

Whole genome sequencing and the *Pseudomonas syringae* pv *actinidiae* outbreak in New Zealand kiwifruit

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Friday 5 November 2010, a bacterial infection was discovered on a New Zealand kiwifruit vine. Testing confirmed the vine was infected with *Pseudomonas syringae* pv *actinidiae* (Psa).



Recorded since 1989 in Japan

First confirmed in 1992 in Korea

Psa was first documented in Italy in 1992

In 2008 the symptoms of Psa in Italy became much more dramatic (attributed to the presence of a more virulent form).

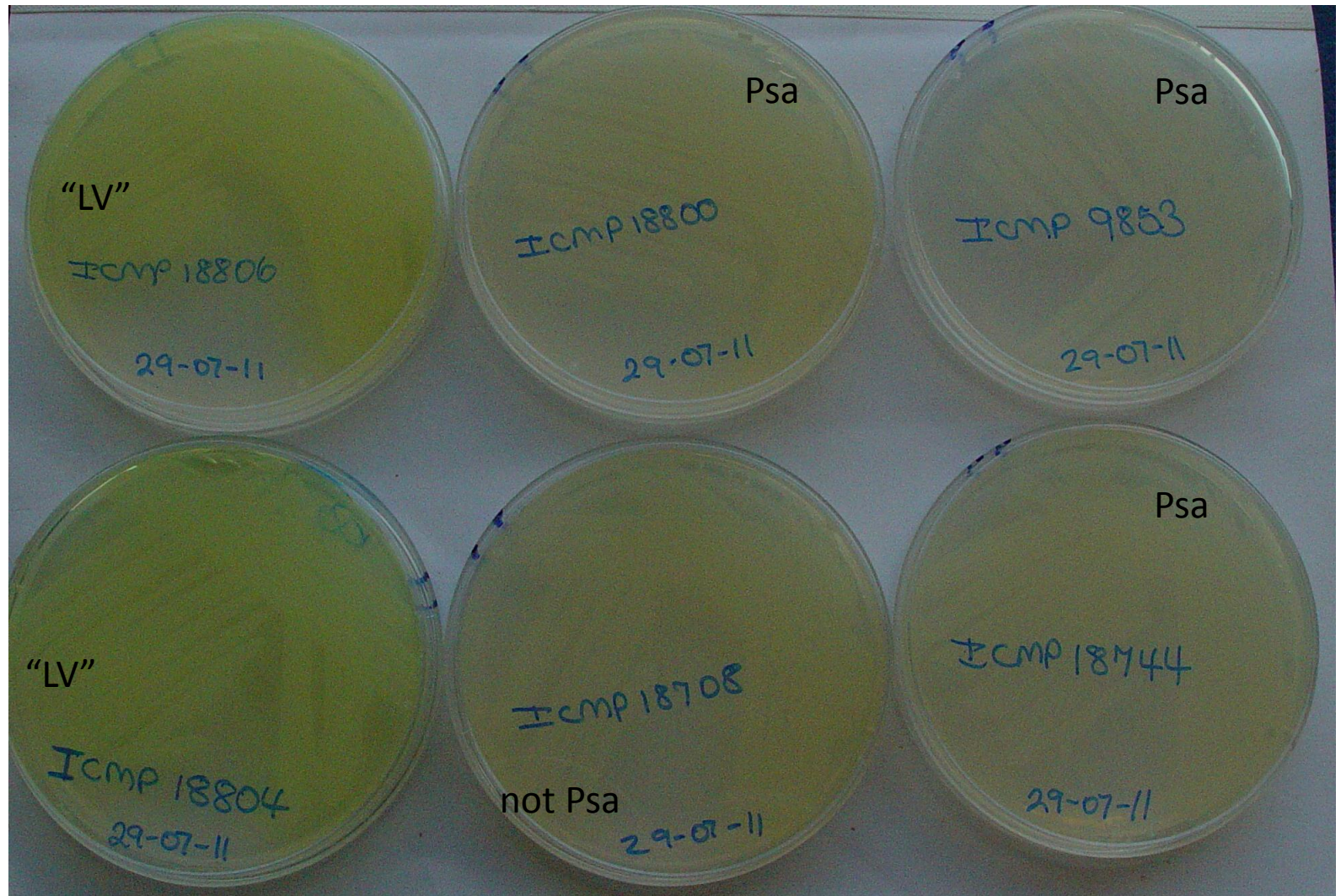
The first Psa infection in France was identified in 2010.

Psa has been reported in Portugal and, more recently, in Chile.

Anecdotal evidence suggests Psa has also been present in China for some time

-KVH information

Fluorescence of various strains on King's B medium



Addressing the question of what are the LV strains?

Multi Locus Sequence Typing (MLST)

amplified 500-600bp regions from 4 different genes with PCR

gltA (or cts, citrate synthase)

gapdh (glyceraldehyde-3-phosphate dehydrogenase)

gyrB (DNA gyrase B)

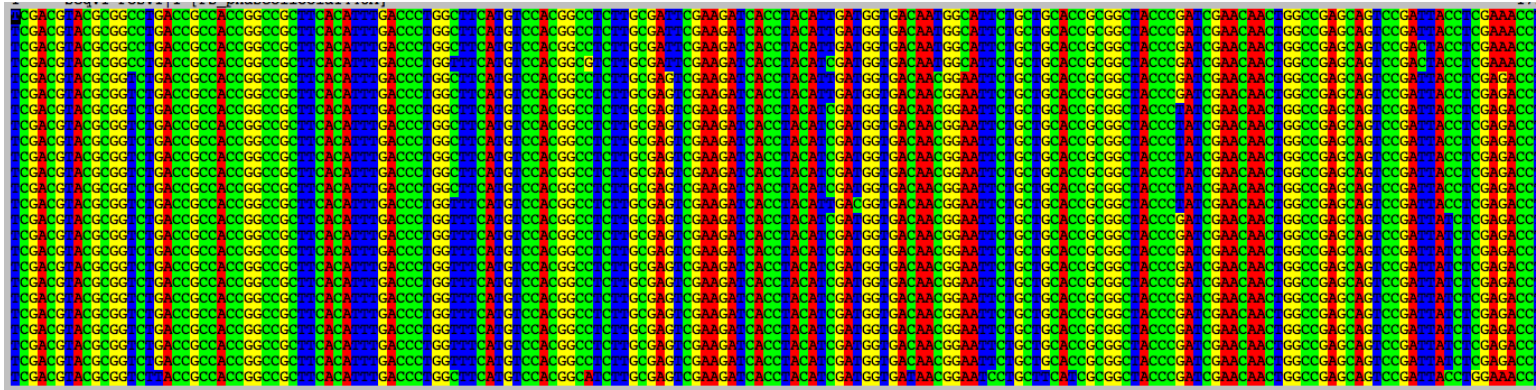
rpoD (sigma factor 70)

-gene regions were those used by others studying *P. syringae* pathovars

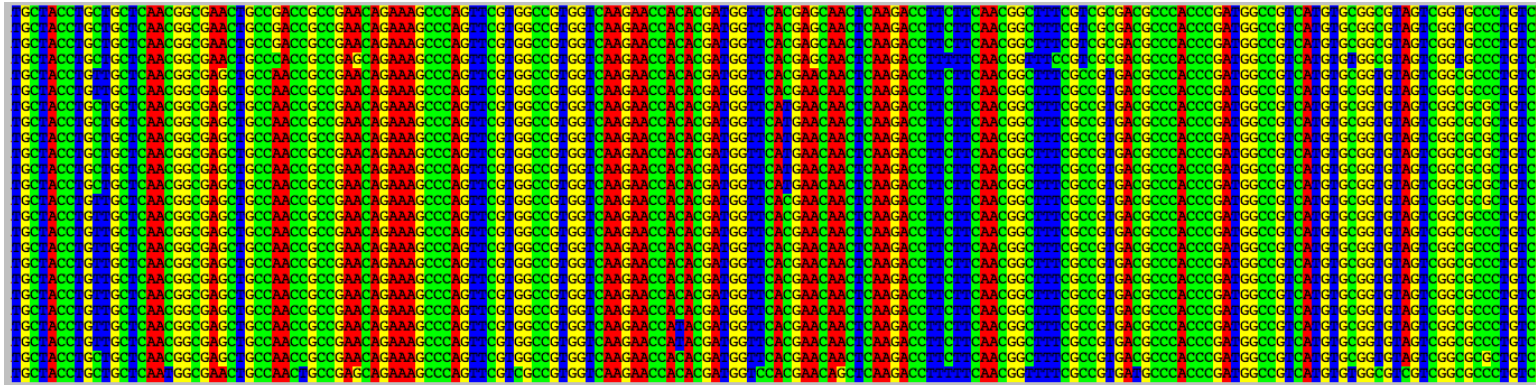


Alignment of gltA gene region

Ps_phaseolicola1448A
Ps_glycinea_race4
Ps_aesculi2250 gltA
Ps_syringaeB728a
Ps_tomatoDC3000
Ps_lachrymansM302278PT
18802
18803
18882
18883
18804
18806
P_avellanaeFN565203
18746
Ps_theaeAY610752
18745
18743
18708_TePuke
18800
18744
9853
Ps_actinidiaeM302091
Ps_morsprunorumM302280
Ps_maculicolaES4326



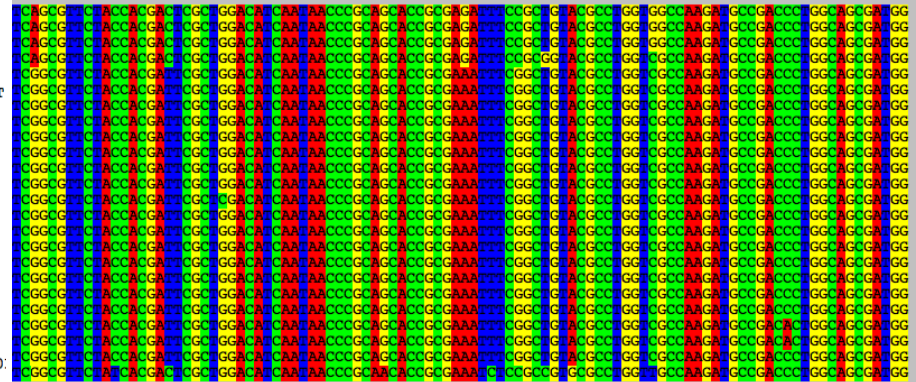
Ps_phaseolicola1448A
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18743
18708_TePuke
18800
18744
9853
Ps_actinidiaeM302091
Ps_morsprunorumM302280
Ps_maculicolaES4326



“LV” or PsD strains

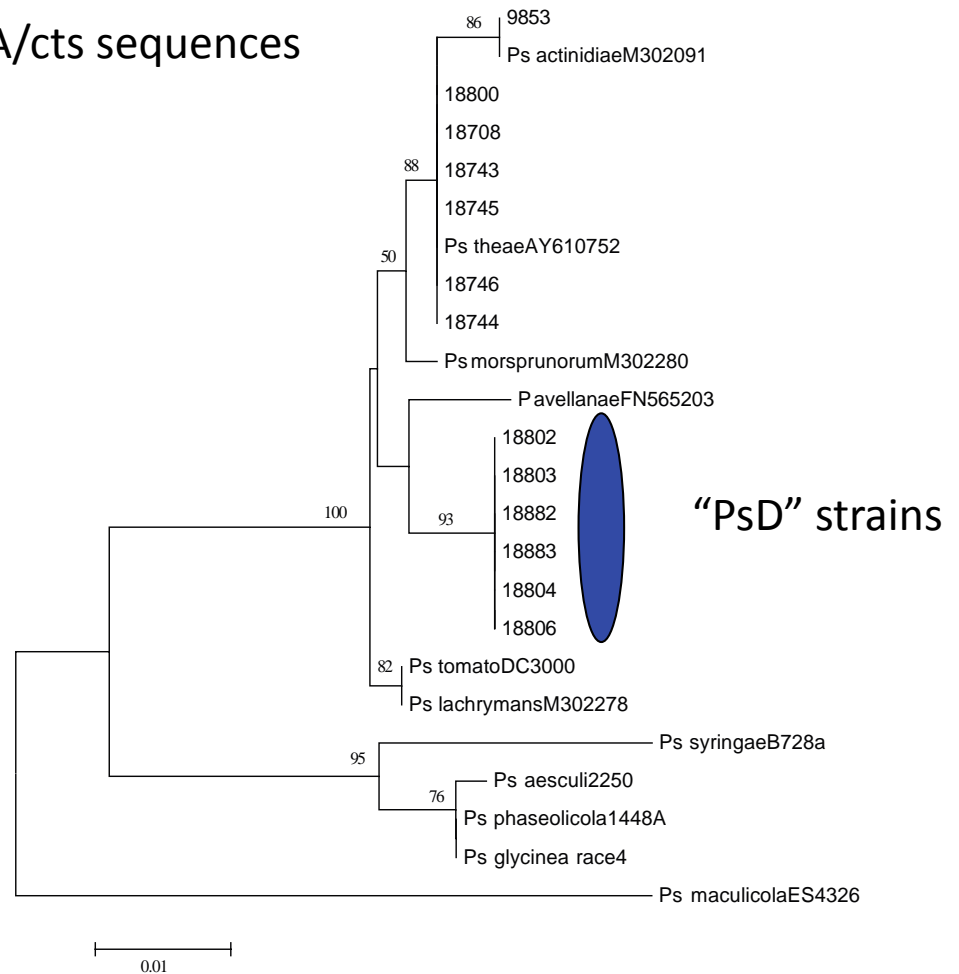
Psa strains

Ps_phaseolicola1448A
Ps_glycinea_race4
Ps_aesculi2250 gltA
Ps_syringaeB728a
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18708_TePuke
18800
18744
9853
Ps_actinidiaeM302091
Ps_morsprunorumM302280
Ps_maculicolaES4326



Phylogenetic tree derived from gltA/cts sequences

Maximum likelihood tree made
using MEGA5
-only gltA locus
100 bootstrap replicates



BLAST®

Basic Local Alignment Search Tool

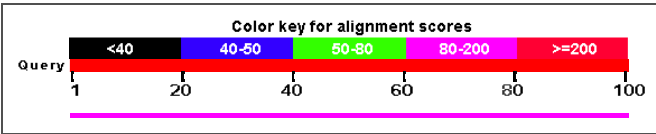
NCBI/ BLAST/ blastn suite/ Formatting Results - 60SESEAX011

s6_2101_11498_36340

Query ID	lcl 41977	Database Name	nr
Description	s6_2101_11498_36340	Description	All GenBank+EMBL+DDBJ+PDB sequences (but no EST, STS, GSS,environmental samples or phase 0, 1 or 2 HTGS sequences)
Molecule type	nucleic acid	Program	BLASTN 2.2.25+
Query Length	101		

Graphic Summary

Distribution of 1 Blast Hits on the Query Sequence



Descriptions

Legend for links to other resources: [U](#) UniGene [E](#) GEO [G](#) Gene [S](#) Structure [M](#) Map Viewer [P](#) PubChem BioAssay

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AE016853.1	Pseudomonas syringae pv. tomato str. DC3000, complete genome	171	171	100%	2e-41	97%	

Alignments

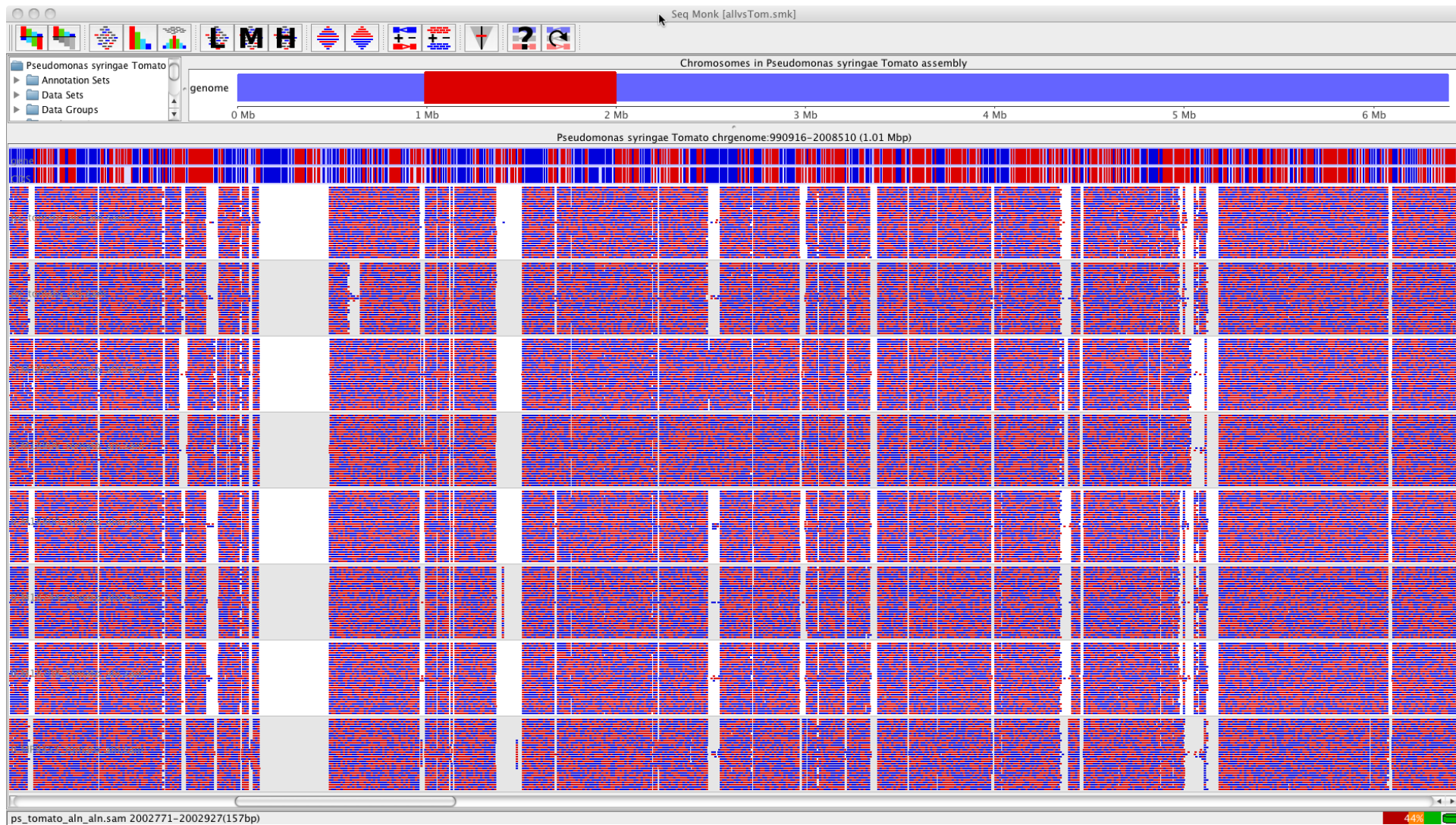
>[gb|AE016853.1|](#) [D](#) Pseudomonas syringae pv. tomato str. DC3000, complete genome
Length=6397126

Features in this part of subject sequence:
ABC transporter, ATP-binding protein

Score = 171 bits (92), Expect = 2e-41
Identities = 98/101 (97%), Gaps = 0/101 (0%)
Strand=Plus/Minus

Query	1	CTGGTAAAAGGCATCGTCGGCTACAGCCCACTGCAATCGGGCAGCATTCTGGTCGACAAC	60
Sbjct	1493312	CTGGTCAAAGGCATCATCGGCTACAGCCCGCTGCAATCGGGCAGCATTCTGGTCGACAAC	1493253
Query	61	CGCGAGCATGCAATTGGCACCCCGCGCGATTTCGCATCAGTT	101
Sbjct	1493252	CGCGAGCATGCAATTGGCACCCCGCGCGATTTCGCATCAGTT	1493212

All 8 new genomes aligned in the region 1-2Mb of the *P. s. tomato* DC3000



ps_tomato_aln_aln.sam 2002771-2002927(157bp)

44%

Searching for copper or antibiotic resistance factors in the Psa genome

Query	Hit	Exp()
AY533312.1 - Psa strA	**No Hit**	
AY997127.1 - Psy strB	**No Hit**	
AEAL01000410.1 - Psa M302091 rpsL	>IL_6_1_24 size=40970 cov=185.182	9.00E-68
AB033419.2 - Psa pPaCu1 copA	>IL_6_1_33 size=60420 cov=136.913	0.00E+00
AB033419.2 - Psa pPaCu1 copB	>IL_6_1_33 size=60420 cov=136.913	2.00E-82
AB033419.2 - Psa pPaCu1 copC	**No Hit**	
AB033419.2 - Psa pPaCu1 copD	**No Hit**	
PSPTO_4303 - Pseudomonas syringae pv. tomato DC3000 mexA	>IL_6_1_23 size=41320 cov=189.599	0.00E+00
PSPTO_4304 - Pseudomonas syringae pv. tomato DC3000 mexB	>IL_6_1_23 size=41320 cov=189.599	0.00E+00
PSPTO_4305 - Pseudomonas syringae pv. tomato DC3000 oprM	>IL_6_1_23 size=41320 cov=189.599	0.00E+00
PSPTO_2646 - P. syringae tomato DC3000 tetR	>IL_6_1_6 size=121055 cov=107.162	2.00E-67
83656969 - gb DQ316137.1 Pseudomonas sp. SO-A5-26 partial oxytetracyclineR (tetC)	**No Hit**	
239829516 - gb FJ950709.1 Pseudomonas sp. c163 partial tetC	>IL_6_1_113 size=16424 cov=110.521	2.00E-03
HM804085.1 - 861-2060 Pseudomonas aeruginosa plasmid R1033, tetA	>IL_6_1_74 size=22455 cov=176.691	1.00E-07
AF133140.1 - 757-1932 Pseudomonas sp. pPSTG2 tetracycline resistance protein (tetG)	>IL_6_1_74 size=22455 cov=176.691	2.00E-07



People:

Russell Poulter
Margi Butler

Iain Lamont
Lois Martin

Becky Laurie
Les McNoe

Mik Black
Peter Stockwell



Funds: Taskforce Green (Michael Franks, Seeka; Grant Eynon, Eastpack)
Department of Biochemistry, University of Otago



With the NZ outbreak, Kiwifruit Vine Health Inc (KVH) set up in December 2010.

KVH is an independent organisation to lead the kiwifruit industry response to the Psa incursion.

Total returns to growers in 2010/11 were \$883.3 million.

In 2010/11 ZESPRI's net profit fell from \$25.9 million to \$7.3 million.

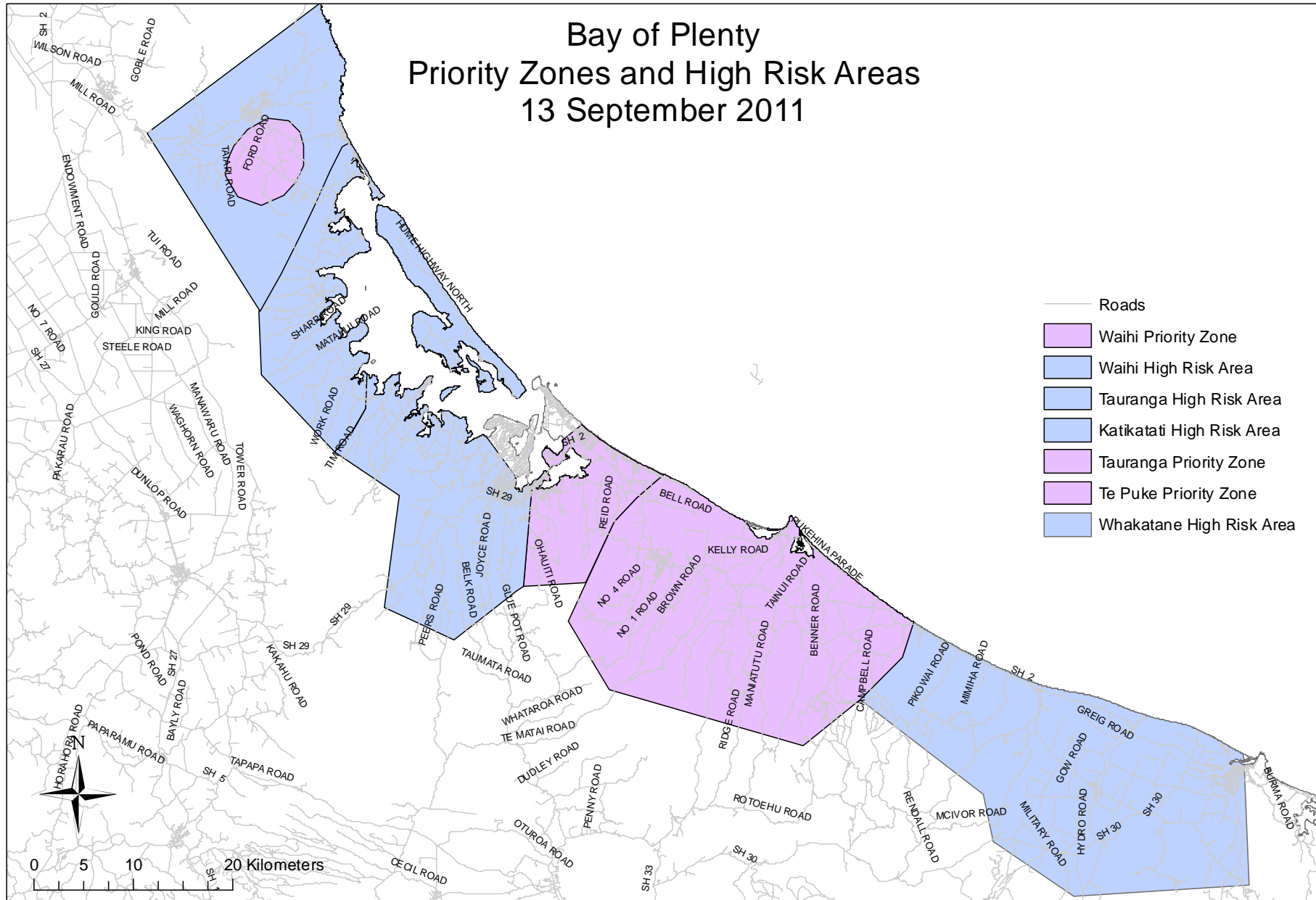
The fall was primarily due to contributing \$12.9 million to the response to Psa in New Zealand.



Canker showing white and red exudate.

<http://www.batteriosi.it/>

Bay of Plenty Priority Zones and High Risk Areas 13 September 2011



Kiwifruit rivals join forces to fight Psa

Seeka Kiwifruit Industries and EastPack launched a research initiative called “Taskforce Green,” beginning with a meeting of scientists in Te Puke in June 2011.

The Taskforce Green group is comprised of a large range of participants from the academic, diagnostic and commercial research fields and hoped to find a novel cure for the disease



Bay of Plenty Times

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Boffins swarm to find Psa answer

22nd June 2011

Seeka Kiwifruit Industries and EastPack have combined efforts to launch a research initiative targeting New Zealand's Psa outbreak.

Leading New Zealand professors and research scientists with areas of expertise including biochemistry, genetics, epidemiology, outbreak intervention and microbiology, elicitor analysis and biotechnology converged on Te Puke last week as part of the project called "Taskforce Green".

Participants from the academic, diagnostic and commercial research fields spent time viewing the VLS laboratory set up in Te Puke to produce fast Psa test results, before seeing first hand the disease's effects on orchards.



A research initiative targeting New Zealand's Psa outbreak has been launched. Photo / File.

Taskforce Green



Michael Franks, Chief Executive, Seeka
Grant Eynon, Deputy Chairman, EastPack
Russell Poulter, University of Otago
Iain Lamont, University of Otago
Margi Butler, University of Otago
Clint Wall, Seeka
David Ackerley, Victoria University
Janine Copp, Victoria University
Matthew Storey, Victoria University
Jerome Demmer, Halcyon Bioconsulting Limited
John Aitken, Christchurch
John MacKay, dnature, Gisborne
Shaline Dowlut, Verified Lab Services (Seeka)
Terry Cooney, Hamilton
Jonathan Cutting, Seeka
Lucia Burt, Seeka
Murray Judd, Seeka
Peter Mourits, Seeka
Peter Weaver, Seeka

PCR detection test available

(Rees-George et al., 2010, Plant Pathology 59, 453–464)

PCR primers complementary to a region of the 16S–23S rDNA internal transcribed spacer (ITS) region

Amplified a DNA fragment from strains of *P. syringae* pv. *actinidiae*, but not from 56 strains of bacteria from six genera and 17 species (except for a strain of the tea pathogen, *P. syringae* pv. *theae*)

Assay was adapted for real-time PCR

Two kinds of Psa described, Psa-V and Psa-LV (both amplified with diagnostic test)
Psa-V at first confined to the Te Puke region

“Psa-LV” found in the South Island and Gisborne
Sometimes referred to as the “Asian” strain





31 August 2011

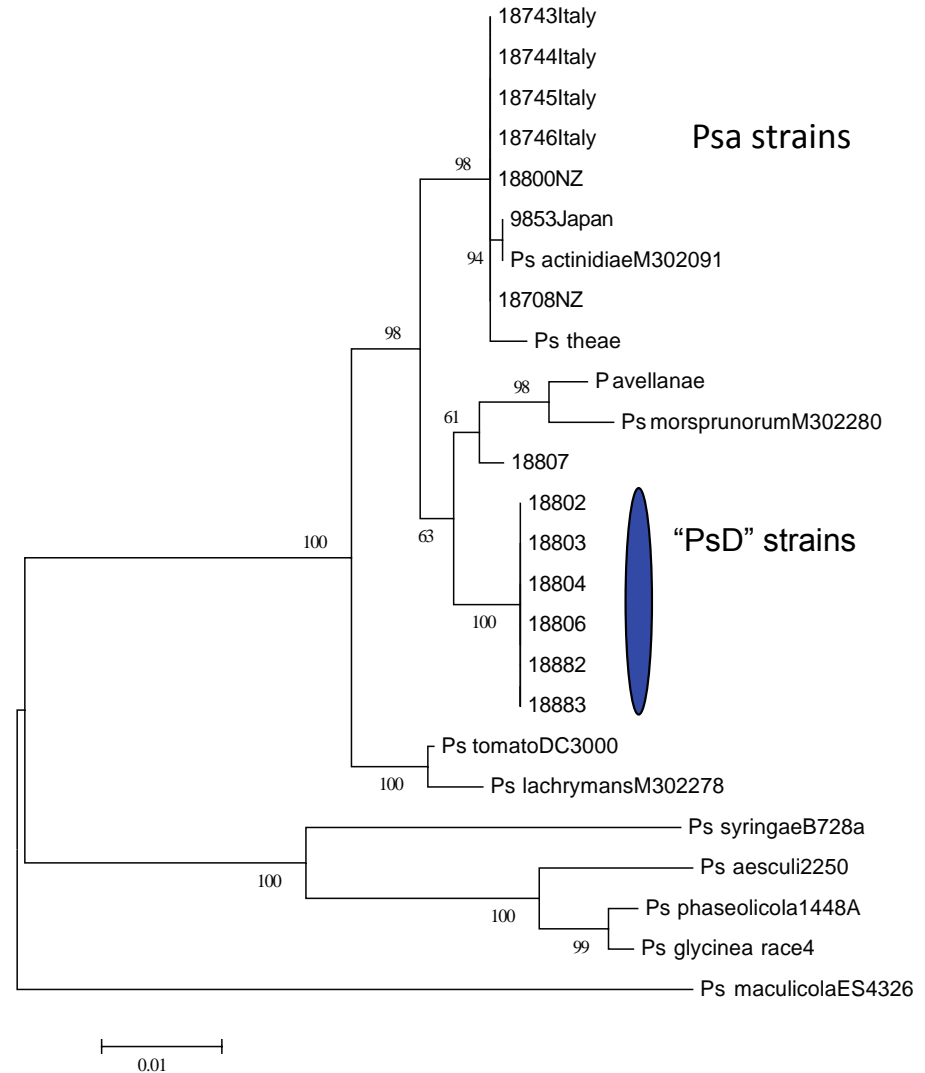
Psa Update

- Psa is confirmed on 319 orchards across New Zealand.
- 279 Psa-affected orchards (87%) are in the Te Puke region.
- 222 orchards are identified with Psa-V (virulent).
- Psa-V orchards are all within the Priority Zones.
- The Priority Zones have Psa and non-Psa affected orchards.

“PsD” strains are not *P. syringae* pv *actinidiae* (Psa)

We now refer to these strains
as “PsD” to emphasise that
they are not part of the
outbreak

Maximum likelihood tree made using MEGA5
All 4 loci (gltA, gyrB, gapdh, rpoD)
100 bootstrap replicates



Relief for kiwifruit industry

[nzherald.co.nz](http://www.nzherald.co.nz)

By [Owen Hembry](#)

5:30 AM Thursday Aug 25, 2011



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Email



Print

The bacterial disease threatening the kiwifruit industry is not as widespread as thought, says listed company Seeka Kiwifruit Industries.

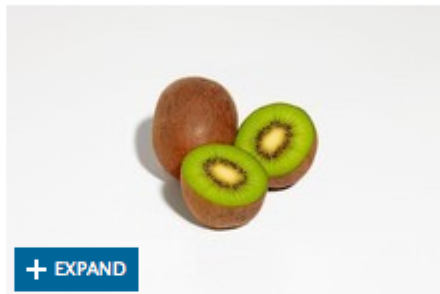
Seeka said research conducted at Otago University under the TaskForce Green Network – a commercially funded research initiative by Seeka and EastPak – had shown that one of the two strains of *Pseudomonas syringae* pv *actinidiae* (Psa) thought to be in New Zealand was only distantly related to the disease and was not a danger to the industry.

It had been thought there were two strains of the disease in New Zealand – one a virulent type restricted to the Bay of Plenty and another which was also in Hawkes Bay, Golden Bay and Motueka. Researchers had found differences in the genomic make-up of the two strains and believed there was now only one strain in the country – the virulent type.

"As the Psa V [virulent strain] is still limited to the Bay of Plenty, it's still worth considering the possibility of completely eradicating the pathogen," Seeka said.

Seeka chief executive Michael Franks said it was a stunning discovery which would help the industry develop a solution.

"Because we've got the genomic sequence just about done, the team should be able to focus on exactly what we've got to do to deal to it."



+ EXPAND

Bacterial disease threatening kiwifruit industry not as widespread as thought, says Seeka Kiwifruit Industries. Photo / Supplied



Kiwifruit disease outbreak spreading

• Antibiotic vine spray 'crazy'

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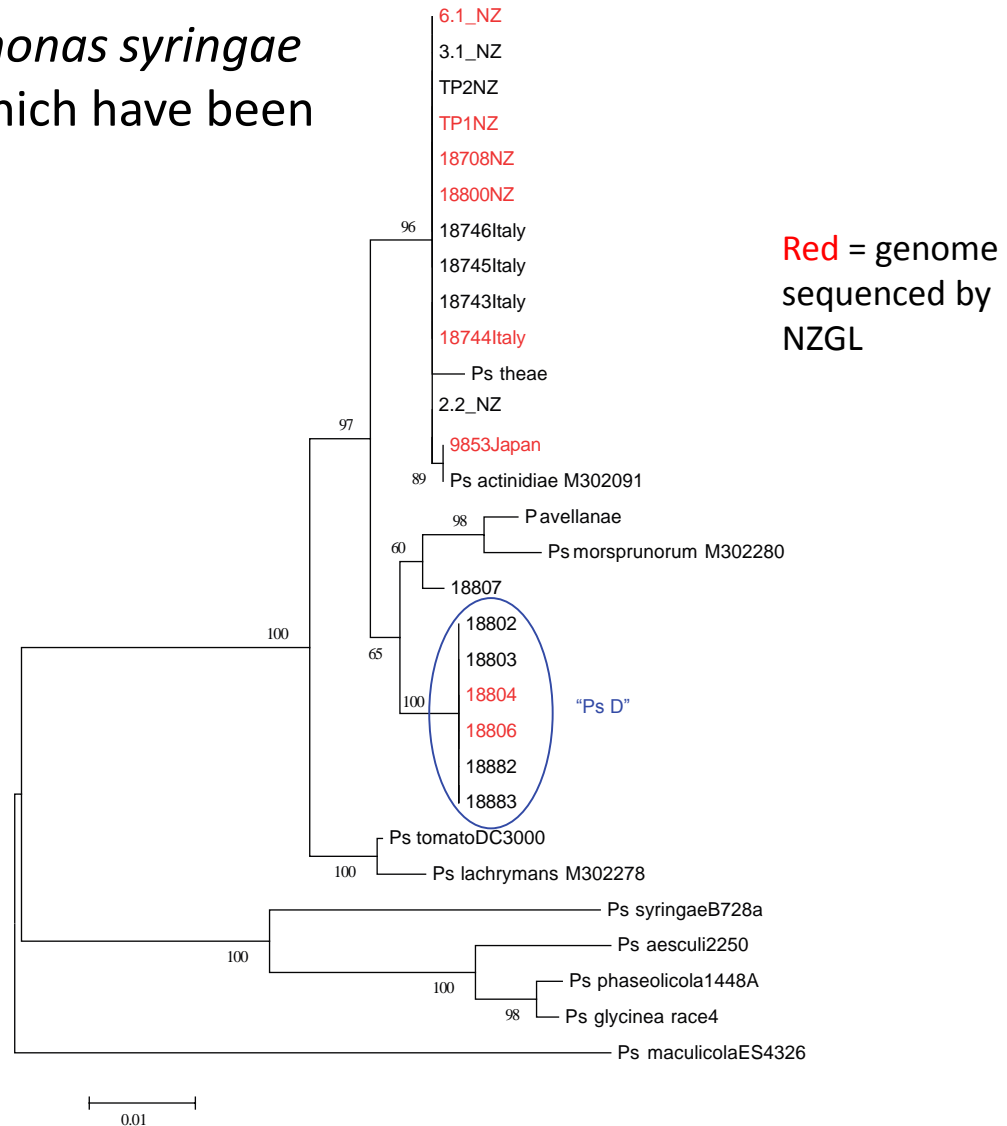
***Pseudomonas syringae* pv. *actinidiae* genome sequencing projects:**

strain name	origin	sequenced by
M302091	Japan	Univ. North Carolina, Chapel Hill
CRAFRU8.43	Italy, Latina, 2008	Università di Udine, Italy
NCPPB3739	Japan, 1984	Università di Udine, Italy
NCPPB3871	Italy, 1993	Università di Udine, Italy

Several strains from Japan, Korea and New Zealand are being sequenced at the University of Toronto by David Guttman in association with Prof. Paul Rainey (Massey) and Plant & Food. They probably include strains of “Psd”.



Phylogenetic tree of *Pseudomonas syringae* pathovars including strains which have been sequenced by NZGL at Otago



4 loci: gltA, gyrB, gapdh, rpoD
concatenated
maximum likelihood method,
100 bootstrap replicates

Strains sequenced by **NZGL** in the Department of Biochemistry, University of Otago

ICMP 9853	Japan 1984	
ICMP 18708	Te Puke, Bay of Plenty Nov. 2010	
ICMP 18800	Paengaroa, Nov. 2010	
ICMP 18744	Italy, 2008	
6-1	Te Puke, July 2011	
TP1	Te Puke, 2011	
ICMP 18804	Te Puke, Nov. 2010	“PsD”
ICMP 18806	Pongakawa, Nov. 2010	“PsD”



<http://www.nzgenomics.co.nz/>

Comparative genomics

Aligning the new genome sequences with the most closely related fully sequenced and annotated *P. syringae* pathovar: *P.syringae* pv *tomato* DC3000

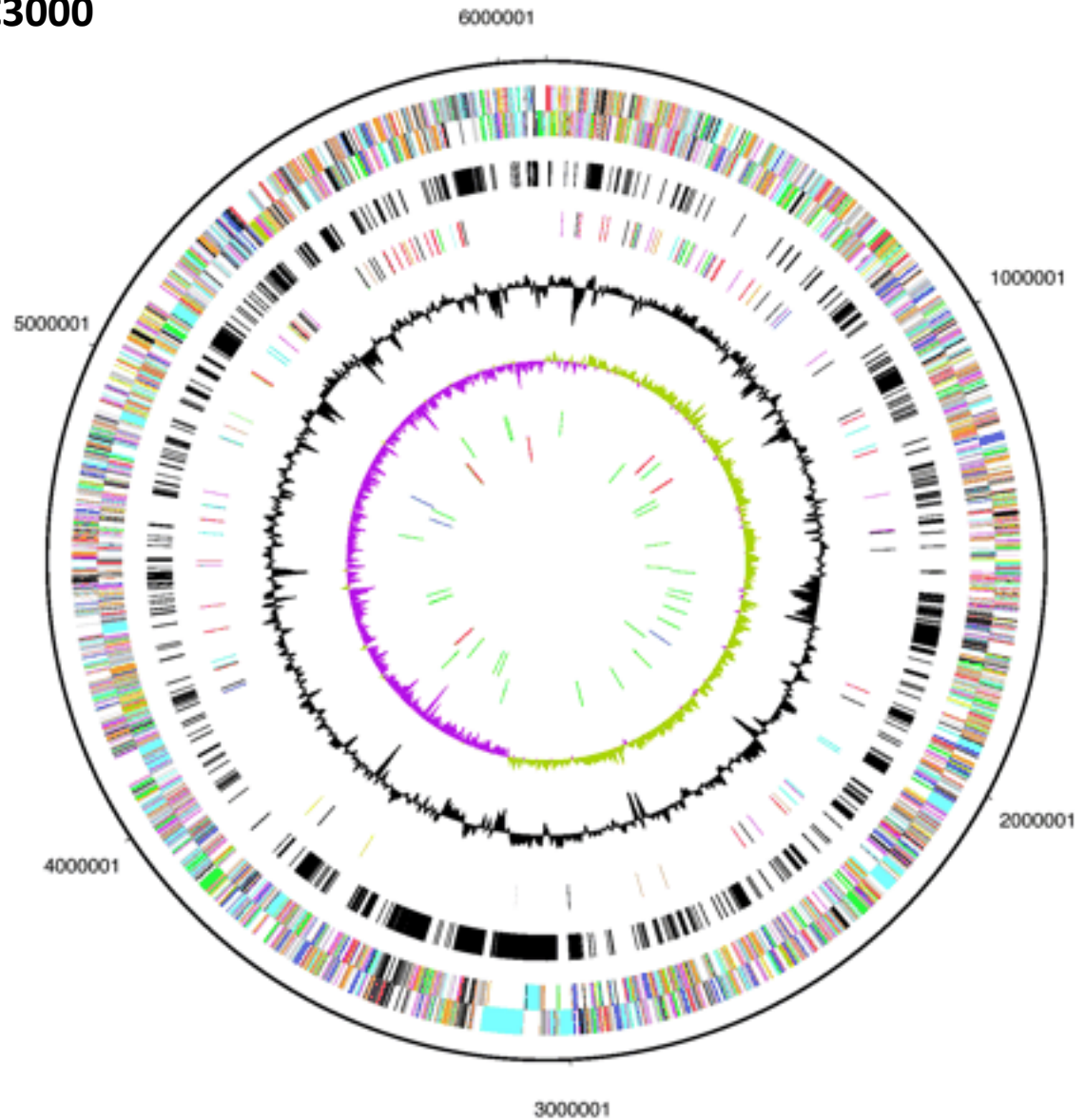
80% of our Psa sequences are found in the Ps tomato DC3000 sequence

Of the 20% not in Ps tomato DC3000, many are represented in M302091, the Japanese Psa strain sequenced in North Carolina

There are some sequences in our dataset that do not occur in either Ps tomato DC3000 or Psa M302091

There are numerous sequences present in our Psa dataset that are absent from the our PsD dataset. These should form good candidates for Psa-specific PCR in the New Zealand context.

Comparison of the complete genome sequences of *Pseudomonas syringae* pv. *syringae* B728a and pv. *tomato* DC3000



Pseudomonas genetic diversity

Table 1. General features from completed *Pseudomonas* genomes

Species/strain	Size*	% G+C*	Genes [†]	% coding	tRNA [†]	rRNA [†]	Released	GenBank	Reference
<i>P. aeruginosa</i>									
PAO1	6 264 404	66.6	5671	89.8	63	13	13/09/2000	AE004091.2	Stover <i>et al.</i> (2000)
PA7	6 588 339	66.4	6396	90.1	65	12	05/07/2007	CP000744.1	Roy <i>et al.</i> (2010)
UCBPP-PA14	6 537 648	66.3	5994	89.8	63	13	06/10/2006	CP000438.1	Lee <i>et al.</i> (2006)
LES858	6 601 757	66.3	6026	88.9	67	13	24/12/2008	FM209186.1	Winstanley <i>et al.</i> (2009)
C3719	6 222 097	66.5	5696	86.6	40	6	04/01/2006	NZ_AAKV000000000 [‡]	Mathee <i>et al.</i> (2008)
PA2192	6 905 121	66.2	6203	85.5	44	2	04/01/2006	NZ_AAKW000000000 [‡]	Mathee <i>et al.</i> (2008)
<i>P. entomophila</i>									
L48	5 888 780	64.2	5293	89.8	78	22	10/05/2006	CT573326.1	Vodovar <i>et al.</i> (2006)
<i>P. fluorescens</i>									
Pf0-1	6 438 405	60.6	5741	90.0	73	19	07/10/2005	CP000094.2	Silby <i>et al.</i> (2009)
Pf-5	7 074 893	63.3	6144	88.7	71	16	30/06/2005	CP000076.1	Paulsen <i>et al.</i> (2005)
SBW25	6 722 539	60.5	6009	88.3	66	16	09/01/2008	AM181176.4	Silby <i>et al.</i> (2009)
<i>P. mendocina</i>									
ymp	5 072 807	64.7	4730	90.7	67	12	20/04/2007	CP000680.1	Unpublished
<i>P. putida</i>									
F1	5 959 964	61.9	5423	89.9	76	20	31/05/2007	CP000712.1	Unpublished
GB-1	6 078 430	61.9	5515	90.2	74	22	05/02/2008	CP000926.1	Unpublished
KT2440	6 181 863	61.5	5481	87.5	74	22	22/01/2003	AE015451.1	Nelson <i>et al.</i> (2002)
W619	5 774 330	61.4	5292	89.8	75	22	11/03/2008	CP000949.1	Unpublished
<i>P. stutzeri</i>									
A1501	4 567 418	63.9	4237	90.3	61	13	20/04/2007	CP000304.1	Yan <i>et al.</i> (2008)
<i>P. syringae</i>									
pv. <i>phaseolicola</i> 1448A	5 928 787	57.9	5436 [§]	86.8 [§]	64	16	01/08/2005	CP000058.1	Joardar <i>et al.</i> (2005a, b)
pv. <i>syringae</i> B728a	6 093 698	59.2	5245	88.7	64	16	12/05/2005	CP000075.1	Feil <i>et al.</i> (2005)
pv. <i>tomato</i> DC3000	6 397 126	58.3	5721 [§]	85.6 [§]	64	16	21/08/2003	AE016853.1	Buell <i>et al.</i> (2003)

From: Silby *et al.*, 2011 FEMSMicrobiolRev35(2011)652–680

Pseudomonas genetic diversity 2

Table 2. Genome diversity – predicted genes unique to single strains*

<i>Pseudomonas</i> isolate [†]	Not present in others of the species [‡]
<i>P. aeruginosa</i> 2192	187
<i>P. aeruginosa</i> C3719	45
<i>P. aeruginosa</i> LESB58	219
<i>P. aeruginosa</i> PA7	660
<i>P. aeruginosa</i> PACS2	29
<i>P. aeruginosa</i> PAO1	54
<i>P. aeruginosa</i> UCBPP-PA14	143
<i>P. fluorescens</i> Pf-5	821
<i>P. fluorescens</i> Pf0-1	657
<i>P. fluorescens</i> SBW25	1195
<i>P. putida</i> F1	272
<i>P. putida</i> GB-1	456
<i>P. putida</i> KT2440	422
<i>P. putida</i> W619	418
<i>P. syringae</i> pv. <i>oryzae</i> str. 1_6	573
<i>P. syringae</i> pv. <i>phaseolicola</i> 1448A	263
<i>P. syringae</i> pv. <i>syringae</i> B728a	216
<i>P. syringae</i> pv. <i>tabaci</i> ATCC 11528	353
<i>P. syringae</i> pv. <i>tomato</i> DC3000	330
<i>P. syringae</i> pv. <i>tomato</i> T1	412

*Data retrieved 23 December 2010.

From: Silby et al., 2011 FEMS Microbiol Rev 35(2011)652–680

Table 1. Draft Genome Sequencing Summary for 14 Phylogenetically Divergent *P. syringae* Strains.

Identifier	<i>Pgy</i> R4	<i>Pmo</i>	<i>Pta</i>	<i>Pae</i>	<i>Pla</i> 107	<i>Cit</i> 7	<i>Pac</i>	<i>Ppi</i> R6	<i>Pja</i>	<i>Ptt</i>	<i>Pma</i>	<i>Pla</i> 106	<i>Pmp</i>	<i>Pan</i>
Pathovar	<i>glycinea</i>	<i>mori</i>	<i>tabaci</i>	<i>aesculi</i>	<i>lachrymans</i>	NA	<i>aceris</i>	<i>pisi</i>	<i>japonica</i>	<i>aptata</i>	<i>maculicola</i>	<i>lachrymans</i>	<i>morsprunorum</i>	<i>actinidiae</i>
Strain	A29-2	MAFF301020	ATCC11528	0893_23	MAFF301315	Cit7	MAFF302273PT	1704B	MAFF 301072 PT	DSM50252	ES4326	MAFF302278PT	MAFF302280PT	MAFF302091
Illumina Reads	4251697	7592243	5030953	15848526	8360650	5319177	6897439	4637560	5122223	5446792	14975328	8177882	16637970	10336469
Illumina Bases	153,061,092	265,728,505	181,114,308	554,698,410	641,057,438	191,490,372	241,410,365	166,952,160	184,400,028	196,084,512	524,136,480	294,403,752	1,247,847,750	361,776,415
454 Reads	432,292	162,625	131,130	130,109	126,287	149,557	155,239	285,725	375,650	144,250	300,835	345,257	141,417	299,744
454 Bases	30,418,492	24,154,020	19,726,747	19,173,310	20,116,024	22,337,212	22,063,131	22,457,735	24,292,249	21,590,755	23,408,095	26,294,110	21,665,692	22,364,744
454 Paired Ends	116,271	51,096	39,842	41,071	33,455	46,380	51,113	104,112	108,436	44,421	110,879	84,301	42,284	112,016
# Contigs	4,430	3,414	1,613	915	791	2,655	1,179	5,099	4,661	3,776	878	798	969	941
Contigs N50	3,723	5,634	16,098	16,806	22,550	6,862	12,409	3,003	4,021	4,753	17,222	15,738	15,161	14,086
# Scaffolds	109	70	32	139	222	57	60	170	60	53	44	90	69	138
Scaffolds N50	111,252	203,999	344,662	81,010	129,539	399,070	176,541	83,352	181,972	165,542	340,783	135,618	175,394	69,188
NCBI Accession # [†]	ADWY	AEAG	AEAP	AEAD	AEAF*	AEAJ	AEAO	AEAI	AEAH	AEAN	AEAK	AEAM	AEAE	AEAL
Included in TTE Screen	Yes	Yes	No	No	Yes	No	Yes	Yes	No	Yes	<i>Pma</i> M4	Yes	Yes	Yes

Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, et al. (2011) Dynamic Evolution of Pathogenicity Revealed by Sequencing and Comparative Genomics of 19 *Pseudomonas syringae* Isolates. PLoS Pathog 7(7): e1002132

Table 2 Sequencing statistics for next-generation *Pseudomonas syringae* sequences

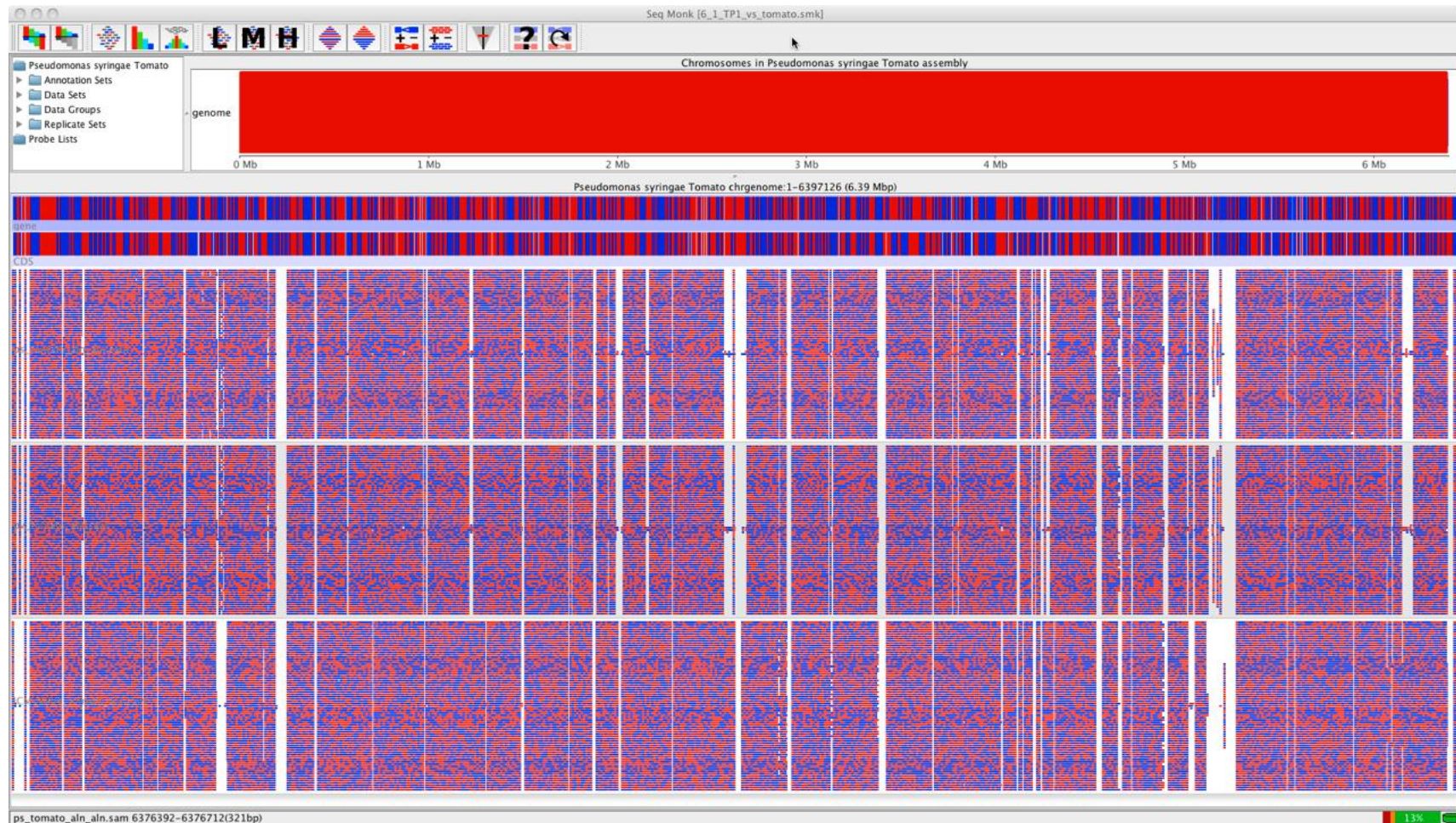
Strain	Sequencing method	Coverage	Assembly	No. of Contigs	No. of Scaffolds	N50	Reference
PtoT1	454 (241 bp)/Illumina (36 bp)	20X/94X	Newbler/Velvet 0.5	134	134	45,878	(65)
Psy642	Illumina PE (42 bp)	120X	Velvet 0.7.18	488	488	26,055	(11)
Pta11528	Illumina PE (36 bp)	145X	Velvet 0.7.18	1,405 ^a	71	317,167	(65)
Pae3681	Illumina PE (36 bp)	116X	Velvet 0.7.48	841	557	26,400	(16)
Pae2250	Illumina PE (36 bp)	133X	Velvet 0.7.48	776	364	42,500	(16)
PaeP6617	Illumina PE (36 bp)	127X	MAQ 0.6.8	NA	NA	NA	(16)
PaeP6623	Illumina PE (36 bp)	106X	MAQ 0.6.8	NA	NA	NA	(16)
Psv3335	454/454 PE (~200 bp)	15X	Newbler	403 ^a	112	87,144 ^a	(56)
PorI_6	454 PE (~200 bp)/ Illumina (36 bp)	3X/24X	Newbler/VCAKE	2855 ^a	130	531,821	(54)

^aCalculated from Genbank sequences.

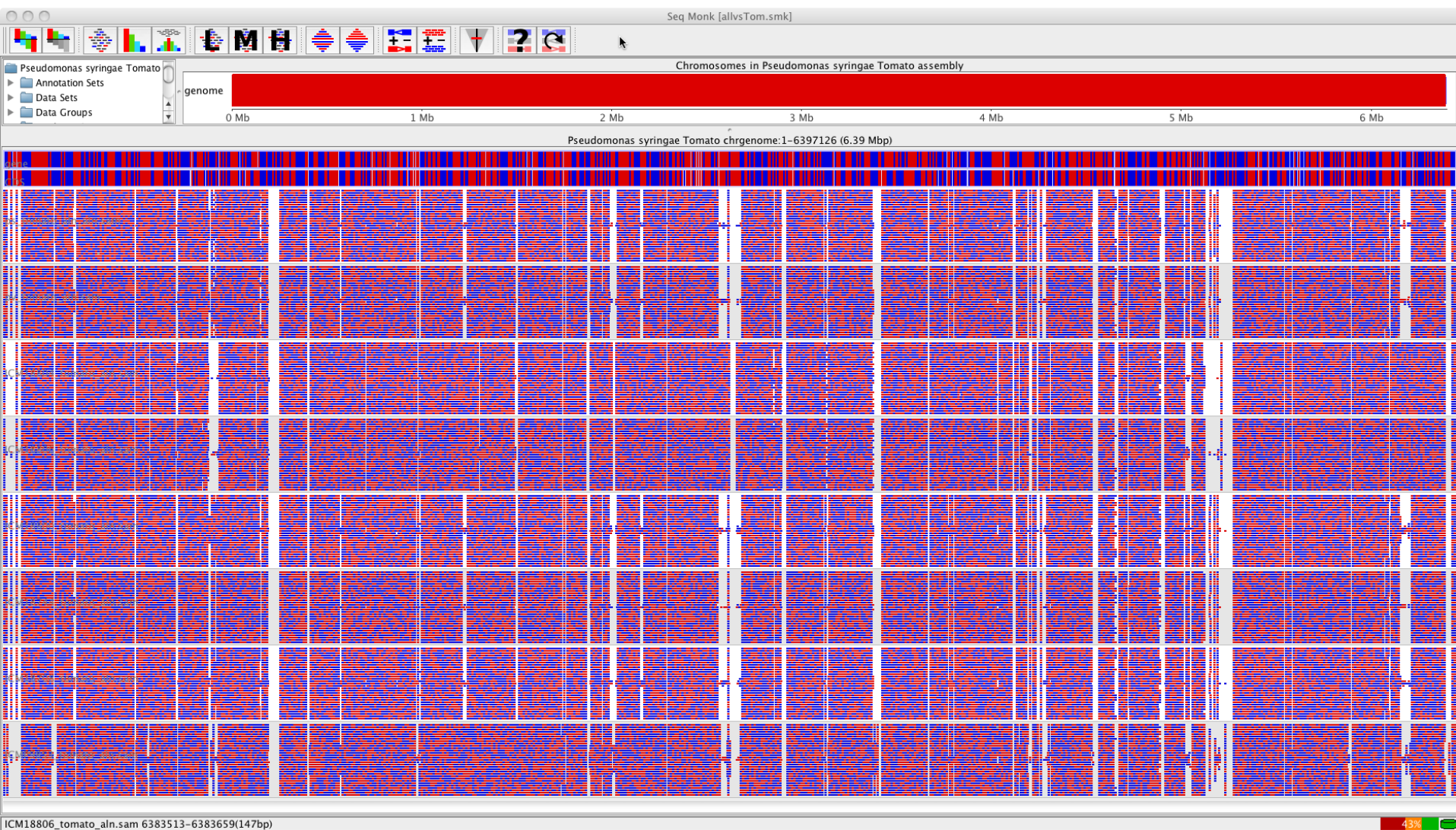
Abbreviations: PE, paired-end; NA, not applicable as this was a reference assembly.

O'Brien et al., 2011. Annu. Rev. Phytopathol. 2011. 49:17.1–17.21

Three genomes aligned with the fully sequenced and annotated
P. syringae pv. *tomato* DC3000 genome



All 8 new genomes aligned with *P. syringae* pv *tomato* DC3000



ICM18806_tomato_aln.sam 6383513-6383659(147bp)

43%

BLAST®

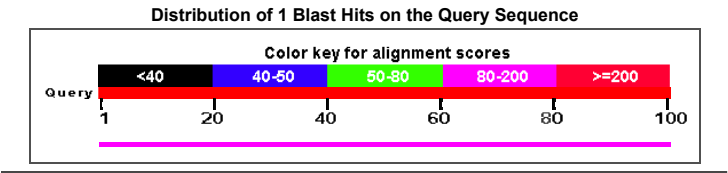
Basic Local Alignment Search Tool

NCBI/ BLAST/ blastn suite/ Formatting Results - 6161XA1E01R

s6_1204_5661_120130

Query ID	lcl 58943	Database Name	wgs
Description	s6_1204_5661_120130	Description	Whole-Genome-Shotgun Sequences
Molecule type	nucleic acid	Program	BLASTN 2.2.25+
Query Length	101		

Graphic Summary



Descriptions

Legend for links to other resources: UniGene GEO Gene Structure Map Viewer PubChem BioAssay

Accession	Description	Max score	Total score	Query coverage	E value	Max ident	Links
AEAL01000690.1	Pseudomonas syringae pv. actinidiae str. M302091 contig167.1, whole genome shotgun sequence	183	183	100%	6e-43	99%	

Alignments

>gb|AEAL01000690.1| Pseudomonas syringae pv. actinidiae str. M302091 contig167.1, whole genome shotgun sequence
Length=985

Score = 183 bits (99), Expect = 6e-43
Identities = 100/101 (99%), Gaps = 0/101 (0%)
Strand=Plus/Plus

Query	1	GGCGCGTTGGCCAGGCGGACCTGCATTCTTCTAGCACTGTCTCATGTTCCAGCGCCNA	60
Sbjct	344	GGCGCGTTGGCCAGGCGGACCTGCATTCTTCTAGCACTGTCTCATGTTCCAGCGCCGA	403
Query	61	ACCTTCTCTCTCGGGCCGGTGTGCATTGCGCCTTTATCGA	101
Sbjct	404	ACCTTCTCTCTCGGGCCGGTGTGCATTGCGCCTTTATCGA	444

College of Plant Protection
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Yang Ling
Shaanxi province



Mt. Huashan



Qin Terra-cotta Warriors



Hukou Waterfall, Yellow River

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