Draft Genome Sequences of 10 Strains of *Pseudomonas syringae* pv. *actinidiae* Biovar 1: A Major Kiwifruit Bacterial Canker Pathogen in Japan

Takashi Fujikawa,[a#] Hiroe Hatomi,[a] Hiroyuki Sawada[b]

[a]Institute of Fruit Tree and Tea Science (IFTS), National Agriculture and Food Research Organization (NARO), Tsukuba, Ibaraki, Japan

[b]Genetic Resources Center (GRC), NARO, Tsukuba, Ibaraki, Japan

Running Head: 10 Genome Sequences of *P. s.* pv. *actinidiae* Biovar 1

[#]Address correspondence to Takashi Fujikawa, ftakashi@affrc.go.jp

Abstract

Several groups (biovars) of the kiwifruit bacterial canker pathogen, *Pseudomonas syringae* pv. *actinidiae*, are found in Japan. Here, we sequenced and compared the 10 genomes of biovar 1, the major group in Japan, which is known as the phaseolotoxin producer.

The kiwifruit bacterial canker pathogen, *Pseudomonas syringae* pv. *actinidiae* (Psa), was first described in Japan in 1989 (1). Subsequently, Psa was found in other kiwifruit-producing countries (2). Based on comparative analyses (2–4), Psa was categorized into several groups (biovars). The first Japanese group was named biovar 1 (Psa1), which was also later found in Italy and Korea. This biovar produces phaseolotoxin (2), a phytotoxin that inhibits arginine biosynthesis in host plants and results in bacterial canker symptom development. On the Psa1 chromosome, a large number of genes involved in phaseolotoxin biosynthesis are accumulated in an approximately 23 kb region (*argK-tox* cluster), which is contained in an exogenous genomic island (*tox* island) that Psa1 acquired in the past (2). However, some Psa1 strains found in Ehime Prefecture, Japan (the Ehime isolates) do not produce phaseolotoxin, although they seem to possess the *argK-tox* cluster (5). On the other hand, several Psa1 strains preserved in the NARO Genebank (https://www.gene.affrc.go.jp/index_en.php) may lack this cluster (2). Here, we selected 10 strains (Table 1) that represent Psa1 diversity and conducted comparative genome analyses.

Genomic DNA was extracted from the strains using the DNeasy mini kit (Qiagen, Hilden, Germany). Genomic DNA was sequenced using an Ion PGM sequencer with an Ion PGM Hi-Q View OT2 kit, an Ion PGM Hi-Q View Sequencing kit, and a 318 Chip

kit v2 (all from Thermo Fisher Scientific Inc., Waltham, MA, USA). The sequence reads were quality controlled (a quality score < 20) and adapter sequences were removed using CLC Genomics Workbench version 12 (Qiagen). Using these reads, contigs (filtered with a size longer than 500 bp) were assembled *de novo* using the same software with default parameters (mapping mode = Create simple contig sequences (fast), automatic bubble size = yes, minimum contig length = 500, automatic word size = yeas, performing scaffolding = yes, auto-detect paired distances = yes). The draft genomes were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) v4. 1 (6).

The guanine and cytosine (G+C) content and genome size for these strains were found to be 58.2–58.8% and 4.9–6.3 Mbp, respectively (Table 1). PGAP identified 5,606–6,432 genes, including multiple rRNA and tRNA genes. In addition, the *argK-tox* cluster of each strain was sequenced (Table 2) and compared with the reference genome (CM002753) of ICMP 9617 (pathotype strain of Psa), indicating that some strains have synonymous substitution (silent mutation) in the cluster. Moreover, in the Ehime isolates (MAFF 211981 and 211983), it was clarified that a frameshift mutation in the fatty acid desaturase gene occurred due to a single G insertion, resulting in the loss of the ability to produce phaseolotoxin. On the chromosomes of MAFF 613017, 613018, and 212324, the *tox* island containing the *argK-tox* cluster could not be found,

suggesting that these strains may not have experienced the island acquisition event. The fact that such diversification has occurred in the *argK-tox* cluster is an important piece of evidence for elucidating the pathogenicity, ecology and evolution of Psa1.

Data availability. All sequences identified in this study have been deposited in GenBank (see Tables 1 and 2 for accession numbers).

Acknowledgments

We are grateful to Ms. M. Taguchi and Ms. A. Sasaki for supporting this work. We also thank the members of IFTS-NARO and GRC-NARO for their helpful discussions. We would like to thank Editage (www.editage.jp) for English language editing. This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

References

1. Takikawa Y, Serizawa S, Ichikawa T, Tsuyumu S, Goto M. 1989. *Pseudomonas syringae* pv. *actinidiae* pv. nov.: the causal bacterial canker of kiwifruit in Japan. Ann Phytopath Soc Japan 55:437–444.
2. Sawada H, Fujikawa T. 2019. Genetic diversity of *Pseudomonas syringae* pv.

actinidiae, pathogen of kiwifruit bacterial canker. Plant Pathol 68:1235–1248.

3. Chapman JR, Taylor RK, Weir BS, Romberg MK, Vanneste JL, Luck J, Alexander BJR. 2012. Phylogenetic relationships among global populations of *Pseudomonas syringae* pv. *actinidiae*. Phytopathology 102:1034-1044.

4. Vanneste JL, Yu J, Cornish DA, Tanner DJ, Windner R, Chapman JR, Taylor RK, Mackay JF, Dowlut S. 2013. Identification, virulence, and distribution of two biovars of *Pseudomonas syringae* pv. *actinidiae* in New Zealand. Plant Dis 97:708-719.

5. Miyoshi T, Shimizu S, Sawada H. 2012. Occurrence and distribution of a defective non-phaseolotoxin-producing mutant of *Pseudomonas syringae* pv. *actinidiae* in Ehime Prefecture, Japan (in Japanese with English summary). Jpn J Phytopathol 78:92-103.

6. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zalsavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI Prokaryotic Genome Annotation Pipeline. Nucleic Acids Res 44:6614-6624.

Table 1. Genome data and accession numbers of 10 strains of *Pseudomonas syringae* pv. *actinidiae* biovar 1.

Strain information				Genome information				PGAP[a] annotation			Reads information			
Strain	Isolation host	Isolation area	Isolation year	GenBank accession no.	Genome size (bp)	G+C content (mol %)	No. of contigs	N_{50}	Total no. of genes	rRNAs (5S, 16S, 23S)	tRNAs	SRA[b] accession no.	No. of reads	Average length (bp)	Genome coverage (x)
MAFF 302091	*Actinidia deliciosa*	Kanagawa Prefecture (Pref.)	1984	JAAEYK000000000	4,916,203	58.2	2,497	2,502	6,374	2, 1, 1	29	SRR11730631	60,196	236	7.7
MAFF 302133	*A. arguta*	Kanagawa Pref.	1987	JAAEYI000000000	5,928,911	58.8	653	14,454	5,654	3, 1, 1	39	SRR11730639	262,576	221	26.4
MAFF 302145	*A. deliciosa*	Wakayama Pref.	1988	JAAEYG000000000	5,164,482	58.4	2,311	3,012	6,432	3, 1, 2	28	SRR11730634	97,845	210	8
MAFF 613024	*A. deliciosa*	Shizuoka Pref.	1995	JAAEYH000000000	4,927,103	58.2	2,470	2,552	6,335	2, 1, 1	28	SRR11730633	57,728	235.6	7.2
MAFF 211985	*A. deliciosa*	Ehime Pref.	2000	SMHD00000000	5,951,025	58.8	475	26,121	5,880	2, 1, 1	44	SRR11730626	286,416	231.9	100.1
MAFF 211981	*A. deliciosa*	Ehime Pref.	2000	JAAEYJ000000000	5,947,905	58.8	524	19,906	5,628	3, 1, 1	41	SRR11730636	527,346	221.5	42.3

MAFF 211983	*A. deliciosa*	Ehime Pref.	2000	JAAEYF00 0000000	5,236,580	58.4	2,155	3,303	6,342	1, 1, 1	34	SRR11730 632	114,619	230.7	8.6
MAFF 613017	*A. deliciosa*	Shizuoka Pref.	1986	JAAEYL00 0000000	5,999,477	58.8	494	23,006	5,606	3, 1, 1	44	SRR11730 635	813,046	215.5	65
MAFF 613018	*A. deliciosa*	Shizuoka Pref.	1986	JAAEYM00 0000000	5,821,751	58.8	1,046	9,663	5,914	1, 1, 1	39	SRR11730 637	151,420	215.5	16.3
MAFF 212324	*A. deliciosa*	Shizuoka Pref.	unknown	JAAEYN00 0000000	6,327,049	58.6	609	17,836	6,120	2, 1, 1	37	SRR11730 638	407,967	237.2	34.9

[a]NCBI Prokaryote Genome Annotation Pipeline

[b]Sequence Read Archive

94 **Table 2. Detail and accession numbers for the *argK-tox* gene cluster of 10 strains of *Pseudomonas syringae* pv. *actinidiae* biovar 1.**

	***argK-tox* gene cluster information**[a]			
Strain	**Phaseolotoxin synthesis**	***argK-tox* gene cluster**	***argK-tox* gene cluster accession no.**	**Detail**
MAFF 302091	+	+	MT551019	Same sequence as ICMP9617
MAFF 302133	+	+	MT551015	Same sequence as ICMP9617
MAFF 302145	+	+	MT551014	Same sequence as ICMP9617
MAFF 613024	+	+	MT551013	Same sequence as ICMP9617

MAFF 211985	+	+	MT551017	Synonymous substitution (silent mutation) in some coding genes against ICMP9617
MAFF 211981	-	+	MT551016	Synonymous substitution (silent mutation) in some coding genes against ICMP9617 Frameshift mutation in the fatty acid desaturase gene due to the insertion of a single 'G '
MAFF 211983	-	+	MT551018	Synonymous substitution (silent mutation) in some coding genes against ICMP9617 Frameshift mutation in the fatty acid desaturase gene due to the insertion of a single 'G'
MAFF 613017	-	-	-	Absence of *tox* island
MAFF 613018	-	-	-	Absence of *tox* island
MAFF 212324	-	-	-	Absence of *tox* island

[a] + denotes presence, - denotes absence