Supplemental Information

for

Isolation, characterization, and pathogenicity of two *Pseudomonas syringae* pathovars from *Populus trichocarpa* seeds

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Supplemental Figures.

Figure S1. Culture conditions of *Pseudomonas syringae* isolates. (A) Degradation analysis of catechol and anthranilate by *Pseudomonas syringae* NP10-3, NP28-5, and tomato pathovar DC3000. (B) Production of indole-3-acetic acid (IAA) in *Pseudomonas syringae* isolates and beneficial endophyte *Pseudomonas fluorescens* GM30. Error bars, standard deviation of three biological replicates, each measured in triplicate.



Figure S2. Toxins detected in *P. syringae* culture supernatants and the biosynthetic gene clusters (BGCs) found in *P. syringae* genomes. (A) Base peak total ion chromatogram (TIC) of *P. syringae* NP10-3. (B) Extracted ion chromatogram (EIC) of coronatine, *m/z* 319.1784, in *P. syringae* NP10-3. (C) EIC of syringolin, *m/z* 493.2900, in *P. syringae* NP10-3. (D) Base peak total ion chromatogram (TIC) of *P. syringae* NP28-5. (E) Extracted ion chromatogram (EIC) of coronatine, *m/z* 319.1784, in *P. syringae* NP28-5. (F) EIC of syringolin, *m/z* 493.2900, in *P. syringae* NP28-5. (G) BGCs of NP10-3 and NP28-5, predicted with antiSMASH 3.0. Clusters with >90% similarity to a known cluster are identified based on biosynthetic class.



Figure S3. *Pseudomonas syringae* isolates cause leaf lesions in 3-week-old *P. trichocarpa* seedlings and kill 3day-old germinated *Arabidopsis thaliana* seedlings. (A) Control-treated *P. trichocarpa* after 3 weeks. (B) *P. trichocarpa* inoculated with *P. syringae* pv. tomato DC3000. (C) *P. trichocarpa* inoculated with *P. syringae* NP10-3. (D) *P. trichocarpa* inoculated with *P. syringae* NP28-5. (E) Control-treated *Arabidopsis* after 2 weeks. (F) *Arabidopsis* inoculated with *P. syringae* NP10-3. (G) *Arabidopsis* inoculated with *P. syringae* NP28-5.



Figure S4. Motility of *Pseudomonas syringae* strains on LB 0.3% agar after 24 h. (A) *P. syringae* pv. *tomato* DC3000. (B) *P. syringae* sp. NP10-3. (C) *P. syringae* sp. NP28-5.



Supplemental Tables.

Table S1. Gene IDs and % identity to the *P. syringae* B728a sequence for *P. syringae* phylogroup II-specific genes.

B728a Gene ID	Gene Name	AA	NP10-3 Gene ID	% ID	NP28-5 Gene ID	%ID
2508861406	DNA-binding transcriptional regulator, LysR family	306	2758144526	99	2757798338	98
2508861408	predicted membrane protein, DUF421	147	2758144524	100	2757798340	100
2508861409	hypothetical protein	152	2758144523	99	2757798341	99
2508862882	sugar phosphate permease	440	2758144016	99	2757795041	99
2508862886	efflux transporter, outer membrane factor lipoprotein, NodT family	490	2758144021	99	2757795046	99
2508862989	DUF1810	141	2758144904	97	2757795154	97
2508863051	uncharacterized conserved protein YegJ	408	2758141542	100	-	-
2508863079	hypothetical protein	80	2758141514	100	2757795849	100
2508863081	hypothetical protein	143	2758141512	100	2757795847	100
2508863082	DUF72	244	2758141511	99	2757795846	99
2508863085	hypothetical protein	78	2758141508	100	2757795843	100
2508863302	AAA domain-containing protein	1945	2758141285	99	2757795616	99
2508863396	uncharacterized iron-regulated membrane protein	464	2758143825	99	2757798743	99
2508863651	GntR transcriptional regulator	239	2758142249	100	2757795301	100
2508863652	ADP-ribosylglycohydrolase	337	2758142250	100	2757795302	100
2508863654	ribokinase family protein	308	2758142252	99	2757795304	99
2508864240	methylase involved in ubiquinone/menaquinone biosynthesis	303	2758141860	99	2757787392	99
2508864241	hypothetical protein	269	2758141859	99	2757797391	100
2508864540	predicted dithiol-disulfide isomerase, DsbA family response regulator, CheY-like receiver domain and HTH	210	2758144162	100	2757798627	100
2508865031	DNA-binding domain	330	2758140915	99	2757798439	99
2508865261	ExbB outer membrane transport energization protein	242	2758141783	100	2757798838	100

Effector	B728 locus	B301D locus	HS191 locus	NP10-3 locus	% ID1	NP28-5 locus	% ID1
avrA1	-	-	-	-		-	
avrB1	-	-	-	-		-	
avrB2	-	-	-	-		-	
avrB3	Psyr_1219	-	-	-		-	
avrB4	-	-	-	-		-	
avrD1	-	-	-	-		-	
avrE1	Psyr_1188	PsyrB_06260	PsyrH_20365	2758140350	98	2757798191	97
avrPto1	Psyr_4919	-	-	-		-	
avrPto2	-	-	-	-		-	
avrPto3	-	-	-	-		-	
avrPto4	-	-	-	-		-	
avrPt05	- Dorr 0729	-	-	-		-	
aviKpiiii avrRnm2	1 Sy1_0738	-	-	-		-	
aviRpii2		-		_		-	
avrRp54	_	-	_	-		-	
hopA1	-	-	-	-		_	
hopA2	-	PsyrB 06425	PsyrH 20205	2758140318 ³	100	-	
hopB1	-	-	-	-		-	
hopC1	-	-	PsyrH_20070	-		-	
hopD1	-	-	-	-		-	
hopE1	-	-	-	-		-	
hopF1	-	-	-	-		-	
hopF2	-	-	-	-		-	
hopF3	-	-	-	-		-	
hopG1	-	-	-	-		-	
hopH1	Psyr_1889	PsyrB_09320	PsyrH_20075	2758143460	93	2757794720	93
hopH2	-	-	-	-		-	
hopH3	- Dorra 4226	- David P. 22470	- DougHL 04610	-	00	-	00
hop11	Povr 1017	PsyrB_05270	PeyrH 21240	2758141012	90 100	2757797125	90 100
hopK1	1 Sy1_1017	1 Sy1D_05270		-	100	-	100
hopM1	Psvr 1186	PsyrB 06250	PsyrH 20375	2758140352	94	2757798193	93
hopN1	-	-	-	-		-	
hopO1	-	-	-	-		-	
hopQ1	-	-	-	-		-	
hopR1	-	-	PsyrH_12260	-		-	
hopS1	-	-	-	-		-	
hopS2	-	-	-	-		-	
hopT1	-	-	-	-		-	
hop12	-	-	-	-		-	
hopU1	-	-	-	-		-	
hop V I	-	-	- DougHL 10025	-	40	-	10
hop V1	Pour 1220	-	rsyrn_12255	2/361409161	49	2/3//96430*	40
hopX1	1 Sy1_1220	-	-	-		-	
hop72	_	-	PsyrH 20105	-		_	
hopZ2	-	-	-	-		-	
hopZ3	Psyr 1224	-	-	-		-	
hopZ4	-	-	-	-		-	
hopZ5	-	-	-	-		-	
hopAA1	Psyr_1183	PsyrB_06235	PsyrH_20390	2758140355	98	2757798196	98
hopAB1	Psyr_4659	-	-	-		-	
hopAB2	-	-	-	-		-	
hopAB3	-	-	-	-		-	

Table S2. Effector protein loci and IMG gene IDs for *Pseudomonas* strains. Effectors from *Pseudomonas* sp. B728, B301D, and H191 are reported in Collmer et al.(1) and Baltrus et al.(2).

hopAC1 hopAD1	Psyr_0527	PsyrB_02970	PsyrH_02665	2758141118	99	2757796037	99
hopAE1	Psyr 4269	PsyrB 22195	PsyrH 04865	2758140918	99	2757798436	99
hopAF1	Psyr 3813	-	PsyrH 07325	-		2757798647	97
hopAG1	Psvr 0778	PsvrB 04160	PsvrH 22420	2758144515	99	2757798349	99
hopAH1	Psyr 0779	PsyrB 04165	PsyrH 22410	2758144514	100	2757798350	99
hopAH2	Psyr 3123	PsyrB 16095	PsyrH 10955	2758144517	99	2757795508	98
hopAI1	Psyr 0785	PsyrB 04195	PsyrH 22380	2758144508	99	2757798356	99
hopAJ2	Psyr_4357	PsyrB_22625	PsyrH_04450	2758141643	99	2757799077	99
hopAK1	Psyr_3839	PsyrB_19950	PsyrH_07200	2758144763	99	2757798675	99
hopAL1	-	PsyrB_08740	-	-		2757794607 ³	99
hopAM1	-	-	-	-		-	
hopAN1	Psyr_0465	PsyrB_02660	PsyrH_02345	27581410562	99	2757795975	99
hopAP1	Psyr_1890	PsyrB_09325	y _	27581434622 ²	98	2757794721	99
hopAO1	-	-	-	-		-	
hopAO2	-	-	-	-		-	
hopAQ1	-	-	-	-		-	
hopAR1	-	-	-	-		-	
hopAS1	-	-	-	-		-	
hopAT1	-	-	-	-		-	
hopAU1	-	-	-	-		-	
hopAV1	-	-	-	-		-	
hopAW1	-	-	-	-		-	
hopAX1	-	-	PsyrH_18845	-		-	
hopAY1	Psyr_2631	-	-	2758143760(6)	95	-	
hopAZ1	-	-	PsyrH_17410	-		-	
hopBA1	-	-	PsyrH_12900	-		-	
hopBB1	-	-	-	-		-	
hopBC1	-	PsyrB_08275	PsyrH_18105	2758142866(2)	99	-	
hopBD1	-	-	-	-		-	
hopBD2	-	-	-	-		-	
hopBE1	-	-	-	2758144790(5)	98	2757799454(5)	99
hopBF1	-	-	-	-		-	
hopBG1	-	-	-	-		-	
hopBH1	-	-	-	-		-	
hopBI1	-	-	-	-		-	
hopBJ1	-	-	-	-		-	
hopBK1	-	-	PsyrH_113064	-		-	
hopBL1	-	-	-	-		-	
hopBL2	-	-	-	-		-	
nopBM1	-	-	-	-		-	
порыл Total	-	-	-	-		-	
	24/9/	19/9/	20/9/	21/9/		20/97	

¹% ID is compared to the Psyr gene from *Pseudomonas sp.* B728a unless otherwise noted.

² gene appears truncated

³% ID is compared to the PsyrB gene from *Pseudomonas sp.* B301D

⁴% ID is compared to the PsyrH gene from *Pseudomonas sp.* H191
⁵% ID is compared to the hopBE1 gene from *Pseudomonas sp. aceris* MAFF302273

⁶% ID is compared to the hopAY1 gene from *Pseudomonas syringae* sp. mori 301020

Effector	N	P10-3	NP28-5				
	gene %GC	%GC vs. genome ¹	gene %GC	%GC vs. genome ¹			
hopH1	47.6%	10.89%	47.6%	10.93%			
hopBE1	48.7%	9.76%	48.7%	9.80%			
hopAF1	-	-	49.0%	9.49%			
hopAL1	-	-	49.2%	9.29%			
hopBC1	50.6%	7.87%	-	-			
hopAP1	51.7%	6.80%	53.7%	4.94%			
hopAY1	52.1%	6.41%	-	-			
hopAN1	65.3%	4.87%	65.2%	4.76%			
hopA2	53.3%	5.28%	-	-			
hopAG1	53.6%	5.00%	53.6%	5.03%			
hopAJ2	62.3%	2.52%	61.9%	2.16%			
hopAA1	62.2%	2.44%	62.1%	2.32%			
hopM1	62.1%	2.36%	62.1%	2.32%			
hopAC1	62.0%	2.28%	62.0%	2.24%			
hopW1	61.9%	2.20%	61.9%	2.16%			
hopAE1	61.9%	2.20%	61.9%	2.16%			
hopAH2	61.1%	1.55%	54.9%	3.84%			
hopAH1	57.7%	1.32%	57.8%	1.26%			
hopI1	58.0%	1.06%	58.1%	1.01%			
avrE1	60.4%	0.97%	60.4%	0.94%			
hopAI1	60.3%	0.89%	60.1%	0.69%			
hopJ1	60.2%	0.80%	60.8%	1.27%			
hopAK1	58.5%	0.63%	58.3%	0.83%			

Table S3. GC content of effector proteins compared to the GC content of the genomes of *P. syringae* NP10-3 and *P. syringae* NP28-5.

¹%GC vs. genome is calculated as |(gene GC) – (genome GC)|/((gene GC) + (genome GC))

Antibiotic		Resistance	DC2000	NID10 2	NID28 E
		gene	DC3000	NF 10-5	INF 20-5
	Ampicillin	ampC	Resistant	Resistant	Resistant
	Apramycin	aac(3) IV	Resistant	Resistant	Resistant
	Cefotaxime		Susceptible	Susceptible	Susceptible
	Chloramphenicol		Susceptible	Susceptible	Susceptible
	Gentamicin		Susceptible	Susceptible	Susceptible
	Kanamycin		Susceptible	Susceptible	Susceptible
	Nalidixic Acid		Susceptible	Susceptible	Susceptible
	Penicillin	ampC	Resistant	Resistant	Resistant
	Rifampicin	rpoB*	Resistant	Susceptible	Susceptible
	Tetracycline		Susceptible	Susceptible	Susceptible

Table S4. Antibiotic susceptibility of *P. syringae* poplar isolates compared to *P. syringae* pv. tomato DC3000.

*mutation confers resistance

Table S5. Physiological effects on *Populus trichocarpa* rooted seedlings after 15 d exposure to *Pseudomonas syringae* isolates.

treatment	final plant mass (g)	final root mass (g)	∆plant mass (g)	stem length (cm)	leaf area (cm²)	leaf lesion coverage (% total leaf surface affected)	Δ chlorophyll (µmol/m² leaf)
Control	0.77 (0.32)	1.04 (0.02)	0.77 (0.32)	11.13 (2.75)	20.68 (7.90)	0.04 (0.01)	-11.33 (4.54)
DC3000	0.84 (0.15)	1.26 (0.08)	0.84 (0.15)	8.32 (3.07)	24.67 (4.43)	0.71 (0.5)	-11.28 (2.74)
NP10-3	0.72 (0.18)	0.41 (0.07)*	0.72 (0.18)	8.45 (3.08)	22.23 (6.30)	2.07 (1.97)	-10.25 (7.37)
NP28-5	0.87 (0.25)	0.35 (0.03)*	0.87 (0.25)	9.17 (1.70)	22.89 (5.66)	6.30 (3.21)*	-6.53 (5.19)

*P<0.05 compared to control

Table S6. Metabolite profile of control and *Pseudomonas*-infected *Populus trichocarpa* rooted plants. Red text indicates metabolite fold change (FC) is positive and the change is significant (Student's t-test reported as P-value); blue text indicates metabolite FC compared to 0 h is decreased and the change is significant.

		Cor	ntrol			NP10-3			NP28-5			
Populus Metabolite ¹		24 h ³ 48 h		24 h 48 h				24 h 48 h				
	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value	FC	p-value
catechol glucoside	11.46	0.035	13.80	0.105	17.03	0.035	21.18	0.068	59.72	0.120	32.47	0.114
threonine	1.74	0.565	0.73	0.616	0.93	0.630	0.25	0.138	6.48	0.293	4.67	0.288
salicyl alcohol	2.28	0.358	1.32	0.242	1.44	0.032	3.03	0.183	29.38	0.217	4.21	0.144
catechin	0.86	0.793	2.49	0.190	8.26	0.149	5.28	0.133	1.38	0.615	4.02	0.017
digalactosylglycerol	1.75	0.156	1.08	0.868	4.26	0.009	2.57	0.033	5.57	0.049	3.92	0.003
salicortin	1.28	0.406	1.23	0.634	2.43	0.002	2.35	0.014	2.96	0.036	3.75	0.001
13.68 [171, 289: glycoside]	1.64	0.022	1.60	0.009	2.65	0.003	2.40	0.009	3.05	0.011	3.64	<0.0001
a-salicyloylsalicin	1.09	0.790	1.24	0.641	2.17	0.027	2.24	0.014	2.95	0.059	3.55	0.004
salicylic acid glucoside	1.91	0.152	1.87	0.020	2.16	0.084	2.67	0.070	2.22	0.191	3.38	0.002
isosalicin	1.97	0.024	2.73	0.046	2.13	0.008	2.76	0.062	5.42	0.148	3.29	0.076
17.68 [476, 386, 296]	1.28	0.552	1.47	0.466	3.15	0.018	2.43	0.046	2.38	0.082	3.06	0.001
citric acid	0.65	0.498	0.79	0.696	3.21	0.126	2.07	0.240	2.33	0.155	2.95	0.082
13.75 [171, 289; glycoside]	1.56	0.078	1.59	0.148	2.35	0.023	2.62	0.010	2.56	0.044	2.83	0.001
2,5-dihydroxybenzoic acid-5-O-glucoside	2.34	0.102	2.20	0.040	6.66	0.011	5.04	0.006	4.87	0.056	2.77	0.029
myo-inositol	1.31	0.423	1.12	0.759	2.38	0.034	2.32	0.042	2.20	0.042	2.72	0.008
fructose	2.95	0.004	2.38	0.104	2.08	0.001	2.20	0.002	2.86	0.004	2.71	0.049
o-cresol-glycoside ²	1.27	0.387	1.38	0.275	1.43	0.146	1.39	0.088	2.23	0.065	2.42	<0.0001
sancyi-sancyic acid-2-O-glucoside	2.37	0.080	0.82	0.102	1.60	0.235	2.15	0.259	2.87	0.262	2.40	0.045
6-hydroxy-2-cyclohexenone-1-carboxylic acid	0.85	0.090	0.82	0.203	1.30	0.190	1.06	0.901	1.51	0.362	2.33	0.063
phytol	0.93	0.812	0.29	0.205	1.2	0.233	1.00	0.280	1.07	0.100	2.33	0.089
galactosylglycerol	1.44	0.285	0.95	0.890	2.82	< 0.0001	2.38	0.030	4.39	0.088	2.29	0.001
trichocarpin	1.17	0.641	1.17	0.732	1.61	0.089	1.69	0.051	1.74	0.087	2.22	0.010
glyceric acid	3.69	0.024	3.05	0.097	1.56	0.441	1.47	0.038	1.19	0.497	2.22	0.265
10.83 [393, 303, 257]	1.76	0.024	1.91	0.002	1.55	0.024	1.82	0.007	1.85	0.020	2.21	0.006
o-cresol ²	1.53	0.121	1.87	0.046	1.28	0.212	1.26	0.193	1.89	0.023	1.97	0.055
12.85 [235; glycoside]	1.07	0.869	0.79	0.591	2.70	0.114	2.66	0.017	1.05	0.930	1.92	0.161
aspartic acid	1.57	0.142	1.13	0.323	1.22	0.243	1.02	0.887	2.23	0.179	1.79	0.246
shikimic acid	0.92	0.838	0.91	0.843	1.38	0.125	1.13	0.605	1.45	0.016	1.79	0.230
quercetin	1.11	0.823	0.96	0.932	3.67	0.165	2.50	0.339	1.36	0.373	1.77	0.264
glucose	2.18	0.035	1.85	0.268	1.10	0.970	0.95	0.865	2.07	0.024	1.71	0.065
unreonic acid	1.08	0.800	0.79	0.007	1.92	0.016	1.00	0.987	2.37	0.067	1.69	0.159
5-oxo-proline	1.10	0.202	1.02	0.330	0.98	0.768	0.99	0.425	1.38	0.000	1.57	0.349
kaempferol	1.19	0.586	0.96	0.912	2.67	0.315	1.91	0.388	1.17	0.372	1.51	0.334
benzyl alcohol	1.00	0.954	0.92	0.512	0.93	0.924	1.28	0.046	1.33	0.209	1.43	0.136
sucrose	1.62	0.008	1.59	0.003	1.16	0.059	1.31	0.010	1.33	0.071	1.41	0.022
succinic acid	1.18	0.589	0.98	0.946	1.10	0.193	1.07	0.732	1.68	0.065	1.40	0.139
salicylic acid	1.24	0.149	1.36	0.156	0.84	0.275	0.89	0.475	1.68	0.127	1.36	0.128
1,2,4-benzenetriol	1.07	0.739	1.10	0.701	1.73	0.230	1.24	0.543	1.03	0.872	1.35	0.191
erythronic acid	1.82	0.040	1.31	0.373	1.31	0.112	1.04	0.844	1.15	0.185	1.33	0.455
glycerol	1.43	0.077	1.15	0.319	0.96	0.634	1.23	0.348	2.14	0.123	1.33	0.200
β-sitosterol	1.44	0.013	1.40	0.027	1.19	0.086	1.10	0.108	1.16	0.202	1.32	0.035
galactosa	2.00	0.025	2.04	0.079	0.53	0.0001	0.59	0.020	1.33	0.103	1.27	0.311
caffeic acid	1 18	0.220	1.16	0.390	1.62	0.009	1.22	0.100	1.41	0.302	1.22	0.309
stearic acid	1.38	0.032	1.29	0.029	0.82	0.033	0.84	0.051	1.24	0.056	1.19	0.041
salicin	1.22	0.212	1.18	0.361	1.36	0.128	1.29	0.250	1.08	0.630	1.17	0.332
glutamic acid	1.20	0.677	0.69	0.409	1.53	0.296	0.90	0.770	1.98	0.314	1.15	0.743
myo-Inositol 1-phosphate	1.70	0.113	1.33	0.373	0.76	0.039	0.82	0.191	1.68	0.041	1.14	0.720
6-hydroxy-2-cyclohexenone (enol)	1.19	0.018	0.98	0.830	1.13	0.498	1.14	0.272	1.26	0.102	1.13	0.188
8.62 [235, 324, 309]	1.21	0.047	1.04	0.649	1.18	0.410	1.27	0.057	1.20	0.124	1.10	0.400
14.50 [254, 289, 268; glycoside]	1.23	0.359	0.99	0.966	1.57	0.016	1.30	0.019	1.10	0.603	1.10	0.506
1-o-p-coumaric acid glucoside	1.18	0.265	1.12	0.502	1.31	0.135	1.28	0.219	1.05	0.700	1.09	0.541
alanine	0.88	0.751	0.72	0.188	0.78	0.113	0.62	0.058	2.08	0.145	1.02	0.954
manc acto catoshol	1.46	0.109	1.51	0.251	0.89	0.293	0.94	0.378	0./1	0.297	1.01	0.959
ovalic acid	1 12	0.105	0.84	0.361	1.39	0.407	0.45	0.048	1.41	0.430	0.95	0.798
adenosine	1.15	0.340	1 12	0.760	1 45	0.000	1 18	0.485	1.50	0.597	0.75	0.544
asparagine	0.75	0.761	0.33	0.274	0.63	0.367	0.23	0.141	1.33	0.656	0.61	0.158
mucic acid	1.08	0.819	0.69	0.215	1.27	0.261	1.04	0.736	1.06	0.891	0.60	0.344
ethyl phosphate	1.43	0.350	1.29	0.526	0.59	0.076	0.72	0.267	0.87	0.779	0.59	0.176
phosphate	1.52	0.420	1.23	0.640	0.62	0.114	0.69	0.211	1.05	0.930	0.56	0.239

11.74 [232, 449, 464, 347] 0.69 0.685 0.47 0.370 0.35 0.154 0.20 0.119 0.43 0.107 0.41 0.057

¹ unidentified metabolite identified by retention time (min) and [key m/z; possible metabolite type]

² metabolite remains to be confirmed

 $^{\rm 3}$ time point FC is compared to same treatment at 0 h

Supplemental References

1. Collmer A, Badel JL, Charkowski AO, Deng W-L, Fouts DE, Ramos AR, et al. *Pseudomonas syringae* Hrp type III secretion system and effector proteins. Proceedings of the National Academy of Sciences of the United States of America. 2000;97(16):8770.

2. Baltrus DA, Nishimura MT, Romanchuk A, Chang JH, Mukhtar MS, Cherkis K, et al. Dynamic evolution of pathogenicity revealed by sequencing and comparative genomics of 19 *Pseudomonas syringae* isolates. PLoS Pathogens. 2011;7(7):e1002132.