## **PROKARYOTES**



# Draft Genome Sequences of Seven 4-Formylaminooxyvinylglycine Producers Belonging to the *Pseudomonas fluorescens* Species Complex

gen@meAnnouncements™

### Rachel A. Okrent,<sup>a</sup> Viola A. Manning,<sup>a</sup> <sup>(D)</sup>Kristin M. Trippe<sup>a,b</sup>

AMERICAN SOCIETY FOR MICROBIOLOGY

USDA-ARS National Forage Seed Production Research Center, Corvallis, Oregon, USA<sup>a</sup>; Department of Crop and Soil Science, Oregon State University, Corvallis, Oregon, USA<sup>b</sup>

**ABSTRACT** Vinylglycines are nonproteinogenic amino acids that inhibit amino acid metabolism and ethylene production. Here, we report the draft genome sequences of seven isolates of *Pseudomonas* that produce 4-formylaminooxyvinylglycine, a compound known to inhibit the germination of grasses and the growth of specific plant-pathogenic bacteria.

The *Pseudomonas fluorescens* species complex is a particularly diverse group of bacteria that play important ecological functions in soil, plant, and animal systems. These ecological and agricultural functions, including plant growth promotion, induction of systemic resistance, and biological control, are often mediated by secondary metabolites (1-4). One compound that contributes to the utility of *P. fluorescens* is the nonproteinogenic amino acid 4-formylaminooxyvinylglycine (FVG) (5, 6). FVG has both herbicidal and antimicrobial properties (7, 8). The activity, production, and regulation of FVG have been studied in the sequenced strain *P. fluorescens* WH6 (9–12). However, little is known about the production of FVG in other strains of *P. fluorescens*.

In order to add to our understanding of the evolution and diversity of FVG biosynthesis, the genomes of seven additional FVG producers have been sequenced. These strains were isolated from environmental sources in the central Willamette Valley of Oregon, including the rhizosphere of grasses (*Poa* spp. and *Lolium perenne* L.) (6, 7, 13) and the surface of a basidiomycete.

Genomic DNA was extracted using PowerLyzer UltraClean Microbial DNA isolation kit (Mo Bio, Inc.). Sequencing libraries were prepared using the Nextera XT DNA library preparation kit (Illumina). High-throughput sequencing MiSeq runs were performed on a standard flow cell version 3, with 300-bp paired-end reads, at the Center for Genome Research and Biocomputing at Oregon State University. The reads were trimmed and assembled into contigs using CLC Genomics Workbench versions 8.0.3 and 8.5.1 (Qiagen) and manually inspected for quality. Contigs less than 500 nucleotides (nt), those less or more than  $\sim 3 \times$  average coverage, or those composed primarily of reads that could not be unambiguously placed were discarded. The remaining contigs were aligned to a reference sequence using the Move Contigs function (14) in Mauve version 2015-02-25 (15). Scaffolds were confirmed and filled in with sequence when possible, and additional contigs were discarded if they did not align to the reference and did not contain coding regions. The reordered multi-FASTA files for the draft genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline. Genes related to FVG production were manually reannotated. The characteristics and accession numbers of the draft genomes are summarized in Table 1. Pairwise average nucleotide identity

# Received 7 March 2017 Accepted 15 March 2017 Published 4 May 2017

Citation Okrent RA, Manning VA, Trippe KM. 2017. Draft genome sequences of seven 4formylaminooxyvinylglycine producers belonging to the *Pseudomonas fluorescens* species complex. Genome Announc 5:e00277-17. https://doi.org/10.1128/genomeA.00277-17.

**Copyright** © 2017 Okrent et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Kristin M. Trippe, Kristin.Trippe@ars.usda.gov.

		Genome size	No. of	N <sub>50</sub>	Mean	No. of	G+C
Strain	Accession no.	(Mb)	contigs	(kb)	coverage (×)	CDSs <sup>a</sup>	content (%)
AH4	MSDE0000000	6.40	41	346	255	5,743	60.5
A3422A	MSDK0000000	6.14	44	292	186	5,539	60.5
TDH40	MSDG0000000	6.14	56	233	104	5,545	60.5
G2Y	MSDJ0000000	6.14	29	345	248	5,534	60.5
P5A	MSDF0000000	6.83	66	241	173	6,091	60.2
E24	MSDI0000000	6.41	42	262	141	5,782	60.3
A342	MSDH0000000	6.12	46	274	217	5,339	59.8

### TABLE 1 Genome statistics

<sup>a</sup>CDSs, coding sequences.

(ANI) analysis was performed using the Microbial Species Identifier tool from the Joint Genome Institute (16).

Based on a comparison of pairwise ANI values, the sequenced isolates are predicted to belong to four different cliques within the *Pseudomonas fluorescens* species complex. Analysis of the gene contents of the strains revealed that all seven strains contain two chromosomal regions linked to the production of FVG (10). One is the *prtl-prtR* sigma factor/anti-sigma pair, which regulates FVG production in the strain *P. fluorescens* WH6 (11). The second is the 13-kb *gvg* cluster, containing multiple genes required for the production and transport of FVG (12).

**Accession number(s).** The whole-genome shotgun projects have been deposited at DDBJ/EMBL/GenBank under the accession numbers listed in Table 1. The versions described in this paper are the first versions of each project.

### **ACKNOWLEDGMENTS**

Research in the laboratory of K.M.T. is supported by ARS project no. 2072-21410-004-00D. Strain P5A was isolated by high school students in Alice Eldridge's microbiology class at Philomath High School in a project supported by the Agriculture and Food Research Initiative competitive grant no. 2012-67012-19868 from the USDA National Institute of Food and Agriculture to R.A.O.

#### REFERENCES

- Gross H, Loper JE. 2009. Genomics of secondary metabolite production by *Pseudomonas* spp. Nat Prod Rep 26:1408–1446. https://doi.org/10 .1039/b817075b.
- Loper JE, Hassan KA, Mavrodi DV, Davis EW, Jr, Lim CK, Shaffer BT, Elbourne LD, Stockwell VO, Hartney SL, Breakwell K, Henkels MD, Tetu SG, Rangel LI, Kidarsa TA, Wilson NL, van de Mortel JE, Song C, Blumhagen R, Radune D, Hostetler JB, Brinkac LM, Durkin AS, Kluepfel DA, Wechter WP, Anderson AJ, Kim YC, Pierson LS, III, Pierson EA, Lindow SE, Kobayashi DY, Raaijmakers JM, Weller DM, Thomashow LS, Allen AE, Paulsen IT. 2012. Comparative genomics of plant-associated *Pseudomonas* spp.: insights into diversity and inheritance of traits involved in multitrophic interactions. PLoS Genet 8:e1002784. https://doi.org/10 .1371/journal.pgen.1002784.
- Pieterse CM, Zamioudis C, Berendsen RL, Weller DM, Van Wees SC, Bakker PA. 2014. Induced systemic resistance by beneficial microbes. Annu Rev Phytopathol 52:347–375. https://doi.org/10.1146/annurev -phyto-082712-102340.
- Garrido-Sanz D, Meier-Kolthoff JP, Göker M, Martín M, Rivilla R, Redondo-Nieto M. 2016. Genomic and genetic diversity within the *Pseudomonas fluorescens* complex. PLoS One 11:e0150183. https://doi.org/10.1371/ journal.pone.0150183.
- Armstrong DJ, Azevedo M, Mills D, Bailey B, Russell B, Groenig A, Halgren A, Banowetz G, McPhail K. 2009. Germination-arrest factor (GAF): 3. Determination that the herbicidal activity of GAF is associated with a ninhydrin-reactive compound and counteracted by selected amino acids. Biol Contr 51:181–190. https://doi.org/10.1016/j.biocontrol.2009.06 .004.
- McPhail KL, Armstrong DJ, Azevedo MD, Banowetz GM, Mills DI. 2010.
  4-Formylaminooxyvinylglycine, an herbicidal germination-arrest factor

from *Pseudomonas* rhizosphere bacteria. J Nat Prod 73:1853–1857. https://doi.org/10.1021/np1004856.

- Banowetz GM, Azevedo MD, Armstrong DJ, Halgren AB, Mills DI. 2008. Germination-arrest factor (GAF): biological properties of a novel, naturally occurring herbicide produced by selected isolates of rhizosphere bacteria. Biol Contr 46:380–390. https://doi.org/10.1016/j.biocontrol .2008.04.016.
- Halgren A, Azevedo M, Mills D, Armstrong D, Thimmaiah M, McPhail K, Banowetz G. 2011. Selective inhibition of *Erwinia amylovora* by the herbicidally active germination-arrest factor (GAF) produced by *Pseudomonas* bacteria. J Appl Microbiol 111:949–959. https://doi.org/10 .1111/j.1365-2672.2011.05098.x.
- Kimbrel JA, Givan SA, Halgren AB, Creason AL, Mills DI, Banowetz GM, Armstrong DJ, Chang JH. 2010. An improved, high-quality draft genome sequence of the germination-arrest factor-producing *Pseudomonas fluorescens* WH6. BMC Genomics 11:522. https://doi.org/10.1186/1471-2164 -11-522.
- Halgren A, Maselko M, Azevedo M, Mills D, Armstrong D, Banowetz G. 2013. Genetics of germination-arrest factor (GAF) production by *Pseudomonas fluorescens* WH6: identification of a gene cluster essential for GAF biosynthesis. Microbiology 159:36–45. https://doi.org/10.1099/mic .0.062166-0.
- Okrent RA, Halgren AB, Azevedo MD, Chang JH, Mills DI, Maselko M, Armstrong DJ, Banowetz GM, Trippe KM. 2014. Negative regulation of germination-arrest factor production in *Pseudomonas fluorescens* WH6 by a putative extracytoplasmic function sigma factor. Microbiology 160: 2432–2442. https://doi.org/10.1099/mic.0.080317-0.
- Okrent RA, Trippe KM, Maselko M, Manning V. 2017. Functional analysis of a biosynthetic cluster essential for production of 4-formylaminooxyvinylglycine, a germination-arrest factor from *Pseudomonas fluore-*

scens WH6. Microbiology 163:207-217. https://doi.org/10.1099/mic.0 .000418.

- Elliott L, Azevedo M, Mueller-Warrant G, Horwath W. 1998. Weed control with rhizobacteria. Soil Sci Agrochem Ecol 33:3–7.
- Rissman Al, 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.
- 15. Darling AC, Mau B, Blattner FR, Perna NT. 2004. Mauve: multiple alignment of conserved genomic sequence with rearrangements. Genome Res 14:1394–1403. https://doi.org/10.1101/gr.2289704.
- Varghese NJ, Mukherjee S, Ivanova N, Konstantinidis KT, Mavrommatis K, Kyrpides NC, Pati A. 2015. Microbial species delineation using whole genome sequences. Nucleic Acids Res 43:6761–6771. https://doi.org/10 .1093/nar/gkv657.