Genome sequencing of *Xanthomonas citri* pv. *malvacearum* and the PCR-based detection of the cotton bacterial pathogen

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Bacterial Blight of Cotton

Upper side: Angular spots

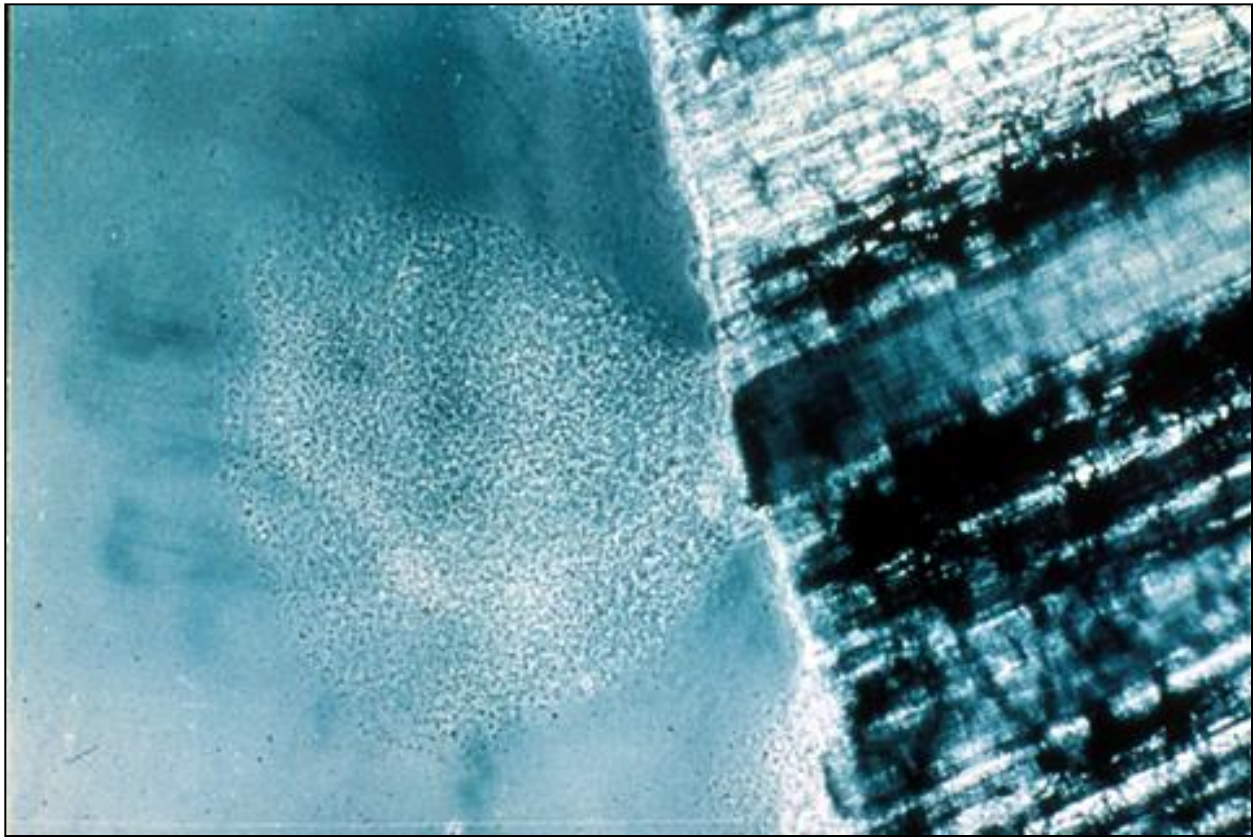
Under side: Water soaked symptom

Boll Rot of Cotton

(Photographed by Tom Allen)

Bacterial streaming

- **The way to differentiate the symptoms from other spots**



Bacterial Blight of Cotton

Pathogen:

- ***Xanthomonas citri* pv. *malvacearum* (Ah-You et al. 2009)**

Hosts:

- ***Gossypium* spp. = cotton (& relatives: okra, swamp hibiscus, etc.)**
- ***Thespesia lambas* = portia tree**
- ***Ceiba pentandra* = kapok tree**

Distribution: worldwide (≈ 20 races)

Yield loss: 0.1-3.4% up to 50-70%

Taxonomy of Bacterial Blight Pathogen of Cotton

- ***Xanthomonas malvacearum*** **(Smith 1901): Physiological and biochemical characteristics**
- ***Xanthomonas campestris*** **pv. *malvacearum* (Dye 1978): Physiological and biochemical characteristics, DNA-DNA hybridization, and host specificity**
- ***Xanthomonas axonopodis*** **pv. *malvacearum* (Vauterin et al. 1995): 16S rDNA, DNA-DNA hybridization**
- ***Xanthomonas citri*** **subsp. *malvacearum* (Schaad et al. 2006): DNA-DNA hybridization; re-PCR, Serology, ITS, SDS-PAGE etc.**
- ***Xanthomonas citri*** **pv. *malvacearum* (Ah-You et al. 2009): DNA-DNA hybridization, amplified fragment length polymorphism (AFLP), and multilocus sequence analysis (MLSA: 16S rDNA, *gyrB*, *atpD*, and *dnaK*)**

The Genus *Xanthomonas* Dowson 1939

- **Type species: *Xanthomonas campestris***
- **Over 100 xanthomonads (species, subspecies and pathovars) have been recognized as distinct plant pathogens.**
- **Practically all-major groups of plants (at least 124 monocot and 268 dicot plant species) suffer from one or more diseases caused by this bacterium.**
- ***Xanthomonas* is a well-defined, homogeneous genus and the strains share a high degree of genetic similarity.**

Genome Comparison of Two Xanthomonads

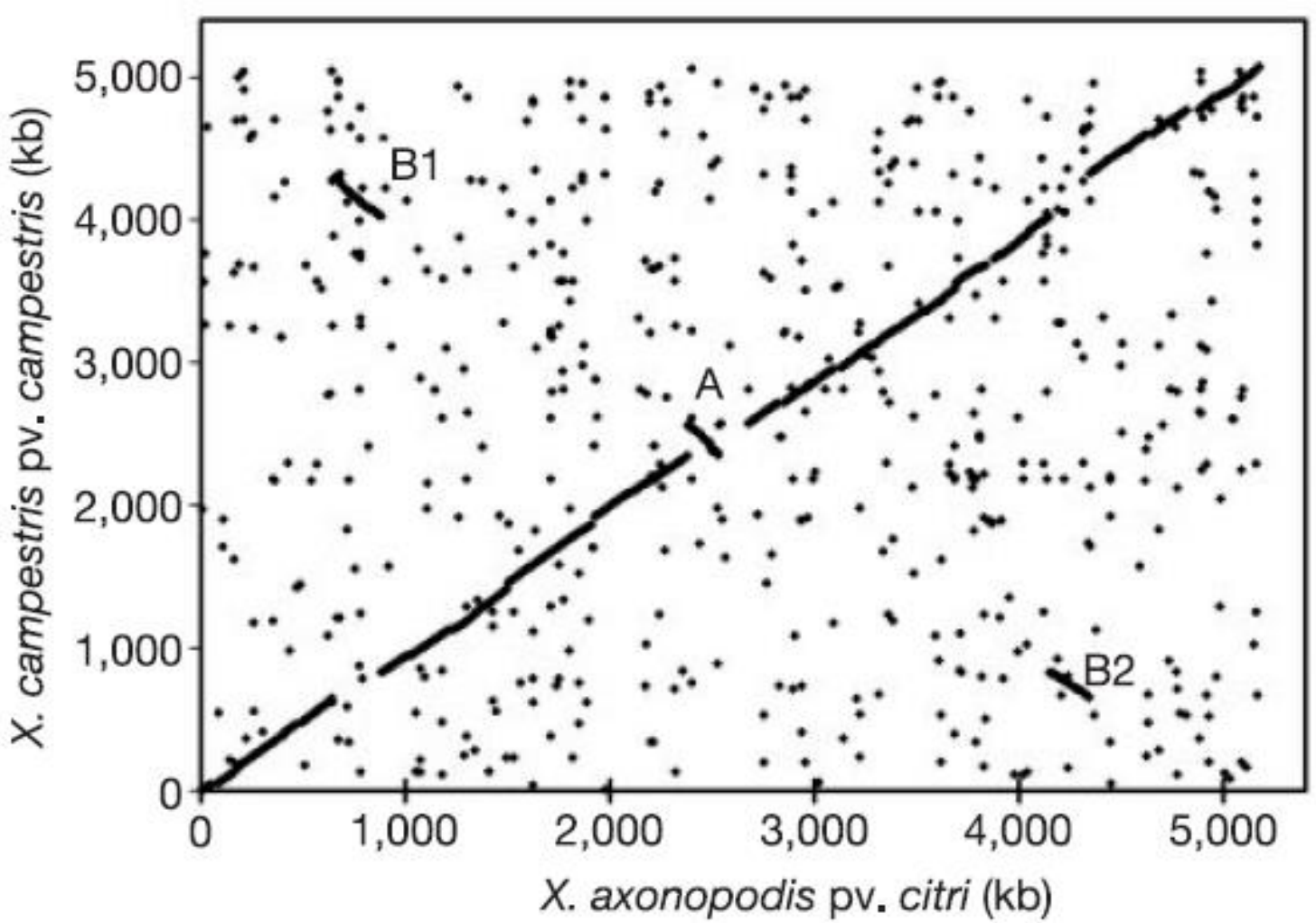
General features of the chromosomes

	Xcc	*Xac*
Length (bp)	5,076,187	5,175,554
G+C content (%)	65.0	64.7
Protein-coding region (% chromosome size)	84.34	85.59
Protein-coding genes		
With assigned function	2,708	2,710
Conserved hypothetical	1,276	1,272
Hypothetical	198	331
Total	4,182	4,313
Transfer RNA	53	54
Ribosomal RNA operons	2	2
Plasmids	0	2
Insertion sequence elements (IS)	109	87

Xcc: Xanthomonas campestris* pv. *campestris
Xac: Xanthomonas axonopodis* pv. *citri

(da Silva et al. Nature 2003)

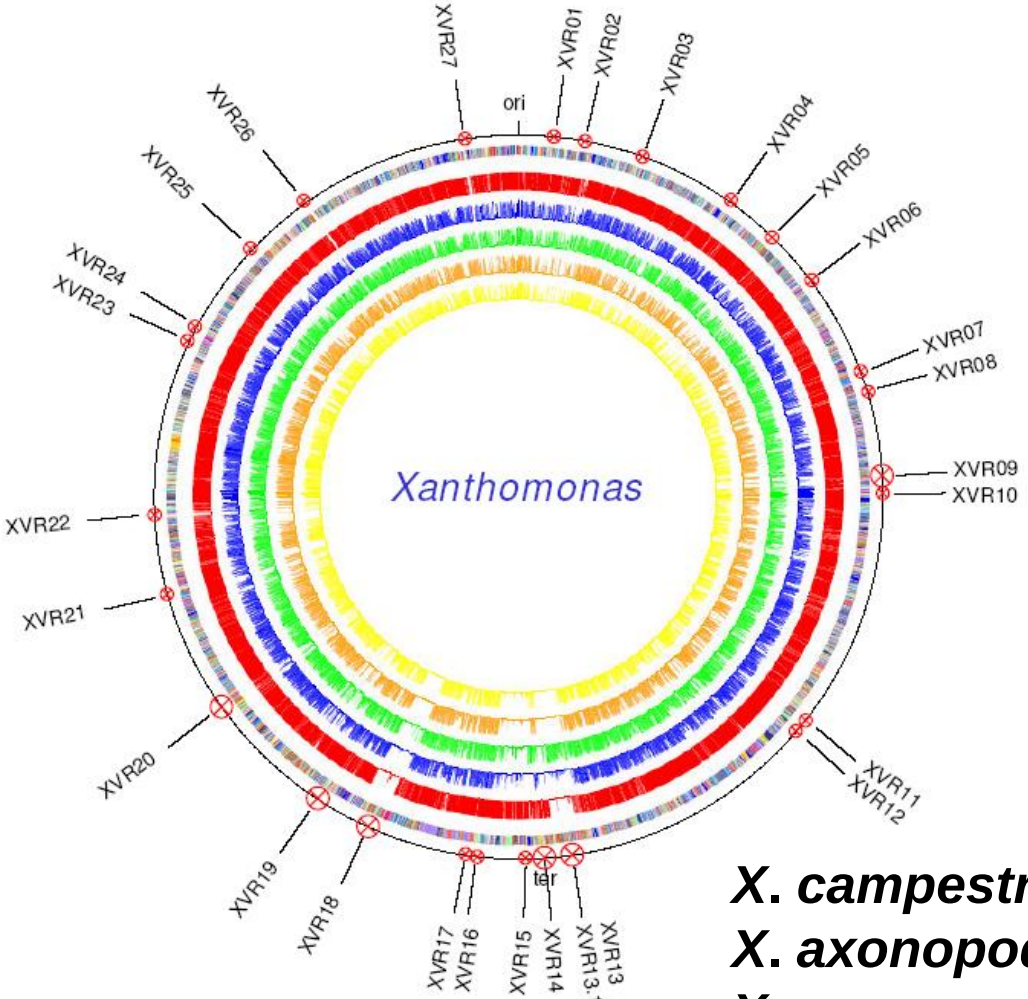
Nucleotide alignment between *Xac* and *Xcc*.



(da Silva et al. Nature 2003)

Comparison of *Xanthomonas* Genomes

(Xanthomonad variable regions)



- Xcc 8004
- Xcc 33193
- Xac 306
- Xcv 85-10
- Xoo10331
- Xoo 311018
- XVR

***X. campestris* pv. *campestris* (*Xcc*)**
***X. axonopodis* pv. *citri* (*Xac*)**
***X. oryzae* pv. *oryzae* (*Xoo*)**
***X. campestris* pv. *vesicatoria* (*Xcv*)**

(He et al. Genome Biology 2007)

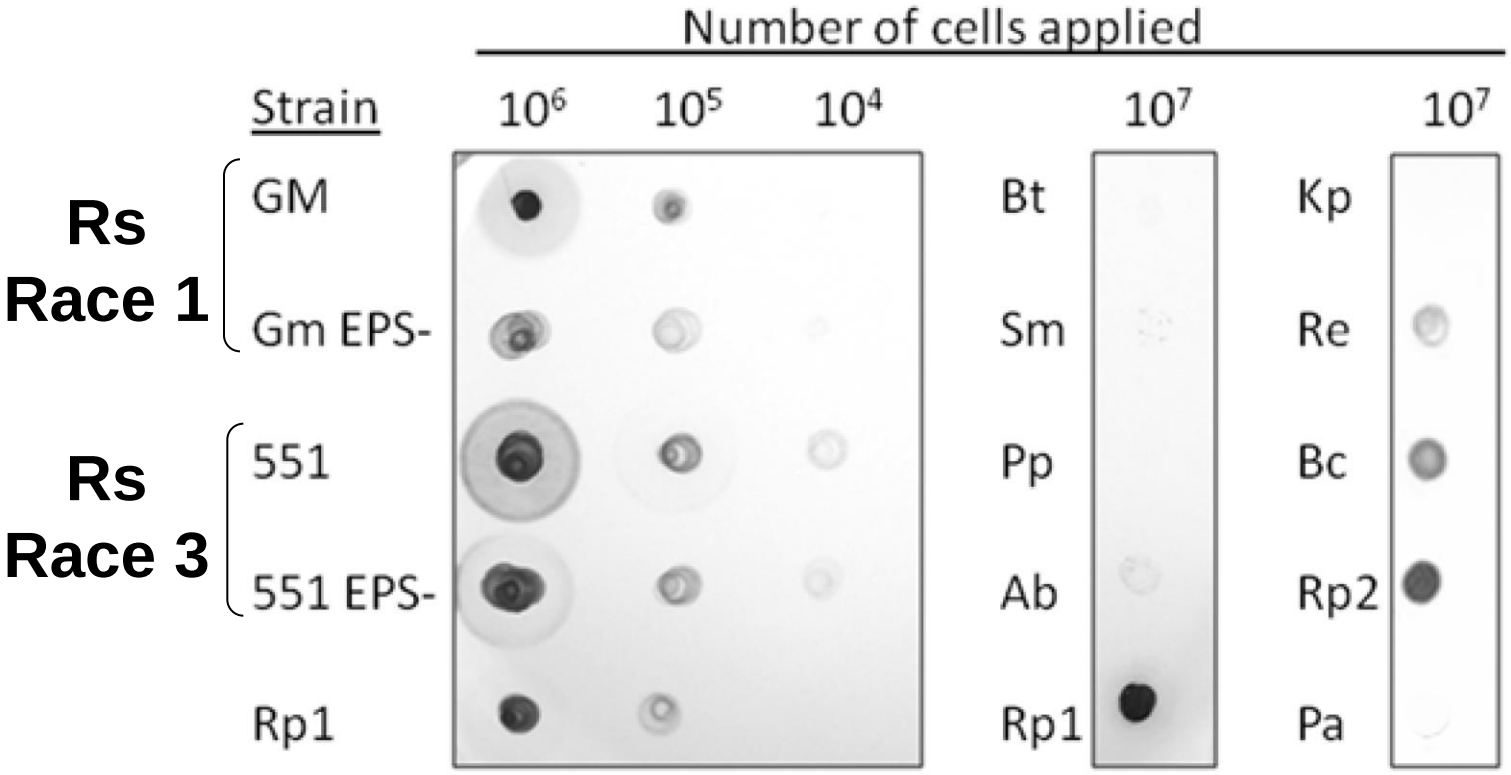
Current Methods for Detection of *Xanthomonas citri* pv. *malvacearum*

- **Semi-selective culture medium (MSSXAN): Culture-dependent isolation and further identification is required (Dezordi et al. 2010)**

- **Random amplified polymorphic DNA (RAPD) technique: Extract DNA and PCR amplification with ITS primers; low reproducibility (Umesha et al. 2010)**

- **Polymerase chain reaction-based detection: pthN – based PCR amplification; the gene *pthN* is > 94% identical with all other sequenced members of the *avrlpth* gene family (Chakrabarty et al. 2005)**

Sensitivity and Cross-Reactions of Antibody of *Ralstonia solanacearum* R3B2



Rs: *Ralstonia solanacearum;* Rp: *Ralstonia pickettii*;
Re: *Ralstonia eutropha*; Bc: *Burkholderia cepacia*

(Denny et al., 2012)

Importance and Difficulty to Detect *Xcm* from Seed

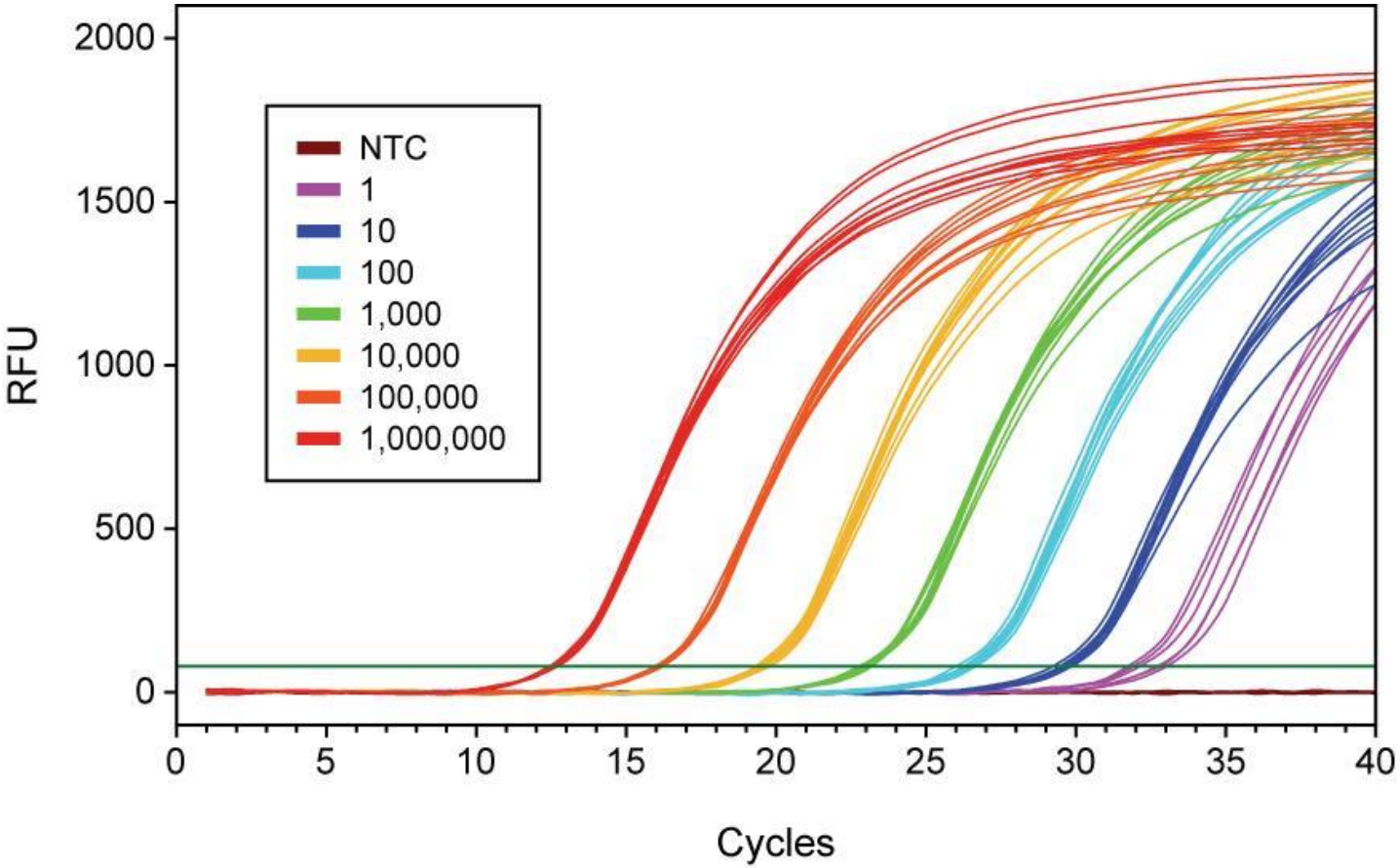
Importance:

- **Seed-borne pathogen (Verma 1986)**
- **Surviving in/on seed at least 22 months and on the lint for at least 4 months (Thaxton and El-Zik, 2001)**

Difficulty:

- **Very low rate of bacterium-carrying seed: ≈0.1% (Verma 1986)**
- **Bacteria are not active (dormancy) in seed (Jones and Lennon 2010).**
- **Endophytic *Xanthomonas* spp. in cotton seed (Misaghi and Donndelinger 1990)**

Real-Time Quantitative PCR



Fast-Cycling Kits for real-time PCR developed using 5 Primers Inc., MD, USA (very sensitive: one cell; and fast: ≈30 minutes)

Bacterial Strain MSCT1

Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Xanthomonas citri subsp. malvacearum strain DSM 3849 16S ribosomal RNA gene, partial sequence	2580	2580	100%	0.0	99%	NR_117146.1

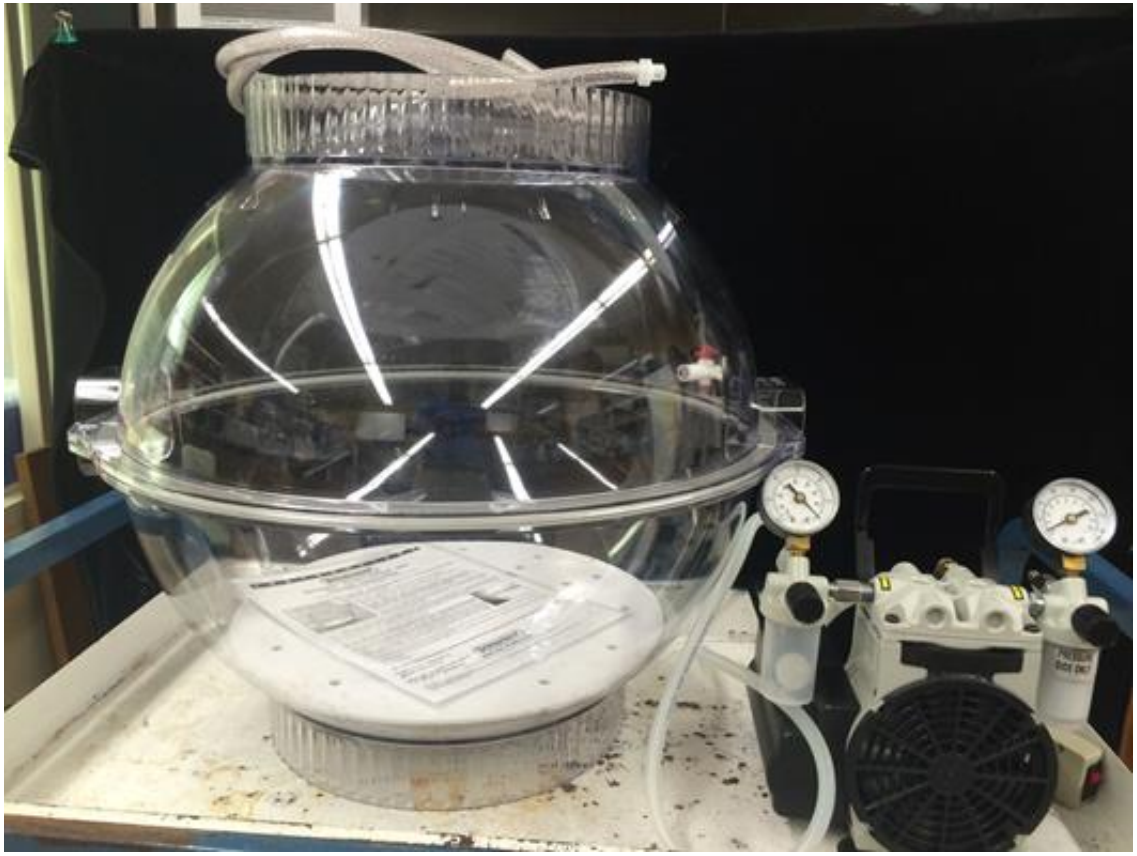
Pathogenicity Tests

***Xcm* MSCT1**

Sterile Distilled Water

Bacterial cells were infiltrated into plant tissue with needleless syringe (Day 6 after inoculation)

Vacuum System for Bacterial Inoculation



Pathogenicity Assays

***Xcm* MSCT1**

Sterile Distilled Water

Bacterial cells were infiltrated into plant tissue with vacuum (20 "Hg for 15 seconds; Day 10 after inoculation)

Sequencing Project Information

MIGS ID	Property	Term
MIGS 31	Finishing quality	Draft
MIGS-28	Libraries used	Illumina PCR free, Nextera Mate Pair (4 kb) (PacBio-not included)
MIGS 29	Sequencing platforms	Illumina Miseq (PE300)
MIGS 31.2	Fold coverage	PCR free (247.7x), Mate Pair (39.5x)
MIGS 30	Assemblers	ALLPATHS-LG
MIGS 32	Gene prediction	Prodigal

Genome Statistics of Strain MSCT

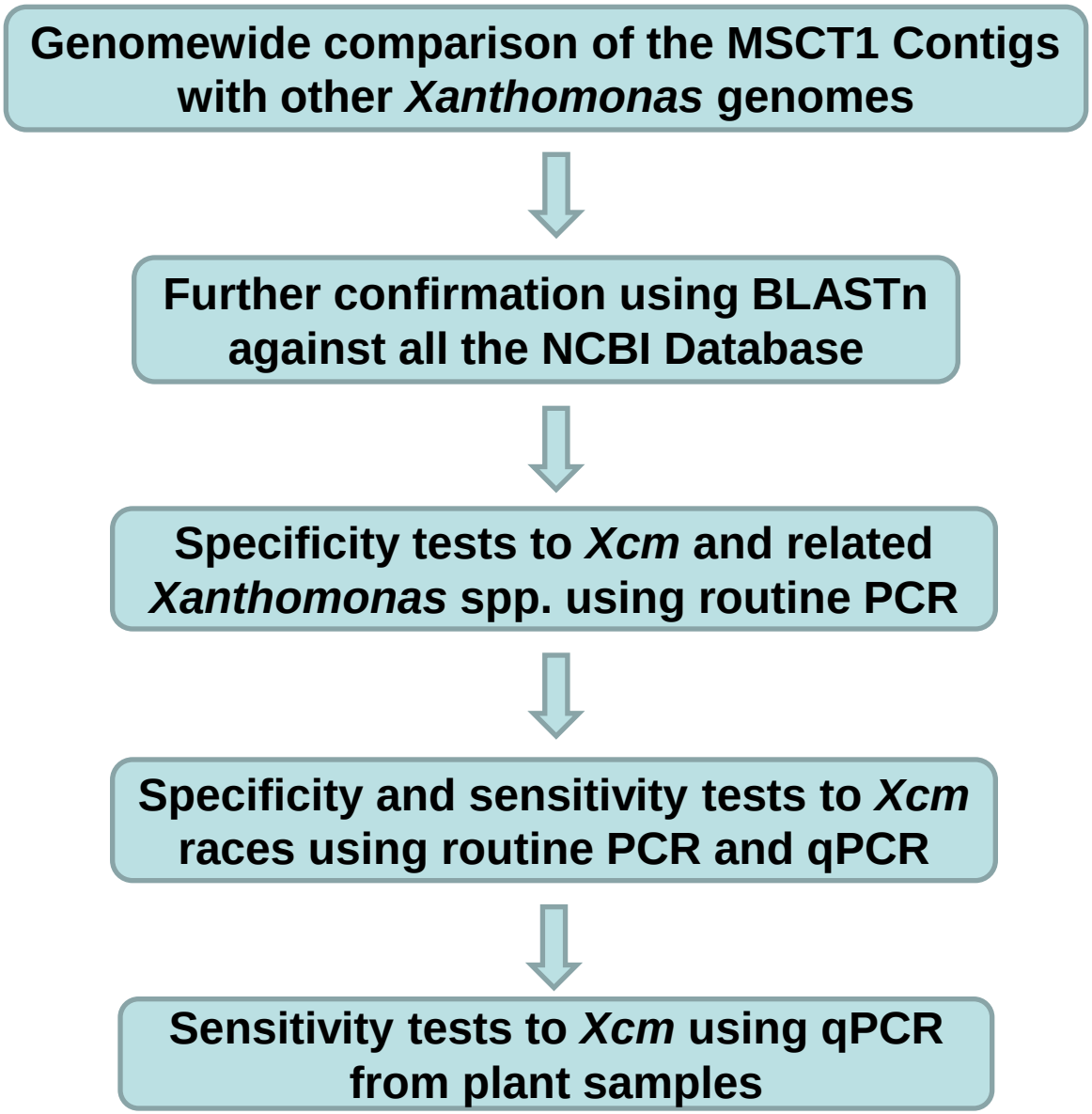
Attribute	Value
Genome size (bp)	5,142,224
DNA coding (bp)	4,295,077
DNA G+C (bp)	3,244,657
DNA scaffolds	14
Total genes	4,307
Protein coding genes	4,247
RNA genes	60
Pseudo genes	1
Genes in internal clusters	NA
Genes with function prediction	3,355
Genes assigned to COGs	3,176
Genes with Pfam domains	3,544
Genes with signal peptides	611
Genes with transmembrane helices	966
CRISPR repeats	1

Average Nucleotide Identity of MSCT1 and Related Strains

	X_cassavae	X_oryzae_oryzae	Xcm_GSPB1386_R18 Nicaragua	Xcm_GSPB2388 HVS Sudan	Xcm_MSCT	Xcm_R18 Burkina_Faso	Xcm_R20 Burkina_Faso
X_cassavae	NA	0.8735	0.8759	0.8685	0.8907	0.876	0.8768
X_oryzae_oryzae	0.8735	NA	0.9107	0.9019	0.9131	0.9053	0.9049
Xcm_GSPB1386_R18_Nicaragua	0.8759	0.9107	NA	0.9941	1	1	0.9988
Xcm_GSPB2388_HVS_Sudan	0.8685	0.9019	0.9941	NA	0.9904	0.9903	0.9937
Xcm_MSCT	0.8907	0.9131	1	0.9904	NA	1	0.9993
Xcm_R18_Burkina_Faso	0.876	0.9053	1	0.9903	1	NA	0.997
Xcm_R20_Burkina_Faso	0.8768	0.9049	0.9988	0.9937	0.9993	0.997	NA

Strain MSCT1 is more close to Race 18 based on calculation of average nucleotide identity of the genome sequences.

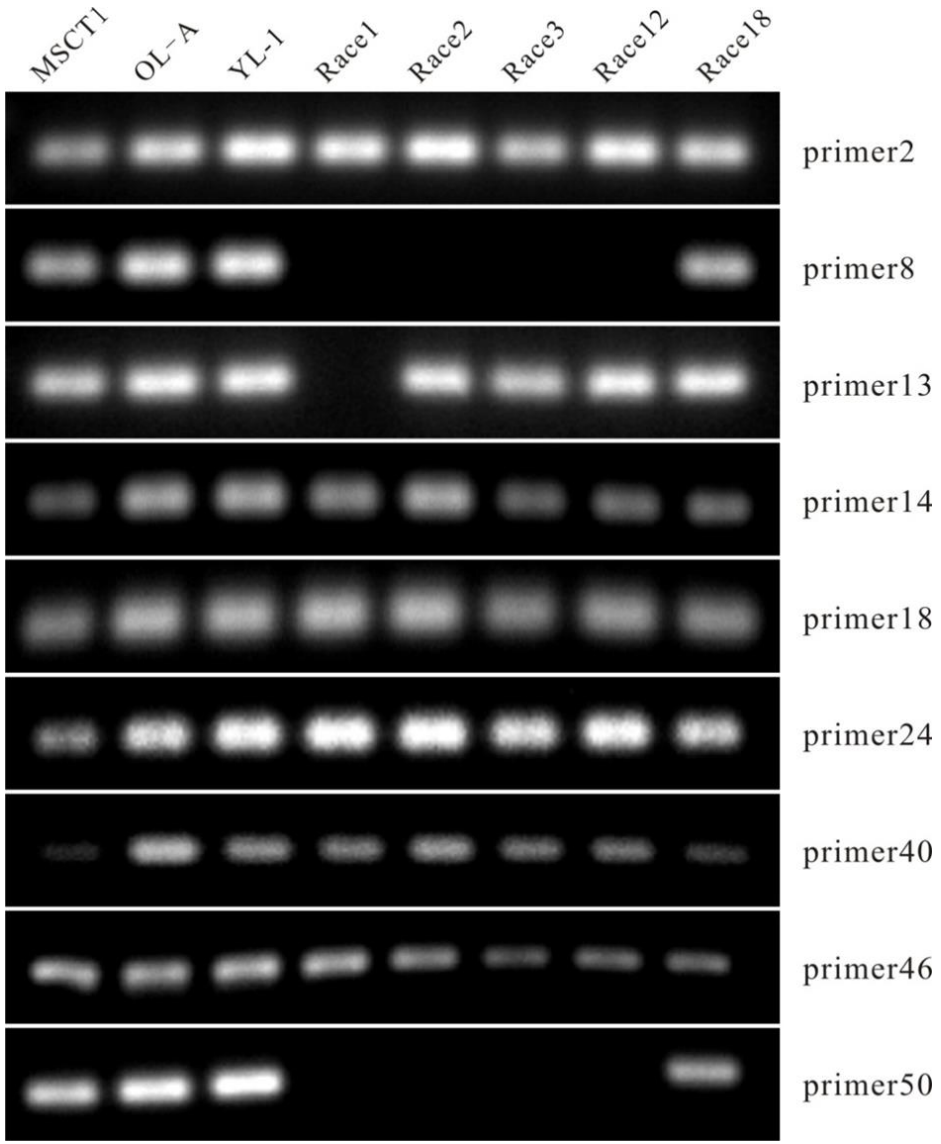
PCR Primer Selection



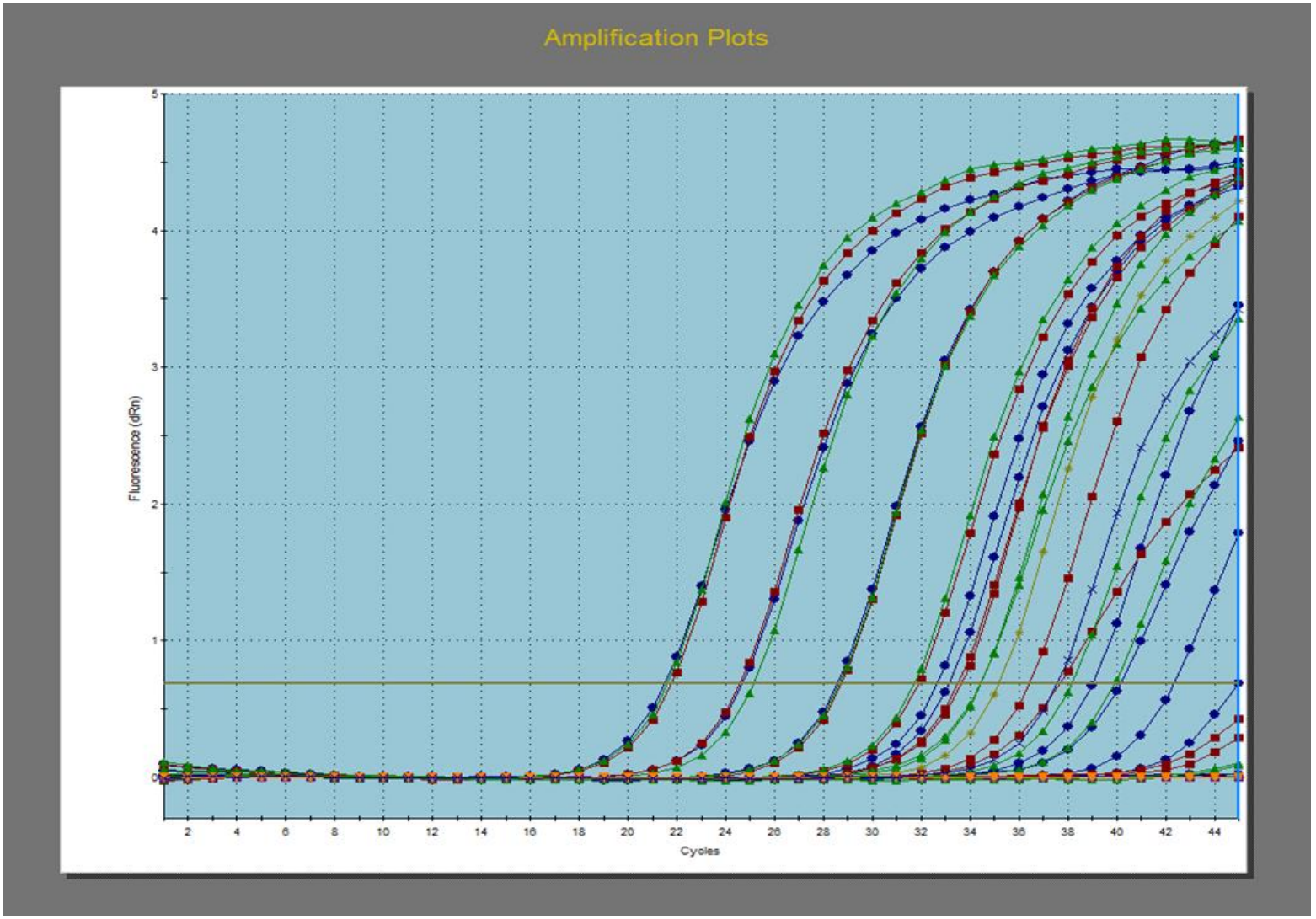
Bacterial Strains Used

Bacterium [a]	Isolate number	Sources
***Xanthomonas citri* pv. *malvacearum* MSCT1**	MSCT1	This study
***Xanthomonas citri* pv. *malvacearum* Race 1**	Race 1	Tom Allen
Xanthomonas citri pv. *malvacearum* Race 2	Race 2	Tom Allen
***Xanthomonas citri* pv. *malvacearum* Race 3**	Race 3	Tom Allen
***Xanthomonas citri* pv. *malvacearum* Race 12**	Race 12	Tom Allen
***Xanthomonas citri* pv. *malvacearum* Race 18**	Race 18	Tom Allen
***Xanthomonas citri* pv. *citri* 306**	XAC306	Dennis Gross
***Xanthomonas campestris* pv. *vesicatoria* 85**	XCV85	Dennis Gross
***Xanthomonas campestris* pv. *campestris* ATCC 33913**	XCC33913	Dennis Gross

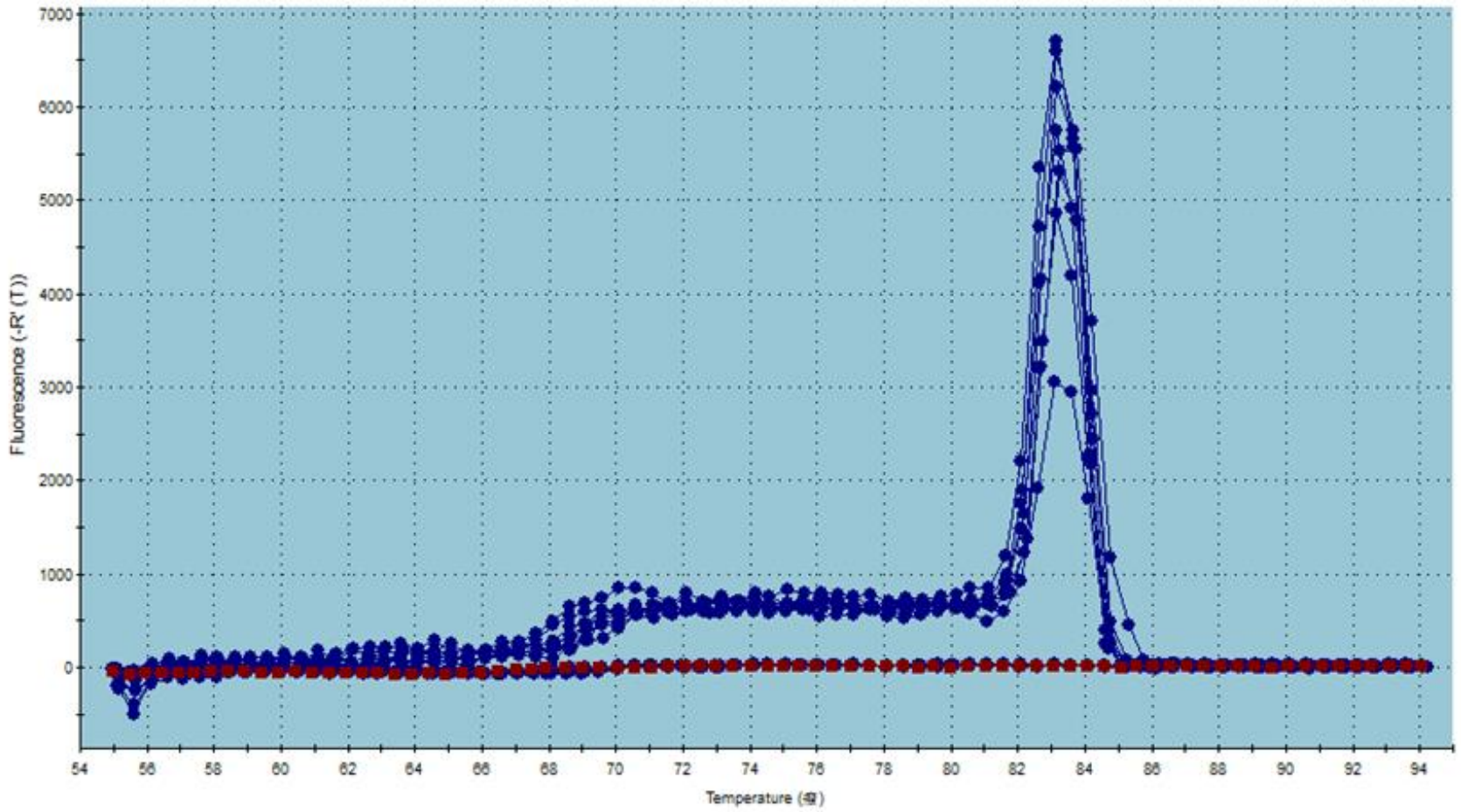
[a] Strain MSCT1 was a local strain isolated from cotton field in Washington County, Mississippi, during 2011.



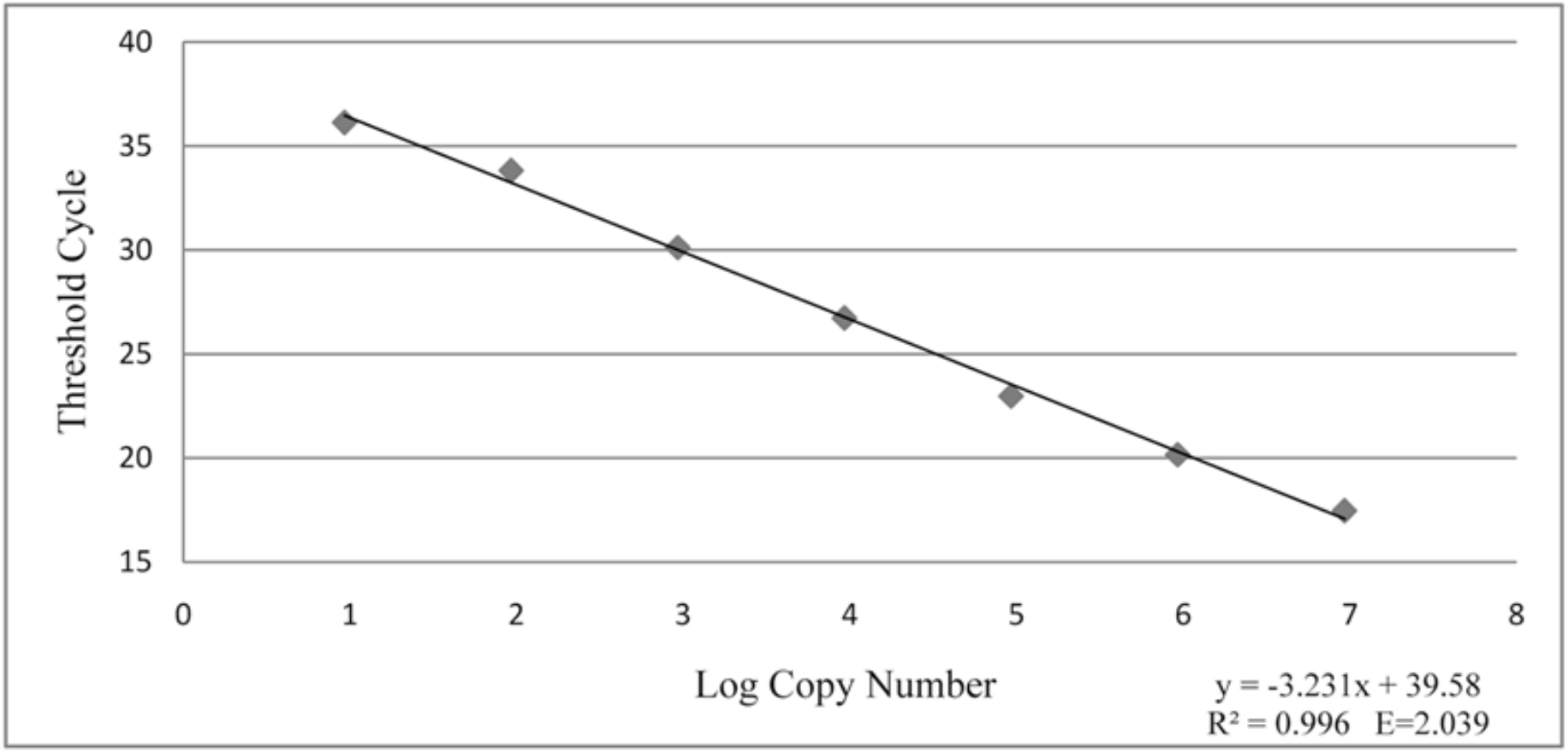
PCR primer candidates for pathogen detection. Bacterial strains of the blight pathogen: MSCT1, OL-A (local isolate), YL-1 (local isolate), Races 1, 2, 3, 12, and 18.



Real-time PCR results of the SYRB method using different amounts of bacterial genomic DNA



Melting curve of the SYBR PCR products to verify primer specificity



TaqMan qPCR standard curve generated using genomic DNA of MSCT1. qPCR efficiencies were 100-104%.

Sensitivity of the qPCR detection of Xcm from Plant Materials

- **Bacterial cells were added to fresh leaves and seed samples;**
- **Total DNA was extracted from the samples, respectively;**
- **TaqMan qPCR was conducted;**
- **The detection limit is 10^2 cells from 1 g fresh leaves and 37 cells from 1g seeds;**
- **No PCR amplicon was observed from the negative control (not inoculated).**

Research Summary

1) **Nearly-complete genome sequence of the cotton bacterial blight pathogen *Xanthomonas citri* pv. *malvacearum* is available.**
2) **The local strain sequenced is Race 18.**
3) **A TaqMan qPCR system has been developed for rapid and accurate detection of Xcm.**
4) **The genome sequence and detection system have many applications:**
   - **Pathogen identification**
   - **Genetic variation**
   - **Seed quality control**
   - **Disease development and epidemiology**
   - **Host range study**

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