




# Whole-Genome Sequences of Two *Pseudoalteromonas piscicida* Strains, DE1-A and DE2-A, with Strong Antibacterial Activity against *Vibrio vulnificus*

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**ABSTRACT** Highly vesiculated *Pseudoalteromonas piscicida* strains DE1-A and DE2-A were isolated from seawater and show bactericidal properties toward *Vibrio vulnificus* and other Gram-positive and Gram-negative bacteria. Here, we report the complete genome sequences of these two *P. piscicida* strains and identify proteolytic enzymes potentially involved in their antibacterial properties.

*Pseudoalteromonas piscicida* strains DE1-A and DE2-A are Gram-negative motile rod-shaped and pigmented gammaproteobacteria that were isolated from coastal seawater along the Delaware Bay (1). Their surfaces are highly vesiculated, and the vessels have been associated with antibacterial properties toward principally Gram-negative marine bacteria, especially vibrios (1). Like our previously fully sequenced strain, DE2-B (2), the vesicles of DE1-A and DE2-A appear to contain and secrete proteolytic enzymes capable of digesting the cell wall of competing organisms (1). Enzymes secreted from DE1-A and DE2-A are especially inhibitory toward *V. vulnificus* (1). *Pseudoalteromonas* spp. are known to produce other antimicrobial compounds, some of which have been associated with their pigments (3, 4). Strains DE1-A and DE2-A are yellow and orange pigmented, respectively. *Pseudoalteromonas* spp. have been found to be useful as probiotics in aquaculture (5–7) and as potential antifouling agents to reduce biofilm formation (8, 9).

The *Pseudoalteromonas* species strains were obtained from freshly collected seawater from the Cape May-Lewes ferry terminal, Lewes, DE (38°46'57.85"N; 75°07'04.73"W) and isolated on lawns of *V. parahaemolyticus* grown on polypeptone peptone medium supplemented with Bacto agar (Becton, Dickinson and Company, Sparks, MD) at 26°C for 72 h. Isolates formed plaques (clearings) in the *Vibrio* lawns with a small yellow (DE1-A) or orange (DE2-A) colony in the center of the plaque. For sequencing, plaque-purified isolates were grown on Difco marine agar (Beckton, Dickinson and Company) at 26°C for 18 h, and total DNA was extracted using a GeneJET total genomic DNA purification kit (Thermo Fisher Scientific, Waltham, MA) according to manufacturer instructions. Sequencing of the genomes was performed by the Genomics Core Facility, Clinical and Translational Research Institute, Drexel College of Medicine, Philadelphia, PA, with a Pacific Biosciences RS II (PacBio, Menlo Park, CA) system using P6-C4 chemistry on a single molecule real-time (SMRT) cell per genome. Coverage was 111× for DE1-A and 119× for DE2-A. *De novo* assembly was initially achieved using the Hierarchical Genome Assembly Process (HGAP) version 2.3 application with assembly polishing using Quiver (10). A custom Python script was used to remove poorly supported contigs. In addition, SMRT resequencing portal protocol RS\_Modification\_and\_Motif\_Analysis version 2.3 (PacBio) was included in the SMRT analysis pipeline. The

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**TABLE 1** Genome and chromosome sizes, annotation results, and GC contents for *P. piscicida* strains DE1-A and DE2-A

<i>Pseudoalteromonas</i> sp. strain and complete genome or chromosome	Size (bp)	No. of genes	No. of pseudogenes	No. of proteins	No. of rRNAs	No. of tRNAs	GC content (%)
DE1-A							
Complete genome	5,427,866	4,863	443	4,281	28	107	44.3
Chromosome 1	4,165,978	3,786	311	3,340	25	106	43.5
Chromosome 2	1,261,888	1,077	132	941	3	1	42.8
DE2-A							
Complete genome	5,317,000	4,752	360	4,250	31	107	43.4
Chromosome 1	4,128,178	3,738	Not listed	3,358	27	106	43.6
Chromosome 2	1,188,822	1,014	Not listed	892	4	1	42.9

assembly was subsequently confirmed and improved using HGAP version 3 and Circlator version 1.5.3. Default settings were used for the assemblies.

Information on the genome and chromosome sizes, annotation data obtained from the NCBI Prokaryotic Genome Annotation Pipeline (Bethesda, MD), and GC content are listed in Table 1. Annotation also revealed that strains DE1-A and DE2-A contain 6 and 7 serine proteases, respectively, which may contribute to this species' antibacterial properties, as previously reported (1).

**Data availability.** The complete genomic sequences of *P. piscicida* DE1-A and DE2-A have been deposited in GenBank under the accession numbers [CP031759](#) and [CP031760](#) (DE1-A) and [CP031761](#) and [CP031762](#) (DE2-A). The Sequence Read Archive (SRA) accession numbers are [SRP158122](#) and [SRP158519](#). The versions described in this paper are the first versions.

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## REFERENCES

- Richards GP, Watson MA, Needleman DS, Uknalis J, Boyd EF, Fay JP. 2017. Mechanisms for *Pseudoalteromonas piscicida*-induced killing of vibrios and other bacterial pathogens. *Appl Environ Microbiol* 83:e00175-17. <https://doi.org/10.1128/AEM.00175-17>.
- Richards GP, Needleman DS, Watson MA. 2017. Complete genome sequence of *Pseudoalteromonas piscicida* strain DE2-B, a bacterium with broad inhibitory activity toward human and fish pathogens. *Genome Announc* 5:e00752-17. <https://doi.org/10.1128/genomeA.00752-17>.
- Offret C, Desriac F, Le Chevalier P, Mounier J, Jégou C, Fleury Y. 2016. Spotlight on antimicrobial metabolites from the marine bacteria *Pseudoalteromonas*: chemodiversity and ecological significance. *Mar Drugs* 14:129. <https://doi.org/10.3390/md14070129>.
- Gram L, Melchiorson J, Bruhn JB. 2010. Antibacterial activity of marine culturable bacteria collected from a global sampling of ocean surface waters and surface swabs of marine organisms. *Mar Biotechnol* 12: 439–451. <https://doi.org/10.1007/s10126-009-9233-y>.
- Fjellheim AJ, Klinkenberg G, Skjermo J, Aasen IM, Vadstein O. 2010. Selection of candidate probiotics by two different screening strategies from Atlantic cod (*Gadus morhua* L.) larvae. *Vet Microbiol* 144:153–159. <https://doi.org/10.1016/j.vetmic.2009.12.032>.
- Goulden EF, Hall MR, Pereg LL, Høj L. 2012. Identification of an antagonistic probiotic combination protecting ornate spiny lobster (*Parulirus ornatus*) larvae against *Vibrio owensii* infection. *PLoS One* 7:e39667. <https://doi.org/10.1371/journal.pone.0039667>.
- Offret C, Rochard V, Laguerre H, Mounier J, Huchette S, Brillet B, Le Chevalier P, Fleury Y. 2018. Protective efficacy of a *Pseudoalteromonas* strain in European abalone, *Haliotis tuberculata*, infected with *Vibrio harveyi* ORM4. *Probiotics Antimicrob Proteins* <https://doi.org/10.1007/s12602-018-9389-8>.
- Klein GL, Soum-Soutéra E, Guede Z, Bazire A, Compère C, Dufour A. 2011. The anti-biofilm activity secreted by a marine *Pseudoalteromonas* strain. *Biofouling* 27:931–940. <https://doi.org/10.1080/08927014.2011.611878>.
- Rodrigues S, Paillard C, Dufour A, Bazire A. 2015. Antibiofilm activity of the marine bacterium *Pseudoalteromonas* sp. 3J6 against *Vibrio tapetis*, the causative agent of brown ring disease. *Probiotics Antimicrob Proteins* 7:45–51. <https://doi.org/10.1007/s12602-014-9173-3>.
- Chin C-S, Alexander DH, Marks P, Klammer AA, Drake J, Heiner C, Clum A, Copeland A, Huddleston J, Eichler EE, Turner SW, Korlach J. 2013. Nonhybrid finished microbial genome assemblies from long-read SMRT sequencing data. *Nat Methods* 10:563–569. <https://doi.org/10.1038/nmeth.2474>.