Analysis of Draft Genome Resources of Thirty-Three Canadian Strains of *Pseudomonas syringae* pv. *tomato* Isolated Between 1992 and 2008 Reveals Achromobactin Virulence Cluster that Is Absent in the Reference Strain DC3000

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Genome Resource Announcement

Pseudomonas syringae pv. *tomato* (Okabe 1933) Young et al. 1978 is the causal agent of bacterial speck disease of field and greenhouse tomato plants, and is an economically important disease of tomato (*Lycopersicon esculentum*). Only one Canadian whole-genome sequence of this economically important pathogen has been publicly available in NCBI GenBank. In 1992 to 2008, annual surveys of tomato fields and greenhouses were conducted in Ontario, Canada, and leaves, stems, and fruit exhibiting typical symptoms of the bacterial disease were collected. The pathogen was isolated from diseased plant material using Vogel-Bonner-tartrate agar, as previously described (Cuppels et al. 1990). Purified strains of the pathogen were stored at -80°C in nutrient broth-yeast extract broth plus 15% glycerol (Cuppels et al. 1990). The bacterial isolates were cultured overnight in Luria-Bertani broth and the genomic DNAs were extracted using the DNeasy UltraClean Microbial kit (Qiagen), with minor modifications. Briefly, 600 μl of microbial culture was used to generate a cell pellet, which was stored at -20°C until used, and 36 μl of RNase Cocktail (Invitrogen) was added to the cell suspension before processing. Also, homogenization was performed using a Tissue Lyser II (Qiagen) set to 20 Hz for 5 min, after which the process was repeated once with plates reoriented at 180° prior to incubation at 37°C for 30 min. The isolates were characterized to the genus *Pseudomonas* using 16S ribosomal RNA (rRNA) sequencing (Lane 1991; Tambong et al. 2017) and BLAST analysis (Altschul et al. 1990). We report, here, draft whole genome sequences of 33 *Pseudomonas syringae* pv. *tomato* (Pst) strains. Libraries were constructed using a Nextera DNA Flex prep kit (Illumina) following the manufacturer's instructions. The draft genome sequences were determined by paired-end sequencing using Illumina NextSeq technology at the Molecular Technologies Laboratory (Ottawa Research and Development Centre, Ottawa, Canada). The mean numbers of paired-end reads of 2,020,245 bp (1,314,234 to 2,955,580 bp), each 150 bp long, were generated (Table 1). The quality of the reads was checked using FastQC (Andrews 2010), trimmed using BBDOUK (Bushnell 2014), if required, and de novo assemblies were performed using SKESA (Souvorov et al. 2018), as implemented in COWBAT ver. 0.5.0.7 pipeline (<https://github.com/OLC-Bioinformatics/COWBAT>). Scaffolds < 500 bp long were discarded. The best assemblies, based

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Table 1. Basic genome statistics of the 33 Canadian strains of *Pseudomonas syringae* pv. *tomato* isolated from 1992 to 2008^a

Strains	Year of isolation	Total reads	Depth ^b	Contigs	N ₅₀ (bp)	L ₅₀	Largest contig (bp)	Total length (bp)	GC (%)	CDSs ^c	Protein-encoding genes ^d	
											With	Without
DC 92-1	1992	2,282,872	53	132	133,701	17	369,737	6,323,733	58.59	6,075	3,933	2,142
DC 92-3	1992	2,282,778	53	123	152,803	15	416,356	6,325,870	58.59	6,081	3,937	2,144
DC 92-7	1992	1,852,375	42	143	176,505	12	574,824	6,358,250	58.59	6,104	3,957	2,147
DC 93-1	1993	1,957,327	45	116	141,037	15	356,731	6,300,490	58.62	6,024	3,910	2,114
DC 94-1	1994	1,871,646	43	119	138,516	15	298,064	6,271,099	58.62	5,993	3,909	2,084
DC 94-2	1994	2,365,268	55	122	138,516	15	298,130	6,273,435	58.62	5,997	3,917	2,080
DC 95-1	1995	2,955,580	69	107	149,283	15	335,638	6,263,138	58.59	5,990	3,899	2,091
DC 95-2	1995	2,850,576	67	100	153,003	13	371,446	6,214,671	58.60	5,913	3,868	2,045
DC 96-2	1996	2,396,612	55	170	159,324	14	574,677	6,381,632	58.55	6,166	3,968	2,198
DC 96-3	1996	2,557,587	60	91	164,584	14	291,886	6,199,279	58.62	5,901	3,878	2,023
DC 97T23A	1997	2,274,160	53	136	138,127	14	390,693	6,304,758	58.62	6,048	3,920	2,128
DC 97T24A	1997	2,540,768	59	149	150,184	15	335,638	6,303,202	58.61	6,050	3,925	2,125
DC 98-1	1998	1,950,553	45	108	168,384	14	401,594	6,363,517	58.56	6,072	3,926	2,146
DC 98-2	1998	1,825,444	42	130	128,336	15	356,719	6,327,833	58.59	6,063	3,926	2,137
DC 99T2A	1999	1,803,495	43	97	128,421	15	297,994	6,195,741	58.62	5,889	3,871	2,018
DC 99T3A	1999	1,953,577	45	121	152,793	14	291,736	6,289,072	58.61	6,012	3,898	2,114
DC 99T5A	1999	1,824,539	43	113	153,087	12	575,537	6,265,515	58.62	5,981	3,901	2,080
DC 99T7A	1999	2,201,810	50	163	138,516	14	416,402	6,390,207	58.55	6,161	3,973	2,188
DC 00T2C	2000	1,955,228	45	121	138,905	14	453,565	6,322,847	58.59	6,044	3,925	2,119
DC 00T3A	2000	2,213,642	51	133	150,137	13	356,719	6,317,806	58.61	6,053	3,921	2,132
DC 00T4A	2000	2,184,112	50	168	127,738	15	369,718	6,390,924	58.55	6,160	3,979	2,181
DC 01T23B2	2001	1,471,655	34	156	149,304	14	389,356	6,341,373	58.56	6,097	3,949	2,148
DC 01T30A	2001	1,639,263	38	156	126,851	16	322,567	6,342,470	58.56	6,044	3,925	2,119
DC 01T31C	2001	1,314,234	30	162	101,231	20	291,627	6,308,123	58.59	6,080	3,938	2,142
DC 02T10A	2002	1,725,363	40	156	118,097	15	575,284	6,326,087	58.59	6,030	3,907	2,123
DC 02T2A	2002	1,548,325	36	144	124,077	17	291,742	6,282,724	58.60	6,085	3,950	2,135
DC 03T12	2003	1,808,136	42	129	126,813	15	385,340	6,284,879	58.59	6,008	3,900	2,108
DC 03T6	2003	1,661,778	38	132	149,283	13	356,698	6,283,754	58.60	6,017	3,915	2,102
DC 04T2A	2004	1,851,271	43	145	126,813	15	385,256	6,323,422	58.59	6,050	3,929	2,121
DC 04T2B	2004	1,910,866	44	135	139,077	14	402,377	6,303,383	58.60	6,014	3,922	2,092
DC 06T1	2006	1,779,614	41	160	118,086	16	356,719	6,348,311	58.56	6,096	3,956	2,140
DC 06T2	2006	1,706,419	39	168	110,683	18	291,481	6,340,210	58.56	6,093	3,955	2,138
DC 08T1	2008	2,151,202	51	108	138,852	14	402,355	6,199,517	58.65	5,913	3,882	2,031

^a Whole-genome sequences were generated using Illumina NextSeq platform and assembled using SKESA. Contigs <500 bp were discarded. Based on CheckM algorithm (Parks et al. 2015) as implemented in PATRIC (Wattam et al. 2014), the completeness of the genomes was 100%, with coarse consistency of 99.3 to 99.7% and fine consistency of 97.3 to 97.7%. Annotation and protein function assignment were implemented in PATRIC (Wattam et al. 2014).

^b Average coverage depth.

^c Total number of protein-coding sequences.

^d Protein-encoding genes with and without functional assignment.

on N₅₀ and L₅₀ values and nearness to the expected genome size, were obtained. The average size of the draft genome sequences is 6.305 Mbp with a range of 6.195 to 6.390 Mbp (average maximum contig = 381,352 bp and N₅₀ = 139,729 bp) (Table 1). The average G+C content of the draft genome sequences is 58.59% (58.55 to 58.65%), with an average estimated coverage of 46.8x (36x to 67x).

The 33 draft genome sequences had a completeness of 100%, average consistency of 97.6%, coarse consistency of 99.6%, and average contamination rate of <0.5% based on the CheckM algorithm (Parks et al. 2015) as implemented in PATRIC pipeline version 3.6.9 (Pathosystems Resource Integration Center) (Wattam et al. 2014). The whole-genome sequences exhibited average nucleotide identity (Jain et al. 2018) values of 98.64 to 98.72% with *P. syringae* pv. *tomato* ICMP 2844^{PT} or DC3000, validating the taxonomic standing of these Canadian strains. The assembled sequences were annotated using the RAST tool kit (RASTtk) (Brettin et al. 2015) as implemented in the PATRIC pipeline, which identified 5,889 to 6,166 protein-coding sequences (CDSs). The 33 genome sequences, each had 50 to 58 transfer RNAs, 2 to 6 rRNAs, and 1 transfer-messenger RNA based on Prokka version 1.14.5 (Seemann 2014). Default parameters were used for all software unless otherwise specified. The Roary pipeline (Page et al. 2015) was used to compute pan and core genomes of 6,808 (Fig. 1A) and 4,993 (Fig. 1B) CDSs, respectively. A pan-genome single-nucleotide polymorphism-based tree clustered the strains into distinct groups (Fig. 1A). Strain Pst DC 98-1

Fig. 1. A, Pan-genome single-nucleotide polymorphism (panSNP) tree and Roary pangenome matrix and **B**, pie chart of core-genomes of the 33 Canadian strains of *Pseudomonas syringae* pv. *tomato* based on BLAST as implemented in Roary pipeline (Page et al. 2015). Taxa names on the panSNP tree are the strain codes without the prefix “Pst”.

showed a unique distribution pattern of the gene clusters, suggesting the presence of several accessory genes (Fig. 1A). Cloud (accessory), shell, and soft-core gene clusters of 786 (<4 strains; for example, Pst DC 98-1), 671 ($4 \leq$ strains < 31), and 358 ($31 \leq$ strains < 32), respectively, were identified (Fig. 1B). Genome mining for virulence factors using the virulence factors of pathogenic bacteria tool (Liu et al. 2019) identified adherence-related genes (e.g., flagella, type IV pili biosynthesis, and lipopolysaccharide O-antigen), CDSs implicated in antiphagocytosis (e.g., alginate biosynthesis or regulation), secretion system apparatuses, and effectors. Also, complete or partial pyoverdine and achromobactin siderophore biosynthetic clusters and iron transport genes were identified in all the strains (Table 2). The achromobactin biosynthetic cluster was not detected in *P. syringae* pv. *tomato* DC3000 (Table 2) or ICMP 2844 (pathotype). This suggests that the Canadian *P. syringae* pv. *tomato* strains might have acquired the achromobactin biosynthetic cluster and iron transport genes from another bacterial group, possibly *P. syringae* pv. *syringae*. An additional iron-chelating siderophore in the Canadian strains could significantly improve their ecological fitness in iron-limiting niches. A complete (20 genes) or partial cluster of genes for the coronatine phytotoxin, reported to be involved in pathogenicity on tomato, was identified in all strains except Pst DC 95-1, Pst DC

Table 2. Achromobactin biosynthetic, coronatine phytotoxin (Cor), lipopolysaccharide O-antigen (LPS), and type I O-polysaccharide (Cap1) genes detected in the 33 whole genome sequences of the Canadian strains of *Pseudomonas syringae* pv. *tomato*^a

Strains	Achromobactin biosynthesis and regulation genes								Other genes		
	acsA	acsB	acsC	acsD	cbrA	cbrB	cbrC	cbrD	Cor	LPS	Cap1
Pst DC 92-1	nd	nd	+	nd	+	+	nd	nd	6	+	1
Pst DC 92-3	+	+	+	+	+	+	+	+	17	+	1
Pst DC 92-7	+	nd	+	+	+	+	+	nd	9	+	1
Pst DC 93-1	+	+	+	+	+	+	+	+	16	+	1
Pst DC 94-1	+	+	+	+	+	+	+	+	17	+	1
Pst DC 94-2	+	+	+	+	+	+	+	+	17	+	1
Pst DC 95-1	+	nd	nd	nd	+	+	+	nd	–	+	1
Pst DC 95-2	nd	nd	+	nd	+	+	+	nd	–	+	1
Pst DC 96-2	+	+	+	+	+	+	+	+	18	+	1
Pst DC 96-3	+	+	+	+	+	+	+	+	–	+	1
Pst DC 97T23A	+	+	+	+	+	+	+	+	18	+	1
Pst DC 97T24A	+	+	+	+	+	+	+	+	18	+	1
Pst DC 98-1	+	+	+	+	+	+	+	+	17	+	–
Pst DC 98-2	+	+	+	+	+	+	+	+	7	+	1
Pst DC 99T2A	nd	nd	+	nd	+	+	+	nd	–	+	1
Pst DC 99T3A	+	+	+	+	+	+	+	+	17	+	1
Pst DC 99T5A	+	+	+	+	+	+	+	+	15	+	1
Pst DC 99T7A	+	+	+	+	+	+	+	+	17	+	1
Pst DC 00T2C	+	+	+	+	+	+	+	+	16	+	1
Pst DC 00T3A	+	+	+	+	+	+	+	+	16	+	1
Pst DC 00T4A	–	+	+	–	+	+	+	+	13	+	1
Pst DC 01T23B2	+	nd	+	nd	+	+	+	nd	9	+	1
Pst DC 01T30A	+	+	+	+	+	+	+	+	16	+	1
Pst DC 01T31C	+	+	+	+	+	+	+	+	18	+	1
Pst DC 02T10A	nd	nd	+	nd	+	+	nd	nd	6	+	1
Pst DC 02T2A	+	+	+	+	+	+	+	+	17	+	1
Pst DC 03T12	+	+	+	+	+	+	+	+	17	+	1
Pst DC 03T6	+	+	+	+	+	+	+	+	18	+	1
Pst DC 04T2A	+	+	+	+	+	+	+	+	15	+	1
Pst DC 04T2B	+	+	+	+	+	+	+	+	17	+	1
Pst DC 06T1	+	+	+	+	+	+	+	+	16	+	1
Pst DC 06T2	+	+	+	+	+	+	+	+	17	+	1
Pst DC 08T1	nd	nd	+	nd	+	+	+	nd	7	+	1
Pst DC 3000	–	–	–	–	–	–	–	–	20	–	1
Psp 1448A	+	+	+	+	+	+	+	+	–	–	–
Pss B728a	+	+	+	+	+	+	+	+	–	–	–

^a Pst = *P. syringae* pv. *tomato*, Psp = *P. syringae* pv. *phaseolicola*, Pss = *P. syringae* pv. *syringae*, + indicates gene is present, – indicates gene not present, and nd = not detected. For coronatine phytotoxin, numbers indicate number of genes present out of the expected 20 genes. Note the absence of achromobactin biosynthetic cluster in the complete genome sequence of strain DC3000. Virulence factors were detected using the virulence factors of pathogenic bacteria tool (Liu et al. 2019).

95-2, Pst DC 96-3, and Pst DC 99T2A (Table 2). These whole-genome data of Canadian strains of *P. syringae* pv. *tomato* could be useful resources in understanding the pathogenicity and evolution of this pathogen over time.

Data Availability

The whole-genome sequences are deposited in DNA Data Bank of Japan/European Nucleotide Archive/GenBank under the accession numbers JAIFYB0000000000 to JAIFZH0000000000. The raw reads are deposited in the NCBI Sequence Read Archive under the accession number PRJNA754202.

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