identified 3,198 protein-coding genes, 48 tRNA-coding regions, and 576 pseudogenes. RAST identified 4,095 protein-coding genes and 381 subsystems. The Y2 genome is ~11% larger and encodes ~16% more genes than that of *P. salmonis* LF-89^T, yet it contains one-sixth the number of virulence, disease, and defense genes, as annotated in RAST (7). A total of 4,266 proteins annotated by Prokka were run in BLASTP against Vir/Dot/Icm proteins of the type IV secretion system (T4SS) in the T346Hunter database (8); 4 proteins related to vir-T4SS assembly, including VirB4, VirB9, VirB11, and PtlG, were initially predicted ($E < 1e^{10}$). The Y2 proteome lacks homologs of most Vir proteins in the vir-T4SS core components, but 69 proteins were initially predicted to be homologs in Dot/Icm T4SS ($E < 1e^{-10}$). Characterization of Y2 and further analysis of the genome will determine if the strain represents the second *Piscirickettsia* species and shed light on the strain's lifestyle and evolution of *Piscirickettsia* species.

Accession number(s). This whole-genome shotgun project has been deposited at DDBJ/EMBL/GenBank under accession number MDTU00000000. The version described here is the first version, MDTU01000000. The 16S ribosomal gene sequence has been deposited at GenBank under accession number KX757850.

ACKNOWLEDGMENTS

Y2 was first isolated and tentatively identified during an undergraduate Marine Microbiology Laboratory class (MICR401L).

FUNDING INFORMATION

This work, including the efforts of Xuehua Wan and Shaobin Hou, was funded by Advanced Studies in Genomics, Proteomics and Bioinformatics.

This work was also supported through A.J.L.'s participation in the NSF REU Site: Undergraduate Research Experiences in DNA-Based Discoveries in Hawaii's Biodiversity under award no. DBI1560491 to S.P.D. and Stephanie Kraft-Terry at the University of Hawai'i at Mānoa.

REFERENCES

1. **Fryer JL, Lannan CN, Giovannoni SJ, Wood ND.** 1992. *Piscirickettsia salmonis* gen. nov., sp. nov., the causative agent of an epizootic disease in salmonid fishes. Int J Syst Bacteriol **42:**120–126. http://dx.doi.org/10.1099/00207713-42-1-120.
2. **Pulgar R, Hödar C, Travisany D, Zuñiga A, Domínguez C, Maass A, González M, Cambiazo V.** 2015. Transcriptional response of Atlantic salmon families to Piscirickettsia salmonis infection highlights the relevance of the iron-deprivation defence system. BMC Genomics **16:**495. http://dx.doi.org/10.1186/s12864-015-1716-9.
3. **Angiuoli SV, Gussman A, Klimke W, Cochrane G, Field D, Garrity G, Kodira CD, Kyrpides N, Madupu R, Markowitz V, Tatusova T, Thomson N, White O.** 2008. Toward an online repository of standard operating procedures (SOPs) for (meta)genomic annotation. Omics **12:**137–141. http://dx.doi.org/10.1089/omi.2008.0017.
4. **Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O.** 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics **9:**75. http://dx.doi.org/10.1186/1471-2164-9-75.
5. **Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, Disz T, Edwards RA, Gerdes S, Parrello B, Shukla M, Vonstein V, Wattam AR, Xia F,**

Stevens R. 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Res **42:**D206–D214. http://dx.doi.org/10.1093/nar/gkt1226.

6. **Seemann T**. 2014. Prokka: rapid prokaryotic genome annotation. Bioinformatics **30:**2068–2069. http://dx.doi.org/10.1093/bioinformatics/btu153.24642063.
7. **Pulgar R, Travisany D, Zuñiga A, Maass A, Cambiazo V**. 2015. Complete genome sequence of *Piscirickettsia salmonis* LF-89 (ATCC VR-1361) a major pathogen of farmed salmonid fish. J Biotechnol **212:**30–31. http://dx.doi.org/10.1016/j.jbiotec.2015.07.017.
8. **Martínez-García PM, Ramos C, Rodríguez-Palenzuela P**. 2015. T346Hunter: a novel Web-based tool for the prediction of type III, type IV and type VI secretion systems in bacterial genomes. PLoS One **10:**e0119317. http://dx.doi.org/10.1371/journal.