The complete genome sequence of *Pseudomonas syringae* pv. *actinidifoliorum* ICMP 18803

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Abstract

The complete genome of *Pseudomonas syringae* pv. *actinidifoliorum* ICMP18803 (Pfm) was sequenced using the Oxford Nanopore minION platform to an average read depth of 123. The genome assembled into a single chromosome of 6,353,853 bp after error-correction with Illumina short reads using Pilon. The complement of effector genes from a *P. syringae* pathovar plays the predominant role in defining its pathogenicity. Automatic gene annotation pipelines often poorly identify and name effector genes, however. Despite Pfm being a relatively weak pathogen of kiwifruit, a set of 31 effectors, 26 of which were full length, was identified by mapping the comprehensive effector library generated by Dillon et al. (2019). The Pfm genome with the effector complement, correctly named and annotated was resubmitted to Genbank (CP081457).

Pseudomonas syringae pv. *actinidifoliorum* (Pfm) was first isolated and characterised from kiwifruit orchards in New Zealand in 2010 (Chapman et al. 2012). It was initially classified as *Pseudomonas syringae* pv. *actinidiae* (Psa) group 4 (or LV for low virulence) due to the high degree of similarity between sequences used for multi-locus sequence analysis (MLSA) between it and other Psa strains (Chapman et al. 2012). Extensive pathogenicity testing of Pfm isolates using several different assay methods determined that this collection of strains caused a distinct and milder set of symptoms on kiwifruit compared to Psa. In contrast to the highly aggressive isolates of Psa biovar 3 (Psa3) which cause leaf-spotting, cane dieback and weeping trunk cankers, particularly on cultivars of *Actinidia chinensis* var *chinensis*, Pfm symptomology is largely restricted to mild leaf spotting (Ferrante and Scortichini 2015; Vanneste et al. 2013). Furthermore, *in planta* bacterial growth and movement assays revealed that Pfm grew to a maximum of 10^5-10^6 colony forming units per square centimetre, as opposed to 10^8 for Psa3 and was capable of only limited systemic movement (Jayaraman et al. 2020; McAtee et al. 2018; McCann et al. 2013). For these reasons, Pfm was given its own pathovar designation (Cunty et al. 2015).

Pfm has been found to have a widespread distribution throughout kiwifruit growing regions in Europe, Australasia and Asia (Abelleira et al. 2015; Cunty et al. 2015; Vanneste et al. 2013). A phylogenetic tree generated from a multi-locus sequence analysis revealed significant genetic variation between these isolates which resolved into four lineages (Cunty et al. 2015). This suggests that there has been a long-term association between Pfm and *Actinidia* spp. that precedes the international outbreak of its more pathogenic relative Psa biovar 3.

Several groups have released short-read assemblies of various Pfm isolates (Butler et al. 2013; Cunty et al. 2016; McCann et al. 2013). In this paper we report the complete sequence of Pfm ICMP18803 isolated from Hawke's Bay, New Zealand in 2010 (PRJNA167409; SAMN13855180). Genomic DNA from Pfm ICMP18803 was purified using a GenePure kit from Qiagen (Hilden, Germany) as described in McCann et al. (2013). Purified DNA was sequenced using the Oxford Nanopore minION platform to a read depth of 123 fold. The genome was assembled into a single contig using Flye 2.7.1, and error-corrected with Illumina short reads using Pilon 1.23 (Kolmogorov et al. 2020; Walker et al. 2014). All suggested short indel corrections were accepted. The resulting single chromosome was 6,353,853 bp in length. The genome was annotated using the PGAAP pipeline (Li et al. 2021). Nanopore and Illumina reads were deposited to the Sequence Read Archive (PRJNA167409).

Although isolates of Pfm presented only weak symptoms on kiwifruit, the genome has 31 effectors, of which 26 are full-length and expected to be active, plus secondary metabolite pathways which might contribute to pathogenicity (Tables 1 and 2). The PGAAP and other automatic annotation pipelines often annotate *P. syringae* effectors incorrectly. The Pfm effector complement was manually curated and annotated using the nomenclature used by Dillon et al. (2019). This was achieved by mapping all effectors from *P. syringae* in supplementary file 3 from Dillon et al. (2019) to the Pfm 18803 genome using the map to reference function in Geneious 10 (Biomatters). Effector alleles with 100% homology to the Pfm ICMP18803 genome were used to accurately annotate the start and stop sites and assign the correct name of the effector family (Table 1). One previously undiscovered effector that formed a new clade in the AvrPto1 phylogeny, AvrPto1r,

was identified. This effector was co-located with HopF1b, HopAR1e, HopAF1b and HopAB1e which may be equivalent to the Exchangeable Effector Locus (EEL) in *P. syringae* pv. *tomato* and Psa, although the EEL is in a different location from the Conserved Effector Locus and the Type Three Secretion System (Alfano et al. 2000; McCann et al. 2013).

The effector complement from different isolates or lineages within a *P. syringae* pathovar often varies, for example within biovars of Psa (McCann et al. 2013). This was also the case for Pfm (Figure 1). Pfm isolates from Europe and New Zealand largely had a conserved effector complement. The more recently discovered isolates from Japan, which form two new lineages, however were missing up to seven of these effectors (Figure 1). Examination of the regions around these effectors suggested that they were lost or acquired via elements associated with horizontal gene transfer.

P. syringae pathovars can produce a range of toxic compounds, some of these, such as phaseolotoxin, coronatine and tabtoxin, have been well-characterised (Bender et al. 1999). Different biovars of Psa have been shown to produce various combinations of phaseolotoxin and coronatine (Fujikawa and Sawada 2019). Pfm does not produce any of the classic *P. syringae* phytotoxins, however analysis using AntiSMASH 6.0 (Blin et al. 2021) revealed nine secondary metabolite pathways (Table 2). The only characterised compound with a potential role in pathogenicity is mangotoxin, also known as Pseudomonas virulence factor (Carrión et al. 2012; Morgan et al. 2019). Other metabolites that might have a role in epiphytic fitness include various siderophores, the surfactant syringafactin and *N*-acetylglutaminylglutamine, which has a role in osmotic stress (Table 2). Of interest is the presence of the novel 3-thiaglutamate biosynthetic pathway, which uses a small

peptide as a scaffold to synthesise a novel small molecule (Ting et al. 2019). This pathway has a limited distribution among *P. syringae* pathovars, with the majority of BLAST hits to *actinidifoliorum* and closely related *thea* pathovars.

The Pfm ICMP18803 genome was annotated using the NCBI genome workbench and resubmitted to GenBank (Kuznetsov and Bollin 2021). The availability of long reads greatly facilitated the correct annotation of effector genes and their relative location in the genome. Although Pfm is a poor pathogen of kiwifruit, a number of its reasonably extensive effector complement are present in Psa (McCann et al. 2013). This makes the comparison between the effector complements of Pfm and Psa a useful model for understanding how bacterial pathogens cause disease.

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Author-Recommended Internet Resources

Oxford Nanopore Technologies: https://nanoporetech.com/

Geneious (Biomatters): https://www.geneious.com/

Flye: https://github.com/fenderglass/Flye

Pilon: https://github.com/broadinstitute/pilon

AntiSMASH: https://antismash.secondarymetabolites.org

125 Table 1

Effector	locus tag	Old name	Full CDS	Chaperone	Hrp boxL
HopY1d	A237_00695	HopY1	premature stop	n/a	yes
HopBO1c	A237_00840	HopX2	yes	n/a	yes
HopAS1b	A237_02165	HopAS1	yes	n/a	yes
HopW1f	A237_04025	HopW1	yes	n/a	yes
HopR1b	A237_04035	HopR1	yes	n/a	yes
HopAG1d	A237_04175	HopAG1	yes	A237_04173	yes
HopAH1k	A237_04180	HopAH1	yes	n/a	yes (HopAG1)
HopAI1e	A237_04185	HopAI1	yes	n/a	yes (HopAG1)
HopF1b	A237_05265	HopF1	yes	A237_05260	yes
HopAR1e	A237_05275	HopAR1	yes	n/a	yes
AvrPto1r	A237_05285	N/A	premature stop	n/a	yes
HopAF1b	A237_05320	HopAF1	yes	n/a	yes (HopAB1)
HopAB1e	A237_05325	HopAY1	yes	n/a	yes
HopN1a	A237_06825	HopN1	yes	A237_06820	yes
HopAA1d	A237_06835	HopAA1	yes	n/a	yes
HopM1f	A237_06850	HopM1	yes	A237_06845	yes
AvrE1d	A237_06865	AvrE1	yes	A237_06855	yes
HopX1d	A237_07010	HopX1	yes	n/a	yes (HrpK1)
HopAZ1a	A237_09620	HopAZ1	yes	n/a	yes
HopAH1a	A237_11260	HopAH2-1	yes	n/a	no
HopAH1i	A237_11265-75	HopAH2-2	Tn inactivated	n/a	no
HopW1c	A237_11645	HopW1	yes	n/a	yes
HopAB1i	A237_12225	HopAB3	yes	n/a	yes
HopE1a	A237_18035	HopE1	yes	n/a	yes
HopAF1f	A237_20845	HopAF1-2	yes	n/a	yes
HopS2c	A237_23320	HopS2	yes	A237_23325	yes
HopT1c	A237_23330	HopT1	yes	n/a	yes (HopO2b)
HopO1a	A237_23335	HopO1	yes	n/a	yes (HopO2b)
HopO2b	A237_23340-50	HopS1	Tn inactivated	A237_23355	yes
HopI1c	A237_23700	HopI1	premature stop	n/a	yes
HopA1a	A237_26495	HopA1	yes	A237_26490	yes

126

127 Table 1. The effector complement of *Pseudomonas syringae* pv. *actinidifoliorum*
128 ICMP18803. Effectors were identified using the comprehensive *P. syringae* effector
129 library compiled by Dillon et al. (2019). These genes were mapped to the
130 *Pseudomonas syringae* pv. *actinidifoliorum* genome in Geneious
131 (https://www.geneious.com) using the map to reference function. This was used to
132 determine the correct stop and start sites and to assign the correct family
133 designation for each effector.

134

135 Table 2

136

Antismash ID	Gene loci	location	Function/product	Reference
1	A237_02300-55	512957-525196	Pyrrolo-quinoline-quinone	(Schnider et al. 1995)
2	A237_13620-75	3078634-3092789	Achromobactin	(Berti and Thomas 2009)
3	A237_13780-805	3114737-3146952	Syringafactin	(Berti et al. 2007)
4	A237_15210-90	3468871-3507069	Yersinabactin	(Jones et al. 2007)
5	A237_17675-780	4049667-4106763	Pyoverdine	(Jones et al. 2007)
6	A237_17945-90	4138999-4150353	3-thiaglutamate	(Ting et al. 2019)
7	A237_20270-90	4633641-4638941	N-acetylglutaminylglutamine	(Beattie et al. 2016)
8	A237_25245-340	5767408-5786589	Unknown	
9	A237_27070-85	6181744-6187662	Mangotoxin	(Carrión et al. 2012)

137

138 Table 2. List of biosynthetic pathways identified using AntiSMASH.

139

Figure 1

MLST UPGMA tree	Isolate	HopY1d_1	HopBO1c_1	HopAS1b_1	HopW1f_1	HopR1b_1	HopAG1d_1	HopAH1k_1	HopAI1e_1	HopF1b_1	HopAR1e_1	AvrPto1r_1	HopAF1b_1	HopAB1e_1	HopN1a_1	HopAA1d_1	HopM1f_1	AvrE1d_1	HopX1d_1	HopAZ1a_1	HopAH1a_1	HopAH1i_1	HopW1c_1	HopAB1i_1	HopE1a_1	HopAF1f_1	HopS2c_1	HopT1c_1	HopO1a_1	HopO2b_1	HopI1c_1	HopA1a_1
	L1a-ICMP18803	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
	L1b-ICMP19497	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100.0	100	100	100	100
	L2-CFBP8043	98.2	100	99.1	99.3	99.1	98.4	93.7	99	100	100	100	99.95	99.9	99.8	100	99.7	99.0	99.8	100	99.1	99.8	99.9	99.7	100	97.9	97.9	99.9	100	98	94.0	99.6
	L4-CFBP8160	98.2	99.9	99.1	99.3	99.1	98.4	93.7	99	100	100	100	99.95	99.9	99.8	99.7	99.8	99.0	99.8	100	99.1	99.8	99.7	99.7	100	97.9	96.8	99.9	99.9	99	94.0	99.5
	L3-ICMP18807	98.4	99.8	99.7	100	99.7	100	100	100	100	100	100	100	100	99.9	98.9	99.8	99.1	99.9	100	99.4	99.9	99.7	99.8	99.7	98.6	100	99.9	100	100	94.0	99.9
	L5-MAFF212156	96.7	0.0	98.4	99.3	98.9	96.6	98.1	99	0	0	0	0	0	99.6	99.7	99.6	98.0	99.9	0	98.9	99.5	99.7	99.5	99.5	98.8	100	99.8	99.7	99	96.1	0
	L6-MAFF212171	97.1	0.0	98.3	99.3	98.8	95.2	92.1	99	0	0	0	0	0	99.6	99.6	99.5	98.8	99.9	0	99.0	99.4	99.6	99.3	0	98.4	97.2	99.9	100	100	95.5	99.7

Figure 1. Comparison of the effector complement from different lineages of *Pseudomonas syringae* pv. *actinidifoliorum*. BLAST was used to search representative genomes from each lineage of *Pseudomonas syringae* pv. *actinidifoliorum* (Pfm) with every effector from Pfm ICMP18803. The first column is an MLST tree using the core genes *gyrB*, *ropD*, *gapA*, *pgi*, *acnB*. The percentage homology at the nucleotide level is given for each result. Green boxes indicate a full-length coding sequence, and red boxes indicate the presence of a premature stop codon or a transposon insertion. Orange boxes indicate that it could not be ascertained whether the gene could produce a full-length protein, usually because the gene spanned two contigs in the short read assembly.

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