# **Supporting Information**

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Fig. S1. Neighbor-joining analysis of Xam strains. Colors indicate geographic origins. Analysis is based on same data as in Fig. 2. CLC Genomics Workbench was used for reference-based assemblies and SNP identification for each Xam strain was compared with the Xam668 genome (Materials and Methods). Xam strains cluster primarily by geographic region with a few outliers. Yellow, Brazil; blue, Colombia; purple, Africa; white, other/geographic outliers. Bootstrap (1,000 replicates) was performed as a test of significance. Tree is unrooted.



Fig. S2. Xam strains contain diverse transcription activator-like (TAL) effector-containing plasmid profiles. Plasmid DNA was isolated and run on a 0.75% agarose gel. Southern analysis was performed to assess the TAL effector sequence content of each plasmid.



**Fig. S3.** Maximum-likelihood phylogeny of sequenced xanthomonads places *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) among its closest relatives. Phylogeny is based on full-genome SNP analysis, 1,442,868 SNPs (*Materials and Methods*). *Xam*, strains NG1, Xam1134, Xam678, Xam668, Xam672, ClO151, and Xam669 cluster together; *Xp, Xanthomonas perforans* 91–118; *Xe, Xanthomonas euvesicatoria* 85–10; *Xac F1, X. axonopodis* pv. *citrumello F1; Xac306, X. a.* pv. *citri; Xoo, Xanthomonas oryzae* pv. *oryzae*, strains Pxo99A, 311018, and 10331 cluster together; *Xcm, Xanthomonas campestris* pv. *musacearum; Xcc, X. c.* pv. *campestris*, strains B100, 8004, and 33913 cluster together; *Xcr, X. c.* pv. *raphani* 756C.



**Fig. S4.** Presence of effectors was determined from de novo and reference-based assemblies. XopE1 and XopE2 have high (~68%) identity. Sequences present in most genomes are more homologous to XopE1, although CIO151 also has a homolog to XopE1 and XopE2. Green, identified through BLAST, confirmed manually; blue, identified through BLAST, sequence polymorphism alters gene model (e.g., frameshift mutation, premature stop codon); white, not identified. Red box outlines conserved effectors with the same gene model as a characterized effector from the effector database. AvrBs3-like sequences were present in all strains of *Xam*; however, they were not completely assembled because of the repetitive central domain.



Fig. 55. Cartoon summarizing our effector prediction and sequence variation analysis pipeline. Python scripts can be found at http://pypi.python.org/pypi/biotools/1.1.2.



**Fig. S6.** (A) Area under the disease progression (AUDPC) measure of virulence does not show a significant trend between effector count and virulence level. Average disease symptoms *in planta* (measured by area under the disease progression curve) of Xam strains are grouped by origin (Africa, purple; Venezuela, orange; Brazil, yellow; Colombia, blue; Indonesia, gray; unknown, white); mean  $\pm$  SD (n = 6) is displayed. (B and C) Effector and TAL count compared with disease symptoms *in planta* (measured by AUDPC) of tested Xam strains.



**Fig. 57.** Sequence variance of select Xam effectors. Sequence variance was calculated as described in *Materials and Methods*. Gene models are displayed immediately above the x axis. In some cases, potential alternate start codons are present 5' to the predicted ATG. In these cases, the sequences have been excluded in these plots.



Fig. S8. Southern analysis of genomic DNA digested with EcoRI. All blots were probed with a <sup>32</sup>P-labeled internal BamHI fragment of TAL 14. TAL effector copy number was compared with virulence level (Fig. 3). Faint bands were confirmed in repeated experiments.

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Table S1. Xam strain and assembly information

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						Ass	embly stat	tistics*		
Strain	Year	Origin	Collector	N50	Length	Contigs	Max	Mean	SD	Reads
AFNC1360	1998	Benin or Nigeria	AFNC	25	4,923,650	150	235,381	32,824	37,800	20,051,776
AT6B	2006	Venezuela	M.S.	22	4,946,093	163	237,170	30,344	39,662	19,151,609
CFBP1851	1974	Columbia	CFBP	17	4,798,436	123	281,156	39,011	52,886	3,310,501
CIO1	1995	Columbia	CIO	19	4,898,899	128	211,998	38,272	45,752	13,719,586
CIO151	1995	Columbia	CIO	24	4,911,246	175	150,154	28,064	35,954	25,616,672
UA226	2008	Columbia	A.B.	18	4,951,862	129	290,136	38,386	49,473	11,330,262
UA303	2008	Columbia	A.B.	23	4,872,968	130	196,821	37,484	39,723	15,222,193
UA306	2008	Columbia	A.B.	23	5,065,231	144	235,368	35,175	42,248	15,102,007
UA323	2008	Columbia	A.B.	23	4,872,007	151	1/3,//6	32,264	37,606	20,612,168
	2008	Columbia	А.В. л.р.	22	4,910,013	140	101,357	33,071	38,915	90,20,217
	2009	Columbia	А.В. л.р.	23	4,800,301	142	183,/5/	34,269	39,350	10,292,031
	2009	Columbia	A.D. A R	22	4,042,000	159	257,275	21 801	41,407	17,000,477
114686	2009	Columbia	A.D. A.R	27	4,037,322	1/13	196 787	3/ 251	10 679	15 9/7 863
IRSRE1182	1995	Brazil	IRSRE	128	4,000,000	648	46 493	7 366	6 968	2 149 549
IBSBF1411	1998	Brazil	IBSBF	15	4 856 132	130	314 799	37 354	55 031	4 275 532
IBSBF1994	2004	Brazil	IBSBF	137	4.774.518	677	45,564	7.052	6.508	2.232.128
IBSBF2345	2006	Brazil	IBSBF	19	4.819.772	128	286.963	37.654	47.959	1.904.256
IBSBF2346	2006	Brazil	IBSBF	18	4,874,593	122	257,932	39,955	48,696	17,994,941
IBSBF2538	2007	Brazil	IBSBF	122	4,833,429	573	50,456	8,435	, 7,446	3,398,164
IBSBF2539	2007	Brazil	IBSBF	24	4,952,612	138	196,970	35,888	39,356	15,572,597
IBSBF2665	2009	Brazil	IBSBF	83	4,836,797	434	111,173	11,144	11,333	1,893,919
IBSBF2666	2009	Brazil	IBSBF	49	4,842,277	311	123,056	15,570	18,537	2,797,254
IBSBF2667	2009	Brazil	IBSBF	21	4,913,060	138	226,291	35,601	42,268	13,781,769
IBSBF2670	2009	Brazil	IBSBF	17	4,902,902	139	28,0128	35,272	50,872	13,653,945
IBSBF2672	2009	Brazil	IBSBF	18	4,856,510	131	257,571	37,072	48,420	8,192,559
IBSBF2673	2009	Brazil	IBSBF	53	4,830,620	305	120,981	15,838	17,359	2,649,843
IBSBF278	1965	Brazil	IBSBF	20	5,020,210	138	239,334	36,378	44,921	12,084,985
IBSBF280	1973	Brazil	IBSBF	171	4,842,752	850	41,005	5,697	5,251	1,750,289
IBSBF2816	2010	Brazil	IBSBF	19	4902619	145	256,960	33,811	46,242	13,153,736
IBSBF2818	2010	Brazil	IBSBF	22	4851686	139	235,402	34,904	42,367	11,4/4,331
IBSBF2819	2010	Brazil	IRZBE	104	4,852,810	557	59,730	8,/12	8,583	2,479,861
IBSBF2820	2010	Brazil	IRZRE	19	4,930,429	150	257,651	32,869	44,768	9,028,899
	2010	Brazil		02	4,900,900	101	237,392	30,470 10 /17	41,909	2 022 714
IBSBF2022	1973	Nigeria	IBSRE	95 21	4,031,349	404 1/17	186 156	33 873	9,343	2,055,714
IBSBF289	7	Brazil	IRSRE	21	4,572,047	155	226 288	31 274	38 049	15 106 454
IBSBF320	1974	Brazil	IBSBE	17	4 803 726	121	257 673	39 700	49 436	14 285 701
IBSBF321	1974	Brazil	IBSBF	183	4,593,056	888	35,503	5,172	4.646	1,869,048
IBSBF356	1980	Brazil	IBSBF	18	4,847,079	130	257,951	37,285	49,019	14,264,479
IBSBF436	1982	Brazil	IBSBF	19	4,903,327	149	257,580	32,908	44,502	13,185,959
IBSBF614	1987	Brazil	IBSBF	136	4,730,788	706	, 51,491	6,700	6,516	2,138,932
IBSBF725	1988	Brazil	IBSBF	19	4,886,517	141	257,945	34,656	45,893	14,295,676
IBSBF726	1988	Brazil	IBSBF	21	4,832,905	140	257,968	34,520	43,377	17,714,917
NCPPB1159	1941	Brazil	NCPPB	17	4,797,980	128	257,906	37,484	48,746	13,904,209
NG1	2011	Nigeria	L.K.	160	4,737,721	785	38,252	6,035	5,419	3,934,608
ORST17	1984	Congo	ORST	23	5,128,104	177	235,384	28,972	40,180	15,379,085
ORST4	1974	Columbia	ORST	161	4,650,210	767	36,169	6,062	5,309	1,942,504
ORST X27	1989	Тодо	ORST	21	4,931,909	133	237,243	37,082	43,419	14,896,651
ThaiXam	1997	Thailand	N.K.	62	4,874,623	324	84,544	15,045	14,840	2,770,771
UG21	2011	Uganda	T.A.	23	5,010,468	148	237,252	33,854	40,078	18,213,584
UG23	2011	Uganda	1.A.	22	4,924,062	133	237,150	37,023	42,562	8,918,645
UG24	2011	Uganda	Т.А. Т.А	20	4,909,909	145	233,940	33,861	43,565	5,036,494
UG27	2011	Uganda	Т.А. Т.А	19	4,903,342	131	268,832	37,430	47,341	3,629,887
	2011	Uganda	Т.А. Т.А	131	4,/22,/34	0/0	54,/51	0,980	0,899	9,803,349
	2011	Uganda	т. <del>д</del> .	21	4,911,002 4 97/ 197	1/5	237,200	33 020	43,922 A1 100	16 650 104
UG44	2011	Uganda	ΤΔ	23 7/	4 895 117	143	237,107	34 710	40 012	19 277 872
UG45	2011	Uganda	т.А	24 59	4,986 397	323	83,811	15,437	15.477	5,338 599
UG51	2011	Uganda	T.A.	22	4,871.289	140	234,400	34,794	41,630	14,764,547
Xam1134	?	?	?	28	4,894,844	158	237,227	30,980	34,575	29,876,966

#### Table S1. Cont.

				Assembly statistics*						
Strain	Year	Origin	Collector	N50	Length	Contigs	Max	Mean	SD	Reads
Xam668	1978	Indonesia	NZTCC	29	4,954,860	195	276,859	25,409	33,003	12,733,850
Xam669	1973	Brazil	NZTCC	21	4,820,297	137	201,435	35,184	40,806	15,193,524
Xam672	1976	Cameroon	NZTCC	25	5,016,075	165	239,253	30,400	37,139	22,340,056
Xam678	1973	DR Congo	NZTCC	47	4,894,142	56	104,760	19,117	19,014	7,734,076

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### Table S2. Illumina sequencing price breakdown

ltem	Price per sample, \$* 15				
Library construction					
Reagents	70				
Sequencing	35				
Total	120				

\*Price assumes use of Apollo 324 automated library prep, IntegenX reagents, sequencing primers ordered through Integrated DNA Technology (PAGE purified), and 48 samples multiplexed in one lane of an Illumina Hi-Seq2000. Prices are current for University of California members as of 2010.

## **Other Supporting Information Files**

Dataset S1 (XLS)

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