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J. M. Lang
Colorado State University-Fort Collins

E. DuCharme
Colorado State University-Fort Collins

J. Ibarra Caballero
Colorado State University-Fort Collins, jorgeiba@colostate.edu

E. Luna
Colorado State University-Fort Collins, emily.peachey@colostate.edu

T. Hartman
University of Nebraska-Lincoln

See last page for additional authors

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Detection and Characterization of *Xanthomonas vasicola* pv. *vasculorum* (Cobb 1894) comb. nov. Causing Bacterial Leaf Streak of Corn in the United States

J. M. Lang,[1] E. DuCharme,[1] J. Ibarra Caballero,[1] E. Luna,[1] T. Hartman,[2] M. Ortiz-Castro,[1] K. Korus,[2,3] J. Rascoe,[4] T. A. Jackson-Ziems,[2] K. Broders,[1] and J. E. Leach[1]

1. Department of Bioagricultural Sciences and Pest Management, Colorado State University, Fort Collins, Colorado, USA
2. University of Nebraska–Lincoln, Lincoln, Lincoln, Nebraska, USA
3. Alachua County Extension, University of Florida, Gainesville, Florida, USA
4. United States Department of Agriculture—Animal Plant Health Inspection Service—Plant Protection and Quarantine—CPHST, Beltsville, Maryland, USA

Corresponding author – J. E. Leach, email jan.leach@colostate.edu

Abstract

Bacterial leaf streak of corn (*Zea mays*) recently reached epidemic levels in three corn-growing states and has been detected in another six states in the central United States. *Xanthomonas vasicola* was identified as the causal agent of this disease. A multilocus sequence alignment of six housekeeping genes and comparison of average nucleotide identity from draft genome sequence were used to confirm phylogenetic relationships and classification of this bacteria relative to other *X. vasicola* strains. *X. vasicola* isolates from Nebraska and South Africa were highly virulent on corn and sugarcane and less virulent on sorghum but caused water-soaking symptoms that are typical of *X. vasicola* infection on the leaves of all three hosts. Based on host range and phylogenetic comparison, we propose the

taxonomic designation of this organism to *X. vasicola* pv. *vasculorum* (Cobb 1894) comb. nov. Polymerase chain reaction-based diagnostic assays were developed that distinguish *X. vasicola* pv. *vasculorum* and *X. vasicola* pv. *holcicola* from each other and from other *Xanthomonas* spp.

Corn (*Zea mays*) is a staple crop worldwide and is the most widely produced feed grain in the United States. In 2014, symptoms of bacterial leaf streak disease were first observed on corn in Nebraska and, by 2016, the disease was reported in Colorado, Iowa, Kansas, Minnesota, Oklahoma, South Dakota, and Texas (Korus et al. 2017). Given similarity of symptoms to those caused by other corn pathogens, it is not known how long the disease has been present in the United States. Bacterial leaf streak was first described in 1949 on corn in South Africa (Dyer 1949), but prior to 2017 it had not been documented in the United States (Korus et al. 2017). Symptoms occur on leaves of field (dent) corn, sweet corn, seed corn, and popcorn crops, appearing as dark, water-soaked, linear lesions with wavy margins confined to the interveinal spaces. Due to the importance of corn in the United States, and the implications of the emergence and spread of a new disease, accurate identification of the causal agent and determination of its relationship to the strains from South Africa are of critical importance to the corn industry. Impacts on yield loss due to this disease are unknown.

The pathogen causing corn bacterial leaf streak was first named *Xanthomonas campestris* pv. *vasculorum* (Dye 1978) in Young et al. 1978, a species and pathovar that also included bacteria causing gumming disease of sugarcane and palm. In early reports, *X. campestris* pv. *vasculorum* strains isolated from sugarcane and palm were pathogenic on corn, sorghum, and sugarcane (Qhobela and Claflin 1992), whereas South African isolates from corn were virulent to corn only and not sorghum or sugarcane (Qhobela et al. 1990). The South African corn bacterial leaf streak isolates were further distinguished from *X. campestris* pv. *holcicola* (causal agent of sorghum bacterial leaf streak) and sugarcane isolates of *X. campestris* pv. *vasculorum* by restriction fragment length polymorphism and, based on these differences, Qhobela et al. (1990) and Coutinho (1988) proposed renaming the corn isolates to *X. campestris* pv. *zeae*, to distinguish them from sugarcane *X. campestris* pv. *vasculorum* isolates and from *X. campestris* pv. *holcicola*. Later, based on DNA-DNA hybridization (Vauterin et al. 1995) and fatty acid profiling (Dookun et al. 2000), the species *X. vasicola* was proposed for *X. campestris* pv. *zeae*, *X. campestris* pv. *vasculorum*, and *X. campestris* pv. *holcicola*, with some sugarcane isolates being separated into a second species, *X. axonopodis*. More recent reports demonstrated that the corn bacterial leaf streak strain National Collection of Plant Pathogenic Bacteria (NCPPB) 206 caused disease on both corn and sugarcane (Karamura et al. 2015). This and other studies that included phylogeny based on multilocus sequence analyses (MLSA) proposed designation of the causal agents of gumming disease of sugarcane and bacterial leaf streak of corn as *X. vasicola* pv. *vasculorum* (Aritua et al. 2008; Coutinho et al. 2015; Dookun et al. 2000; Harrison and Studholme 2014; Karamura et al. 2015; Rademaker et al. 2005; Studholme et al. 2010; Vauterin et al. 1995; Wasukira et al. 2014). Although there is consensus among the reports, the pathovar naming was not proposed according to the rules of the International Society for Plant Pathology Committee on the Taxonomy of Plant-Pathogenic Bacteria International Standards for Naming Pathovars of Plant-Pathogenic Bacteria (Bull et al. 2008; Lapage et al. 1992).

Thus, nomenclature of the *X. vasicola* strains that cause bacterial leaf streak of corn and gumming disease of sugarcane were still unresolved.

MLSA and whole-genome comparisons are now a regular accessory for classification of bacteria (Almeida et al. 2010; Jacques et al. 2016; Langlois et al. 2017; Young et al. 2008). Calculations of average nucleotide identity (ANI) from draft genomes are a widely accepted baseline for taxonomic placement of prokaryotes (Bull and Koike 2015). Although these tools are useful for placement of organisms into a common species (Konstantinidis and Tiedje 2005; Richter and Rosselló-Móra 2009), the assignment of plant pathogens as pathovars still requires determining an organism's capacity to cause disease on reciprocal hosts compared with pathotype strains of the various pathovars (Young et al. 2001). This is complicated if a pathogen can infect multiple hosts, as in the case of the *X. vasicola* complex.

In addition to resolving nomenclature, reliable and robust tools for accurate and rapid identification of the corn bacterial leaf streak pathogen were needed to confirm the presence of the pathogen, monitor its spread, and develop management practices. Polymerase chain reaction (PCR)-based diagnostic assays are available to identify *X. vasicola* to species level but these tests do not differentiate *X. vasicola* pv. *vasculorum* from *X. vasicola* pv. *holcicola* (Adriko et al. 2012; Lewis Ivey et al. 2010). Using the rich genome sequence data available for members of the genus *Xanthomonas*, unique sequences identified through comparative genomic approaches have enabled development of diagnostic assays for various *Xanthomonas* spp. and, in some cases, even pathovars (Ash et al. 2014; Lang et al. 2010, 2014; Langlois et al. 2017; Verdier et al. 2011). Thus, leveraging genomics is a powerful approach to developing diagnostic tools for the rapid and accurate disease diagnosis needed to inform disease mitigation strategies and regulatory entities.

In this study, we address the identity of the pathogen causing the newly found bacterial leaf streak of corn in the United States. We used MLSA and comparative genomic approaches to compare corn bacterial leaf streak isolates from the United States and South Africa to determine phylogenetic relationships to other *Xanthomonas* spp. and pathovars. We performed greenhouse inoculations of corn, sugarcane, and sorghum to determine host range of the U.S. strains. Based on this work, we propose the U.S. strains causing bacterial leaf streak of corn, formerly named *X. campestris* pv. *vasculorum*, *X. campestris* pv. *zeae*, or *X. vasicola*, be named *X. vasicola* pv. *vasculorum* (Cobb 1894) comb. nov. Finally, we developed primers for PCR-based diagnostic assays that distinguish the corn bacterial leaf streak pathogen *X. vasicola* pv. *vasculorum* and the sorghum bacterial leaf streak pathogen *X. vasicola* pv. *holcicola* from each other and from other *Xanthomonas* spp.

Materials and Methods

MLSA

Bacterial strains (Table 1) were grown overnight at 28°C on nutrient agar (Becton, Dickinson and Company, Franklin Lakes, New Jersey) to reduce production of extracellular polysaccharides that impact quality of DNA extractions. Genomic DNA for all strains in this study was prepared using the Easy DNA kit (Life Technologies, Grand Island, New York) ac-

cording to the manufacturer's recommendations, except that the final product was recovered in 50 µl of sterile, molecular-grade water. If public genome or partial sequence were not available (Tables 1 and 2), six genes (*atpD*, *dnaK*, *gyrB*, *fusA*, *lepA*, and *rpoD*) were amplified from genomic DNA using previously described primers (Triplett et al. 2015; Young et al. 2008). PCR products were purified using the Qiaquick PCR Purification kit (Qiagen, Valencia, California) according to the manufacturer's instructions but eluted in 30 µl of sterile water, then directly sequenced at Genewiz (San Francisco, California). Generated sequences were aligned, trimmed, and concatenated in MEGA7 (Kumar et al. 2016). Partial sequences totaling 3,927 bp were used for MLSA.

Phylogenetic analysis

Evolutionary history was inferred by using the maximum-likelihood method based on the equal input model (Tajima and Nei 1984). Bootstrap values were generated from 1,000 replicates. Phylogenetic trees were constructed using the neighbor-joining method (Saitou and Nei 1987). All phylogenetic analyses were performed in MEGA7 (Kumar et al. 2016). Comprehensive sequences and phylogenetic data were submitted to TreeBase under study number S20566.

Genome sequencing, assembly, and comparison

Draft genome sequences were generated for *X. vasicola* pv. *vasculorum* strains NE744 (Holt County, Nebraska) and the historic South African strain *X. vasicola* pv. *vasculorum* SAM119 (Qhobela et al. 1990) using DNA extracted as described above. Genomic libraries were prepared at Michigan State University's Research Technology Core Facility (East Lansing) using the Illumina TruSeq Nano DNA Library Preparation kit. Completed libraries were quality checked and quantified using a combination of Qubit dsDNA HS, Caliper LabChipGX HS DNA, and Kapa Illumina Library Quantification qPCR assays. Libraries were pooled and loaded on an Illumina MiSeq standard v2 flow cell; sequencing was performed in a 2-by-75-bp paired-end format for NE744 and a 2-by-250-bp paired-end format for SAM119 using a v2 500 cycle MiSeq reagent cartridge. Base calling was done by Illumina Real-Time Analysis (RTA) v1.18.64 and output of RTA was demultiplexed and converted to FastQ format with Illumina Bcl2fastq v1.8.4. Genomes were assembled using SPAdes v3.9.0 (Bankevichet al.2012). QUAST was used to assess assembly qualities (Gurevich et al. 2013). Assembled genomes were uploaded to PATRIC (Wattam et al. 2014) for preliminary annotation using a RASTtk pipeline (Brettin et al. 2015).

Table 1. Bacterial strains used in this study and confirmed specificity of diagnostic primers for *Xanthomonas vasicola* pv. *holcicola* (Xvh) and *X. vasicola* pv. *vasculorum* (Xvv)

					Xvv				Xvh		
Species	Strain[a]	Origin	Host	Source	3	5	7	8	1	2	3
Burkholderia andropogonis	3549		*Zea mays*	L. E. Claflin	—	—	—	—	—	—	—
Clavibacter michiganensis pv. *nebraskensis*	CO428	United States	*Z. mays*	K. Broders	—	—	—	—	—	—	—
Clavibacter sp.	CO-4	United States	*Z. mays*	K. Broders	—	—	—	—	—	—	—
Enterobacter sp.	CO-3	United States	*Z. mays*	K. Broders	—	—	—	—	—	—	—
Enterobacter sp.	CO-22	United States	*Z. mays*	K. Broders	—	—	—	—	—	—	—
Excherichia coli	DH5α	…	…	…	—	—	—	—	—	—	—
Pantoea sp.	CO-2	United States	*Z. mays*	K. Broders	—	—	—	—	—	—	—
Pantoea agglomerans	B55	United States	*Triticum aestivum*	N. Tisserat	—	—	—	—	—	—	—
Pseudomonas fuscovaginae	SE-1	Philippines	*Oryza sativa*	G. Ash	—	—	—	—	—	—	—
P. syringae pv. *syringae*	M108	United States	*Solanum lycopersicum*	H. F. Schwartz	—	—	—	—	—	—	—
Xanthomonas sp.	M136	Mali	*O. sativa*	V. verdier	—	—	—	—	—	—	—
Xanthomonas sp.	SHU100	Philippines	*O. sativa* seed	C. M. Vera Cruz	—	—	—	—	—	—	—
Xanthomonas campestris											
pv. *campestris*	X1910	United States	*Brassica oleracea*	N. Dunlop	—	—	—	—	—	—	—
pv. *leersiae*	NCPPB4346	China	*Leersia hexandra*	V. Verdier	—	—	—	—	—	—	—
pv. *pennamericanium*	ATCC49152	Nigeria	*Pennisetum glaucum*	L. E. Claflin	—	—	—	—	—	—	—
pv. *pennisetum*	PMS91	Senegal	*P. glaucum*	L E. Claflin	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB2005[b,e]	Ethiopia	*Ensete ventricosum*	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB2251	Ethiopia	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4378	Uganda	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4381[c,e]	Uganda	*Musa* sp.	A Bogdanove	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4386	Uganda	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4387	Democratic Republic of Congo	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4388	Democratic Republic of Congo	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—

pv. *musacearum*	NCPPB4389	Rwanda	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4390	Rwanda	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4393	Tanzania	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4394	Tanzania	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4433	Burundi	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
pv. *musacearum*	NCPPB4434	Kenya	*Musa* sp.	E. Wicker	—	—	—	—	—	—	—
zeae and *vasculorum*[d]	SAM113	South Africa	*Z. mays*	L E. Claflin	+	+	+	+	—	—	—
zeae and *vasculorum*[d]	SAM118	South Africa	*Z. mays*	L E. Claflin	+	+	+	+	—	—	—
zeae and *vasculorum*[d]	SAM119[PTb,c,e]	South Africa	*Z. mays*	L E. Claflin	+	+	+	+	—	—	—
X. euvesicatoria	85-10	United States	*Capsicum frutescens*	A Bogdanove	—	—	—	—	—	—	—
X. euvesicatoria	KX-1	United States		L E. Claflin	—	—	—	—	—	—	—
X. euvesicatoria	0177	United States	*Allium cepa*	H. F. Schwartz	—	—	—	—	—	—	—
X. hortorum pv. *pelargonii*	X5	United States	*Geranium* sp.	L E. Claflin	—	—	—	—	—	—	—
X. oryzae											
pv. *oryzae*	A3857	India	*O. sativa*	J. E. Leach	—	—	—	—	—	—	—
pv. *oryzae*	BAI3	Burkina Faso	*O. sativa*	V. Verdier	—	—	—	—	—	—	—
pv. *oryzae*	R3	Australia	*O. sativa*	J. E. Leach	—	—	—	—	—	—	—
pv. *oryzae*	Xoo4	Thailand	*O. sativa*	J. E. Leach	—	—	—	—	—	—	—
pv. *oryzae*	MAI1	Mali	*O. sativa*	V. Verdier	—	—	—	—	—	—	—
pv. *oryzae*	NAI8	Niger	*O. sativa*	V. Verdier	—	—	—	—	—	—	—
pv. *oryzae*	PXO86	Philippines	*O. sativa*	C. M. Vera Cruz	—	—	—	—	—	—	—
pv. *oryzae*	PXO99A[e]	Philippines	*O. sativa*	J. E. Leach	—	—	—	—	—	—	—
pv. *oryzae*	X11-5A	United States	*O. sativa*	C. Gonzalez	—	—	—	—	—	—	—
pv. *oryzae*	Xoo199	Korea	*O. sativa*	S. H. Choi	—	—	—	—	—	—	—
pv. *oryzicola*	BLS98	Philippines	*O. sativa*	C. M. Vera Cruz	—	—	—	—	—	—	—
pv. *oryzicola*	BLS105	Philippines	*O. sativa*	C. M. Vera Cruz	—	—	—	—	—	—	—
pv. *oryzicola*	BlS256	Philippines	*O. sativa*	C. M. Vera Cruz	—	—	—	—	—	—	—
pv. *oryzicola*	BLS305	Philippines	*O. sativa*	C. M. Vera Cruz	—	—	—	—	—	—	—
pv. *oryzicola*	MAI4	Mali	*O. sativa*	V. Verdier	—	—	—	—	—	—	—
pv. *oryzicola*	MAI10	Mali	*O. sativa*	V. Verdier	—	—	—	—	—	—	—

X. translucens											
pv. *cerealis*	NCPPB1943	United States	*T. aestivum*	L E. Claflin	—	—	—	—	—	—	—
pv. *cerealis*	NCPPB1944	United States	*Bromus inermis*	V. Verdier	—	—	—	—	—	—	—
pv. *phleipratensis*	ICMP5744	United States	*Phleum pretense*	L E. Claflin	—	—	—	—	—	—	—
pv. *translucens*	B76	United States	*Hordeum vulgare*	N. Tisserat	—	—	—	—	—	—	—
pv. *translucens*	NCPPB2389	India	*H. vulgare*	C. Bragard	—	—	—	—	—	—	—
pv. *translucens*	UPB787	Paraguay	*H. vulgare*	C. Bragard	—	—	—	—	—	—	—
pv. *undulosa*	UPB513	Mexico	*T. aestivum*	C. Bragard	—	—	—	—	—	—	—
X. vasicola											
pv. *holcicola*	66	Kansas	*Sorghum bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	86	Kansas	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	93	Kansas	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	107	Lesotho	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	114	Lesotho	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	123	Lesotho	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	124	Lesotho	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	Mex-1[b,c]	Mexico	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	NCPPB989[e]	Texas	*Holcus* sp.	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	NCPPB1241[b,c]	Australia	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	NCPPB2417[Tb,c,e]	New Zealand	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	SAS211[c,e]	South Africa	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *holcicola*	Z-5	Zimbabwe	*S. bicolor*	L E. Claflin	—	—	—	—	+	+	+
pv. *vasculorum*	201600017x	Nebraska	*Z. mays*	T. Jackson-Ziems	+	+	+	+	—	—	—
pv. *vasculorum*	201600018x	Nebraska	*Z. mays*	T. Jackson-Ziems	+	+	+	+	—	—	—
pv. *vasculorum*	201600039x	Nebraska	*Z. mays*	T. Jackson-Ziems	+	+	+	+	—	—	—
pv. *vasculorum*	201600068x	Nebraska	*Z. mays*	T. Jackson-Ziems	+	+	+	+	—	—	—
pv. *vasculorum*	NE744[b,c,e]	Nebraska	*Z. mays*	K. Korus	+	+	+	+	—	—	—
pv. *vasculorum*	NE181[e]	Nebraska	*Z. mays*	K. Korus	+	+	+	+	—	—	—
pv. *vasculorum*	CO-5[c]	Colorado	*Z. mays*	K. Broders	+	+	+	+	—	—	—
pv. *vasculorum*	KS444	Kansas	*Z. mays*	J. Chaky	+	+	+	+	—	—	—

pv. *vasculorum*	NCPPB206[c,e]	South Africa	*Z. mays*	L. E. Claflin	+	+	—	—	—	—	—
pv. *vasculorum*	NCPPB1326[b,c,e]	Zimbabwe	*Saccharum officinarum*	L. E. Claflin	+	+	+	+	—	—	—
pv. *vasculorum*	NE429	Nebraska	*Z. mays*	T. Jackson-Ziems	+	+	+	+	—	—	—
pv. *vasculorum*	NE442	Nebraska	*Z. mays*	L. Appel	+	+	+	+	—	—	—
pv. *vasculorum*	UVZ 411[c]	South Africa	*S. officinarum*	R. A. Bailey	+	+	+	+	—	—	—
pv. *vasculorum*	ZCP611[c]	Zimbabwe	*S. officinarum*	P. Sinai	+	+	+	+	—	—	—

a. NCPPB = National Collection of Plant Pathogenic Bacteria (http://ncppb.fera.defra.gov.uk/), ICMP = International Collection of Micro-organisms from Plants (http://landcareresearch.co.nz/resources/collections/icmp), and UPB = Unité de Phytopathologie Bacterial, Université Catholique de Louvain. Superscript letters: PT = proposed pathotype strain; T= type strain.
b. Strains tested for pathogenicity to corn and sorghum.
c. Strains tested for pathogenicity to sugarcane.
d. *X. campestris* pv. *zeae* and *X. vasicola* pv. *vasculorum*.
e. Strains used in multilocus sequence analyses. PXO99A is a 5-azacytidine resistant strain of race 6 PXO99 (Mew et al. 1992; Salzberg et al. 2008).

Table 2. Average nucleotide identity (ANI) values calculated by whole-genome comparison using draft genomes

Species	Strain[b]	Origin	Host	GenBank accession	Reference	ANI (%) with[a] NE744	ANI (%) with[a] SAM119
Stenotrophomonas maltophilia	K279a	United Kingdom	*Homo sapiens*	NC_010943	Crossman et al. 2008	75.68	75.71
Xanthomonas albilineans	GPE PC73	Guadeloupe	*Saccarum officinarium*	GCA_000087965.1	Pieretti et al. 2009	79.17	79.17
X. axonopodis pv. *vasculorum*	NCPPB 900	La Reunion	*S. officinarium*	GCA_000724905.2	Harrison and Studholme 2014	89.28	89.17
X. campestris pv. *musacearum*	NCPPB 4381	Uganda	*Musa* sp.	ACHT 00000000	Studholme et al. 2010	98.62	98.63
X. campestris pv. *musacearum*	NCPPB 2005	Ethiopia	*Ensete ventricosum*	AKBE 01000000	Wasukira et al. 2012	98.81	98.81
X. oryzae pv. *oryzae*	PXO99[A]	Philippines	*Oryza sativa*	NC_010717	Salzberg et al. 2008; Booher et al. 2015	90.96	90.9
X. vasicola							
pv. *holcicola*	NCPPB 989	United States	*Holcus* sp.	JSCA 01000000		98.58	98.55
pv. *holcicola*	NCPPB 1241	Australia	*Sorghum vulgare*	JSBV 01000000		98.58	98.58
pv. *holcicola*	NCPPB 2417	New Zealand	*S. vulgare*	JSBW 02000000		98.61	98.57
pv. *vasculorum*	NCPPB 702	Zimbabwe	*S. officinarium*	ACHS 00000000.1	Studholme et al. 2010	99.38	99.38
pv. *vasculorum*	NCPPB 206	South Africa	*Zea mays*	AKMB 00000000	Studholme et al. 2010; Wasukira et al. 2012	99.50	99.47
pv. *vasculorum*	NCPPB 890	South Africa	*S. officinarium*	AKBN 01000000	Wasukira et al. 2014	99.53	99.52
pv. *vasculorum*	NCPPB 895	Madagascar	*S. officinarium*	AKBO 01000000	Wasukira et al. 2014	99.51	99.53
pv. *vasculorum*	NCPPB 1326	Zimbabwe	*S. officinarium*	AKBK 01000000	Wasukira et al.	99.34	99.36
pv. *vasculorum*	NCPPB 1381	Zimbabwe	*S. officinarium*	AKBL 00000000.1	Wasukira et al.	99.40	99.42
pv. *vasculorum*	NE744	Holt County, Nebraska	*Z. mays*	MVYW00000000	This study	n.a.	99.98
X. campestris pv. *zeae*/ *X. vasicola* pv. *vasculorum*	SAM 119	Klerksdrop, South Africa	*Z. mays*	MVYX00000000	Qhobela et al. 1990	99.98	n.a.

a. Percent ANI calculated using draft genome sequence in JSpecies V1.2.1 relative to strain NE744 or SAM119; n.a. = not applicable.
b. NCPPB = National Collection of Plant Pathogenic Bacteria (http://ncppb.fera.defra.gov.uk/)

Disease phenotyping

Corn (hybrid 'DKC 61-88') and sorghum ('Mycogen IG588') were grown in a 1:1 mix of Promix-BX Mycorrhizae (Quakertown, Pennsylvania) and Greens Grade (Profile Products, LLC, Buffalo Grove, Illinois), then inoculated 4 weeks after planting with eight selected bacterial strains (Table 1). Sugarcane (L-99-226) nodes were grown in a 1:1 mix of Promix-BX Mycorrhizae and sand for 2 weeks, then transplanted to 100% Promix-BX Mycorrhizae. Each bacterial strain was cultured in peptone sucrose agar (Karganilla et al. 1973) for 24 h at 28°C, then suspended to 108 CFU ml^{-1} in sterile, distilled water. Bacterial suspensions were infiltrated into the intercellular spaces of corn, sorghum, and sugarcane leaves on either side of the abaxial main vein with a needleless 1-cm^3 syringe (Reimers and Leach 1991) and by leaf clipping, as previously described (Kauffman et al. 1973). For stem inoculations, 500 µl of a bacterial suspension was injected into stems using a 21-gauge needle (Vidaver 1977). Distilled water was included as a negative control in all inoculations. At least two leaves were inoculated on three to six individual plants. All inoculations with each isolate-host combination were repeated at least two times. Plants were maintained in a greenhouse (27 ± 1°C, 16-h day length, and 80% relative humidity). At 7 days postinoculation (dpi), lesions on infiltrated and stab-inoculated plants were measured; the rating scale proposed by Coutinho (1988) was applied for stab-inoculated plants.

Molecular diagnostic assay development

Draft genomes were collected from the National Center for Biotechnology Information (NCBI) Genome database for all strains listed in Table 2, with the exception of two sequenced genomes generated in this study. Unique loci that were conserved in all *X. vasicola* pv. *vasculorum* genomes but either not present in *X. vasicola* pv. *holcicola* or *X. campestris* pv. *musacearum* or polymorphic in these strains relative to *X. vasicola* pv. *vasculorum* were targeted for development of diagnostic primers. Design was done using an in-house genome alignment-based computational pipeline: UniqPrimer (L. Triplett and J. E. Leach, unpublished data, code available upon request). UniqPrimer runs a comparative analysis to identify unique loci in the included genomes that are absent in excluded genomes and then designs primers for these regions. Draft genome sequences of all publicly available *X. vasicola* pv. *vasculorum* and genomes generated in this study were set as "include" genomes in UniqPrimer whereas *X. vasicola* pv. *holcicola*, *X. campestris* pv. *musacearum*, other xanthomonads, as well as phylogenetic outliers were set as "exclude" genomes. Default settings were used in this design and yielded over 50 potential diagnostic primer sets; however, outputs are not ranked or curated. Specificity of primer targets was validated in silico by Primer-BLAST (Ye et al. 2012) against the NCBI whole-genome shotgun (wgs) database where any non–*X. vasicola* pv. *vasculorum* hits were discarded. Next, a robust set of primers located across the genome were synthesized (IDT, Coralville, Iowa) and screened in the lab first with a small panel of DNA from positive and negative control strains. Primer sets that successfully amplified only positive controls were then evaluated on the remaining *X. vasicola* pv. *vasculorum* isolates from diverse hosts and a set of negative control isolates comprising several different *Xanthomonas* spp. and other genera of phytobacteria by conventional PCR, as previously described (Lang et al. 2010, 2014). Negative

controls pools were also separately spiked with a positive control DNA to ensure detection. Additionally, genome comparisons revealed variation among *X. vasicola* pv. *vasculorum* isolates in the presence or absence of the *xopAF* gene that encodes the bacterial effector XopAF (Studholme et al. 2010; Wasukira et al. 2014). Primers were designed using Geneious 8.0.5 (http:// www.geneious.com/) to amplify a 362-bp fragment of *xopAF* by aligning *xopAF* sequences of five *X. vasicola* pv. *vasculorum* strains (NCPPB accessions 702, 890, 895, 1326, and 1381) retrieved from the GenBank wgs database. Each 25-µl reaction included 1 µl of each primer at 10 µM (Table 3), 15.7 µl of water, 5 µl of 5× GoTaq reaction buffer, 0.75 µl of 25 mM $MgCl_2$, 0.5 µl of 10 mM dNTP, and 0.05 µl of GoTaq DNA polymerase (Promega Corp., Madison, Wisconsin). Optimized cycling conditions were an initial denaturation at 94°C for 3 min; followed by 30 cycles of 94°C for 30 s, annealing temperature specific to each primer (Table 3) for 30 s, and 72°C for 1 min; with a final elongation at 72°C for 10 min.

Table 3. Primers developed with specificity to *Xanthomonas vasicola* pvs. *vasculorum* and *holcicola* and the effector XopAF

Target	Name	Sequence (5′–3′)	Product size (bp)	Temp (°C)[a]
X. vasicola pv. *vasculorum*				
Putative membrane protein	Xvv3_F	CAAGCAGAGCATGGCAAAC	207	55
	Xvv3_R	CACGTAGAACCGGTCTTTGG		
Putative exported protein	Xvv5_F	CCGTCGAAATGGTCTCAACT	200	55
	Xvv5_R	CGGAAGAGTTGGAAGACAGC		
Hypothetical protein	Xvv7_F	CTACTACGCCCAGCGACTTC	205	53
	Xvv7_R	ACGTCGAGCCATTCTGAAAC		
Hypothetical protein	Xvv8_F	GGGTTATTGACGGCACTCTC	206	53
	Xvv8_R	GGGCAGCCTGTAACGAATTA		
X. vasicola pv. *holcicola*				
Putative acetyltransferase	Xvhl_F	GCAGATTGTCAGCATCAGGA	201	55
	Xvhl_R	GATCTTACGCACAGCACCAA		
Putative membrane protein	Xvh2_F	CGAATTTTGTGTGACCAGGA	200	53
	Xvh2_R	GAATTCACCAAATGGGCATC		
Hypothetical protein	Xvh3_F	ATCCCATGGGTCTGAGTCTG	200	53
	Xvh3_R	AGTCCATTGCAGGAGTTTG		
XopAF	XopAF_F	CCATTGCCATTGCTAGCACC	362	60
	XopAF_R	TATTCGACGGTTCCCACTGC		

a. Annealing temperature

PCR-based detection of X. vasicola *pv.* vasculorum *in corn leaves*

To detect the presence of *X. vasicola* pv. *vasculorum* in corn leaf tissue, a small section (approximately 3 cm) with a characteristic lesion was excised to include tissue beyond the lesion margin. The leaf tissue was surface disinfested in 5 ml of fresh 10% bleach for 30 s, with vigorous shaking. Samples were rinsed three times with 10 to 15 ml of sterile distilled water for 30 s Using flame-sterilized forceps, the tissue was placed in 1 ml of sterile distilled water in a sterile 1.5-ml microcentrifuge tube. The tissue was cut several times to

promote release of bacteria with flame-sterilized scissors, then incubated at room temperature for at least 1 h. For bacterial isolation, one loopful (10 μl) of solution was spread onto nutrient agar and incubated at 28°C for 2 days. Single-characteristic yellow colonies were selected and restreaked for isolated colonies on nutrient agar; then, a single colony was selected and suspended in water for use in colony PCR. Alternatively, *X. vasicola* pv. *vasculorum* was directly detected using 1 μl of leachate from the cut tissue as the DNA template for direct PCR.

Results

MLSA

DNA gyrase B is an accepted benchmark to delineate *Xanthomonas* spp. (Parkinson et al. 2009, 2007). A preliminary alignment of the DNA gyrase B gene (*gyrB*) of *Xanthomonas* strains representing over 27 different described species and *Stenotrophomonas maltophilia* K279a as an outgroup was used to evaluate the placement of recent isolates of *X. vasicola* pv. *vasculorum* from corn in the genus *Xanthomonas* (Supplementary Fig. S1). Strains NE744 and NE181, isolated in 2015 from dent corn in two different counties of Nebraska (Holt and Cedar, respectively), grouped with *X. vasicola* pv. *holcicola* and *X. vasicola* pv. *vasculorum* from corn and sugarcane as well as *X. campestris* pv. *musacearum* from banana, consistent with its identification as *X. vasicola* (Korus et al. 2017).

MLSA using six different housekeeping genes (*atpD*, *dnaK*, *gyrB*, *fusA*, *lepA*, and *rpoD*) established genotypic relationships of the U.S. corn strains with other *Xanthomonas* spp. *S. maltophilia* K279A and *X. albilineans* GPEPC73 (causal agent of leaf scald of sugarcane) were included in the analysis as outgroups (Fig. 1). *X. vasicola* pv. *vasculorum* and *X. campestris* pv. *musacearum* group together in one clade, with *X. vasicola* pv. *holcicola* next in proximity (Fig. 1). The sequences for the six genes were identical for the South African strain SAM119 and all other *X. vasicola* pv. *vasculorum* strains; thus, they grouped into a single clade. Three single-nucleotide polymorphisms (SNP) were identified in the six concatenated genes between the *X. vasicola* pv. *vasculorum* and *X. campestris* pv. *musacearum* strains. Fifteen SNP differentiated *X. vasicola* pv. *holcicola* strains from *X. vasicola* pv. *vasculorum* and *X. campestris* pv. *musacearum*. Curated sequences and alignments are in TreeBASE under study number S20566.

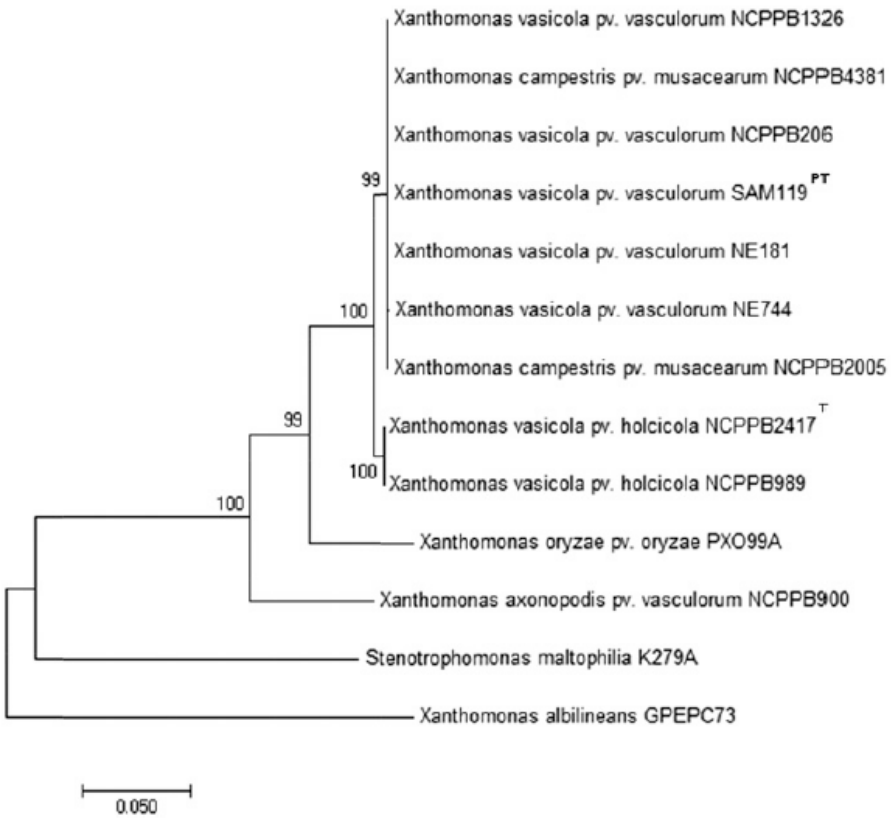

Figure 1. Phylogeny based on partial *atpD*, *dnaK*, *fusA*, *gyrB*, *lepA*, and *rpoD* sequence alignment. The evolutionary history was inferred by using the maximum-likelihood method based on the equal input model in MEGA7 (Kumar et al. 2016). Bootstrap values generated from 1,000 replicates are shown at nodes. Branch lengths measure in the number of substitutions per site. The final dataset contained 3,967 bp. Superscript letters: T = type strain and PT = pathotype strain.

Genomics

Draft genomes of *X. vasicola* pv. *vasculorum* NE744 and SAM119 were assembled using SPAdes v3.9.0 (Bankevich et al. 2012), yielding 104 and 84 contigs ≥ 500 bp (N_{50} between 139,700 and 169,893), for total lengths of 4,869,712 and 4,856,397 bp for *X. vasicola* pv. *vasculorum* NE744 and SAM119, respectively. Assembled contigs and raw reads for *X. vasicola* pv. *vasculorum* NE744 and SAM119 were deposited to GenBank and the Sequence Read Archive with accession numbers MVYW00000000 and MVYX00000000. GC contents for these strains were 63.25 and 63.22%, which is consistent with other members of the genus *Xanthomonas*.

Genome assemblies were used to calculate ANI to delineate species relative to other *Xanthomonas* spp. at a 95% cut off using JSpecies v1.2.1 (Konstantinidis and Tiedje 2005;

Richter and Rosselló-Móra 2009) (Table 2). The genomes of the causal agent of corn bacterial leaf streak isolated in Nebraska are 99% identical to *X. vasicola* pv. *vasculorum* from Madagascar, South Africa, and Zimbabwe, whether isolated from corn or sugarcane. The highest ANI percentage was between *X. vasicola* pv. *vasculorum* NE744 and SAM119. Both genomes generated in this study were, on average, 98% similar to *X. campestris* pv. *musacearum* from banana (Uganda or Ethiopia) and *X. vasicola* pv. *holcicola* isolated from sorghum or *Holcus* spp. (United States, Australia, or New Zealand), including the *X. vasicola* type strain NCPPB2417.

Evidence of type III and type IV secretion systems was found in preliminary annotations of the U.S. (NE744) and South African (SAM119) genomes (data not shown), similar to predictions from other *X. vasicola* pv. *vasculorum* genomes (Wasukira et al. 2014, 2012). A TBLASTN search for transcription activator-like (TAL) effector gene sequences using conserved features (N and C termini) of this protein family from *X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola* did not reveal TAL effectors in the *X. vasicola* draft genomes. Thus, these strains do not appear to contain genes encoding TAL effectors. However, the highly repetitive sequence of TAL effectors cannot be fully resolved by draft sequence alone and further investigation by DNA hybridization or long-read sequencing is necessary.

Pathogenicity

To determine pathogenicity, a panel of historical and recently collected strains (Table 1) were inoculated to corn, sorghum, and sugarcane using two previously reported assays, infiltration into leaves, or stab injection into the stems (Coutinho 1988; Coutinho et al. 2015; Goszczynska et al. 2007; Karamura et al. 2015; Qhobela and Claflin 1988; Qhobela et al. 1990). Phenotypes after stem injection across corn and sorghum were variable (Supplementary Figs. S2 and S3); therefore, we focused our analysis on results from quantitative leaf infiltration assays (Fig. 2). When introduced into corn leaves (hybrid DKC 61-88) by infiltration, *X. vasicola* pv. *holcicola* and *X. vasicola* pv. *vasculorum* strains were all moderate to highly virulent (lesions of 5.6 to 10.6 cm), with two exceptions: *X. vasicola* pv. *vasculorum* strains NCPPB206 (corn) and NCPPB1326 (sugarcane), which were weakly virulent (average lesion lengths ≤ 1.6 cm). However, *X. vasicola* pv. *vasculorum* isolates caused less water soaking and shorter lesions on sorghum than on corn. Prolific bacterial exudate was observed after inoculation of either *X. vasicola* pv. *vasculorum* or *X. vasicola* pv. *holcicola* to corn or *X. vasicola* pv. *holcicola* to sorghum regardless of the inoculation technique used (Supplementary Fig. S4).

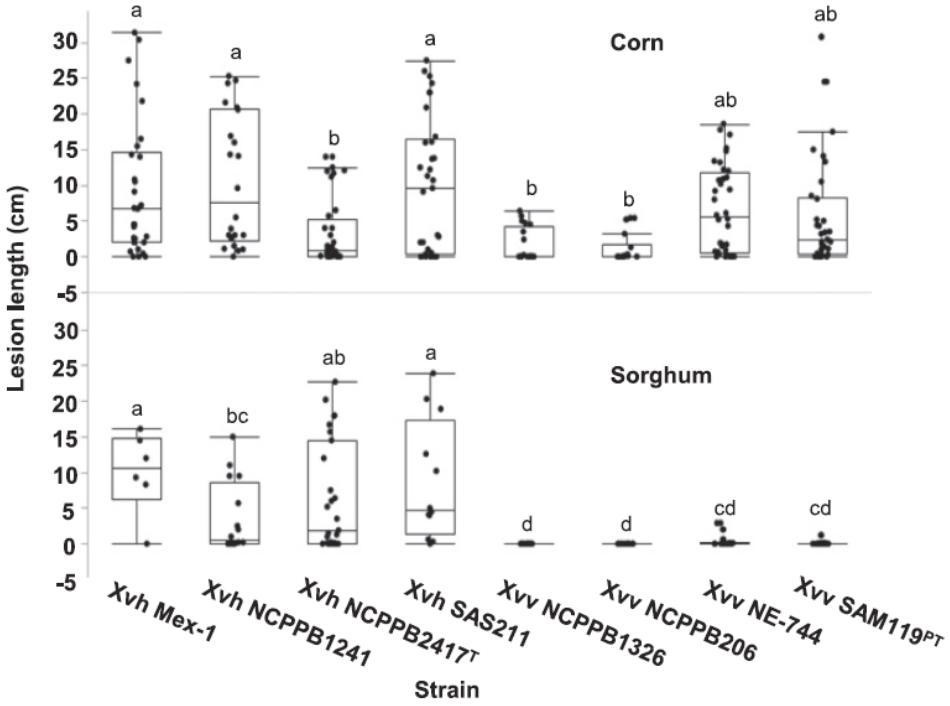

Figure 2. Disease caused by *Xanthomonas vasicola* pv. *vasculorum* (Xvv) and *X. vasicola* pv. *holcicola* (Xvh) on corn (hybrid DKC 61-88) and sorghum (Mycogen IG588). Leaves of 4-week-old plants were infiltrated with each strain at 10^8 CFU ml^{-1}, and disease was assessed at 7 days postinoculation. Lesion lengths indicate expansion beyond the infiltration site. The entire experiment was replicated four times and combined data from all replications is shown here. Letters designate significance at $P < 0.0001$. Superscript letters: T = type strain and PT = pathotype strain.

Sugarcane was inoculated by leaf infiltration only, but similar to corn and sorghum, lesion lengths were variable between plants and even on the same leaf (Supplementary Table S1). Representative phenotypes at 4 dpi with selected strains are shown in Figure 3. Strains of *X. vasicola* pv. *vasculorum* and *X. vasicola* pv. *holcicola* produced a deep purple response in sugarcane leaves over time but *X. vasicola* pv. *holcicola* caused the earliest reaction, starting at 3 dpi. Regardless of original host, *X. vasicola* pv. *vasculorum* strains caused water soaking at 4 dpi; after 5 dpi, infiltration sites turned deep purple and all lesions continued to expand. Although not as abundant as on corn or sorghum, bacterial exudate was observed on sugarcane after inoculation by all *X. vasicola* pv. *vasculorum* strains. *X. campestris* pv. *musacearum* was not pathogenic and caused no water soaking on sugarcane and, again, *X. vasicola* pv. *vasculorum* NCPPB 1326 was not highly virulent.

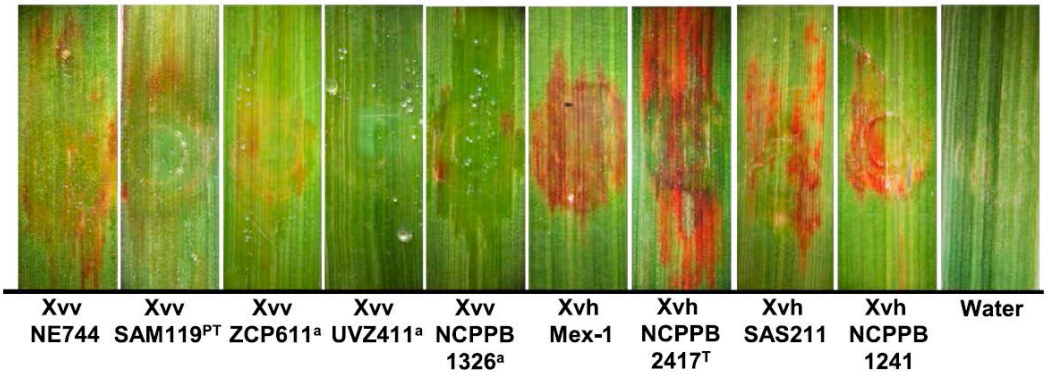

Figure 3. Qualitative disease assessment of *X. vasicola* pv. *vasculorum* (Xvv) and *X. vasicola* pv. *holcicola* (Xvh) on sugarcane (L-99-226). Six-week-old plants were infiltrated with each strain at 10^8 CFU ml^{-1}, and reactions were photographed at 4 days postinoculation. Superscript letters: a = strains isolated from sugarcane, T = type strain, and PT = pathotype strain.

Based on our pathogenicity tests, *X. vasicola* pv. *vasculorum* strains from either corn or sugarcane cause disease on corn, sorghum, or sugarcane, but they are usually most aggressive to the host from which they were originally isolated. The disease phenotypes caused by the U.S. corn isolate on all three hosts are most similar to those caused by *X. vasicola* pv. *vasculorum* from South Africa. *X. vasicola* pv. *holcicola* strains are virulent to all three hosts, but are more aggressive to sorghum than the sugarcane and corn isolates.

Diagnostics

Four primer sets (Xvv 3, Xvv5, Xvv7, and Xvv8) were generated that specifically amplify *X. vasicola* pv. *vasculorum* from isolated DNA, heat-killed cells, or infected, macerated tissues, and that did not amplify any nontarget strains. Gene targets, primer sequences, and recommended annealing temperatures are listed in Table 3. In total, 17 strains positively amplified with the four sets of *X. vasicola* pv. *vasculorum* primers and were differentiated from the *X. vasicola* pv. *holcicola*, *X. campestris* pv. *musacearum*, and other nontarget strains by these assays. No false positives (i.e., amplification of nontarget strains) were detected. Furthermore, if the primers amplified DNA from plant exudates, if leaf tissue was intact and not exceptionally decomposed, *X. vasicola* pv. *vasculorum* was isolated from the leaves (9 of 13 samples). *X. vasicola* pv. *vasculorum* NCPPB206 did not amplify with primers Xvv7 or Xvv8. Primers specific for *X. vasicola* pv. *holcicola* were also generated and tests with the same sets of target and nontarget strains revealed consistent amplification of target strains and no false positives (Tables 3 and 1). Predicted gene function of loci identified for primer design from draft genome sequences are included in Table 3.

XopAF

The effector gene *xopAF* was reported in *X. euvesicatoria*, *X. translucens* pv. *translucens*, *X. citri* subsp. *citri*, and *X. vasicola* pv. *vasculorum* from sugarcane but was absent in the

only other strain of *X. vasicola* pv. *vasculorum* publicly available from corn, NCPPB206, and also absent in *X. campestris* pv. *musacearum* (Jalan et al. 2013; Studholme et al. 2010; Wasukira et al. 2014). To determine whether newly isolated corn strains from the United States contained this effector or close relatives, we tested for the effector gene using *xopAF*-specific primers (Table 3). No strain of *X. vasicola* pv. *vasculorum* isolated from corn in the United States, no *X. vasicola* pv. *holcicola* or *X. campestris* pv. *musacearum* contained *xopAF*, whereas all sugarcane strains of *X. vasicola* pv. *vasculorum* did possess this gene regardless of geographic region of isolation (Fig. 4).

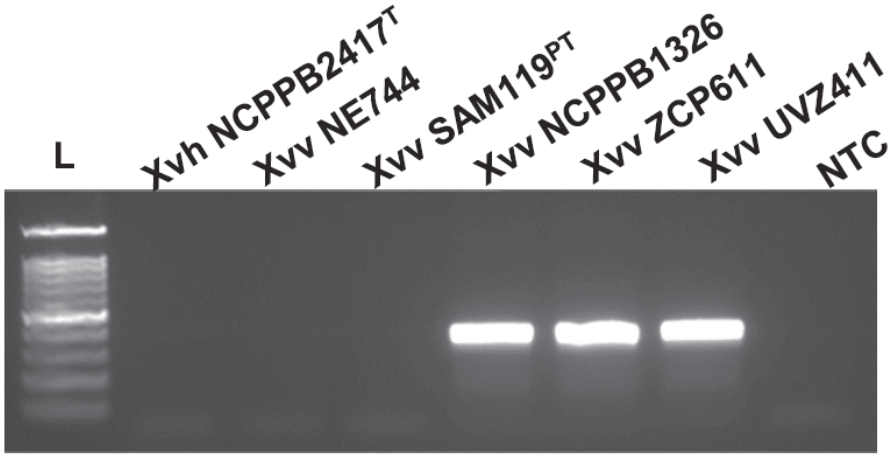

Figure 4. Targeted amplification of *xopAF* in *Xanthomonas vasicola* pv. *vasculorum* (Xvv) and *X. vasicola* pv. *holcicola* (Xvh). Lane L = 100-bp DNA ladder (GoldBio, St. Louis) and NTC = no template control. Superscript letters: T = type strain and PT = pathotype strain.

Discussion

The causal agent of the bacterial leaf streak that recently emerged on corn in the United States was reported as *X. vasicola* (Korus et al. 2017). In this study, we performed phylogenetic analyses and compared genome sequences and host ranges of *X. vasicola* isolated from corn, sugarcane, and sorghum to refine the taxonomic designation of the U.S. corn isolates. We confirm that the pathogen causing bacterial streak of corn in the United States is *X. vasicola* and propose the designation *X. vasicola* pv. *vasculorum* (Cobb 1894) comb. nov. Furthermore, we propose strain SAM119 (Qhobela et al. 1990) as the pathotype strain. Below, we summarize our results and rationale for these conclusions.

X. campestris pv. *vasculorum* groups A and B were initially proposed based on sodium dodecyl sulfate polyacrylamide gel electrophoresis of proteins, gas chromatography of fatty acid methyl-esters, and DNA-DNA hybridization (Vauterin et al. 1992). Vauterin et al. (1995) then proposed renaming and reclassification of the species based on DNA-DNA hybridization; this report separated group A, composed of *X. axonopodis* pv. *vasculorum*

from sugarcane, and group B, composed of *X. vasicola* pv. *vasculorum* from corn or sugarcane and *X. vasicola* pv. *holcicola* from sorghum. Our MLSA supports placement of corn strains from the United States and the previously reported South African strain SAM119 into *X. vasicola* group B (Fig. 1). The corn strains form a distinct clade from *X. vasicola* pv. *holcicola* but, interestingly, do not separate from *X. campestris* pv. *musacearum*. Using comparisons of genomic similarities as measured by ANI to provide further taxonomic context (Vinatzer et al. 2017), we found that the corn and sugarcane isolates that clustered in MLSA phylogenetic trees (Fig. 1) had ANI of > 99.3% (Table 2). In addition, ANI values for *X. vasicola* pv. *holcicola* and *X. vasicola* pv. *vasculorum* from corn in the United States indicated approximately 98.6% genome similarity, and phylogenetic analyses showed these organisms branching into two distinct groups.

Based on MLSA, *X. campestris* pv. *musacearum* and *X. vasicola* pv. *vasculorum* were indistinguishable; however, previous whole-genome comparisons (Wasukira et al. 2012, 2014), the ANI values, and the diagnostic primers in this study (Tables 2 and 3) did differentiate the two pathovars. Because MLSA compares only variation in housekeeping genes while ANI detects variation across the entire genome sequence, differences in predicted relationships might be expected. Therefore, host range studies are necessary to tease apart this complex. We note that neither pathogenicity of *X. vasicola* pv. *vasculorum* to banana nor *X. campestris* pv. *musacearum* to corn were assessed in this study. However, in previous studies, *X. vasicola* pv. *vasculorum* from corn or sugarcane were not pathogenic to banana, and *X. campestris* pv. *musacearum* did not cause symptoms on corn (Aritua et al. 2008; Karamura et al. 2015). The African strain of *X. campestris* pv. *musacearum* (NCBBP4381) is not pathogenic to sugarcane.

Ideally, a combination of MLSA, comparison of whole-genome sequence, and ecology are integrated to define a prokaryotic species (Gevers et al. 2005; Whitman 2015). For plant-pathogenic bacteria, pathovars are distinguished based on differences in host range, although differences in symptomology on the same plant species can also warrant separate pathovar designations (Jacques et al. 2016; Young et al. 2001). Previous reports showed that corn *X. vasicola* pv. *vasculorum* isolates were pathogenic to corn when reintroduced, but they did not cause disease on sorghum or sugarcane (Coutinho and Wallis 1991; Qhobela et al. 1990). These authors also indicated that *X. vasicola* pv. *holcicola* strains were pathogenic to both corn and sorghum, while a sugarcane isolate of *X. vasicola* pv. *vasculorum* caused disease on all three hosts. However, our results and those from other pathogenicity studies (Karamura et al. 2015), which compare some of the same isolates, conflicted with these results, possibly due to the use of different cultivars or experimental conditions. In our studies using established protocols, *X. vasicola* pv. *vasculorum* strain NE744 from corn in Nebraska was pathogenic to corn, sorghum, and sugarcane, and the symptoms (water-soaking with spreading lesions) were most similar to those caused by corn and sugarcane isolates of *X. vasicola* pv. *vasculorum* from other countries. Based on our pathogenicity tests, we recommend that the U.S. and South African corn strains be included in *X. vasicola* pv. *vasculorum*.

X. vasicola pv. *holcicola* strains were also pathogenic to all three hosts but were more aggressive to sorghum than the corn and sugarcane isolates. We note that, when bacterial leaf streak was first observed in corn in the United States, it was thought to be caused by

X. vasicola pv. *holcicola* because in early literature based on greenhouse inoculations, *X. vasicola* pv. *holcicola* caused symptoms on corn (Bradbury 1986; Qhobela and Claflin 1988). However, to date, *X. vasicola* pv. *holcicola* has never been associated with bacterial leaf streak symptoms on corn in the field. The lack of evidence for occurrence of this pathovar on corn in the field as well as the genomic differences between the two pathovars support the current separate pathovar designation for these members of *X. vasicola*. Further phenotyping with more strains, more hosts, and different host varieties would improve resolution of the pathovar designation.

Both of the sugarcane isolates of *X. axonopodis* pv. *vasculorum* and *X. vasicola* pv. *vasculorum* are reported to cause gumming disease of sugarcane (Bradbury 1986; Dookun et al. 2000; Vauterin et al. 1995), creating confusion for pathologists. Unfortunately, little information is available on the etiology of sugarcane gumming disease, and resolution of this issue will require comparisons of host range and symptomology that include both species in optimized disease assays. A plausible hypothesis is that *X. axonopodis* pv. *vasculorum* is a vascular pathogen and causal agent of gumming disease whereas *X. vasicola* pv. *vasculorum* is a nonvascular and causal agent of leaf streak.

The emergence of corn bacterial leaf streak and its spread throughout the midwestern United States created an urgent need for diagnostic tools that could be used to accurately identify the causal agent and to track its distribution. By comparing the draft genomes generated as part of this study with the large number of *Xanthomonas* genomes now available, we identified unique regions and used these to develop sets of diagnostic primers that distinguish *X. vasicola* pv. *vasculorum* (four primer pairs) from *X. vasicola* pv. *holcicola* (three primer pairs) and from other bacteria. The primers Xvv3, Xvv5, Xvh2, and Xvh3 have been tested in our labs and in collaboration with colleagues in Iowa (C. Block, A. Robertson, and G. Munkvold, Iowa State University) and Kansas (D. Jardine, Kansas State University). The primers are currently being used for disease diagnosis and for epidemiological surveys across the United States in private, public, and federal institutions, including the United States Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Plant Protection and Quarantine (PPQ). Future integration of multiple loci variable number of tandem repeat analysis to specifically type populations of the *X. vasicola* complex from corn and sorghum in the United States would be valuable for epidemiological surveillance and could also help determine geographic lineages of these organisms (Poulin et al. 2015; Pruvost et al. 2014; Zhao et al. 2012).

Consistent with previous reports, of the *X. vasicola* pv. *vasculorum* bacteria only those from sugarcane contain the gene *xopAF*, an effector that may contribute to host range and symptomology (Studholme et al. 2010; Wasukira et al. 2014). No evidence of TAL effectors was found in the draft genomes of *X. vasicola* pv. *vasculorum* strains NE744 and SAM119 but Harrison and Studholme (2014) did predict their presence in *X. axonopodis* pv. *vasculorum* NCPPB900. TAL effectors are found in diverse *Xanthomonas* spp. but are not present in all (Jacques et al. 2016). In many systems, these proteins contribute to pathogenicity. Given the absence of *xopAF* and genes for TAL effectors in the corn *X. vasicola* pv. *vasculorum*, these organisms must rely on a distinct set of effectors for virulence.

In the phylogenetic tree, the most closely related *Xanthomonas* spp. to *X. vasicola* is *X. oryzae*, which includes two pathovars, *oryzae* and *oryzicola*, causal agents of bacterial

blight and bacterial leaf streak of rice, respectively. An interesting possibility is that these xanthomonads diverged from an ancestral group adapted to monocots. Rice and sugarcane are grown in proximity to one another in some tropical areas, which may have historically fostered evolution of these distinct groups. Although *X. vasicola* pv. *vasculorum* was previously thought to only infect monocots, including palms, it was recently identified on Eucalyptus in association with *Pantoea ananatis* (Coutinho et al. 2015). An outbreak of brown stalk rot on corn caused by *P. ananatis* was also reported in South Africa in 2004 (Goszczynska et al. 2007). Intriguingly, we frequently isolated *P. ananatis* from corn samples in the United States that exhibited bacterial leaf streak symptoms; however, when these *P. ananatis* were inoculated to corn, they did not cause disease (J. M. Lang, unpublished results). It is unknown whether a synergistic relationship exists between *X. vasicola* pv. *vasculorum* and *P. ananatis* and, if so, whether this interaction is related to the emergence of bacterial leaf streak in the United States.

The origin of the U.S. corn bacterial leaf streak pathogen is unknown. Based on ANI values, *X. vasicola* pv. *vasculorum* strain NE744 is highly similar to strain SAM119 (99.9%) that was isolated from corn in South Africa over 20 years ago (Qhobela et al. 1990). Although it is tempting to speculate that *X. vasicola* pv. *vasculorum* was introduced via international germplasm movement, given its distribution in at least seven states, pinpointing if, when, or how it was introduced would be difficult.

It is not known whether *X. vasicola* pv. *vasculorum* is seed transmitted, or how it is moved within and across fields. Because the disease has been confirmed on various hybrids of popcorn, seed, dent, and sweet corn (T. A. Jackson-Ziems and K. Broders, personal communication), it is unlikely that the rapid spread is due to widespread planting of one or a few susceptible varieties. The disease occurs in both irrigated and nonirrigated areas and in a variety of corn production systems, including various tillage and crop rotation regimes (T. A. Jackson-Ziems and K. Broders, personal communication). A deeper examination of the *X. vasicola* pv. *vasculorum* genomes from geographically diverse locations and hosts as well as epidemiological investigations are important to understanding how and why this recent epidemic in the United States has occurred.

In conclusion, the phylogenetic analyses, genome comparisons, and pathogenicity studies reported here support classification of the U.S. corn bacterial leaf streak pathogen as *X. vasicola* pv. *vasculorum* (Cobb 1894) comb. nov. (Korus et al. 2017). This classification is consistent with previous groupings of the South African corn isolates as *X. vasicola* pv. *vasculorum* (Aritua et al. 2008; Rademaker et al. 2005; Studholme et al. 2010; Vauterin et al. 1995; Wasukira et al. 2014). We propose a new pathotype strain, *X. vasicola* pv. *vasculorum* SAM119, based on historical precedence and the availability of genome sequence data (Qhobela et al. 1990; Whitman 2015). SAM119 has 99% genomic identity with recent isolates from the United States and unlike NCPPB206, the oldest publicly available strain from corn (1949), SAM119 remains highly virulent on corn.

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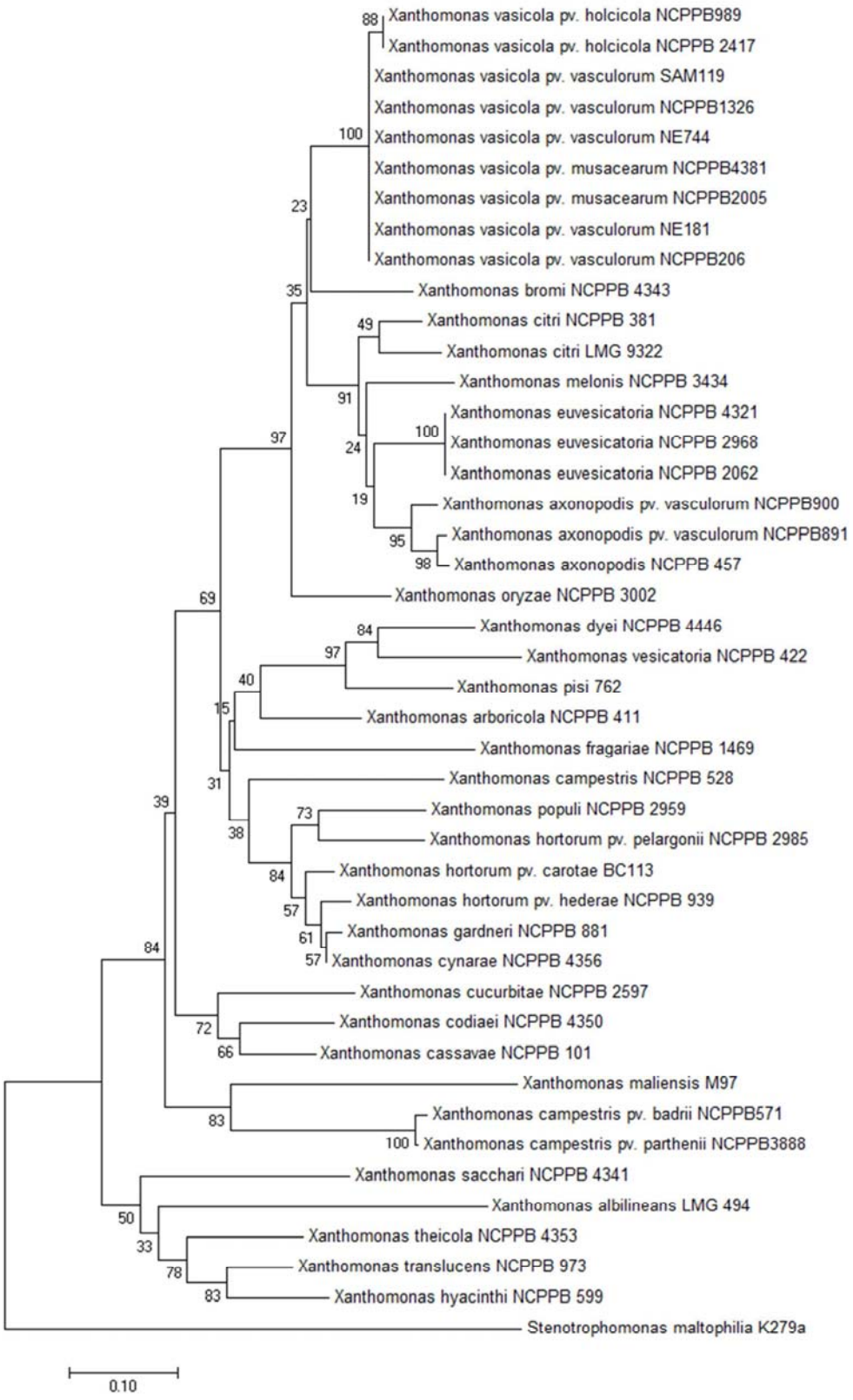
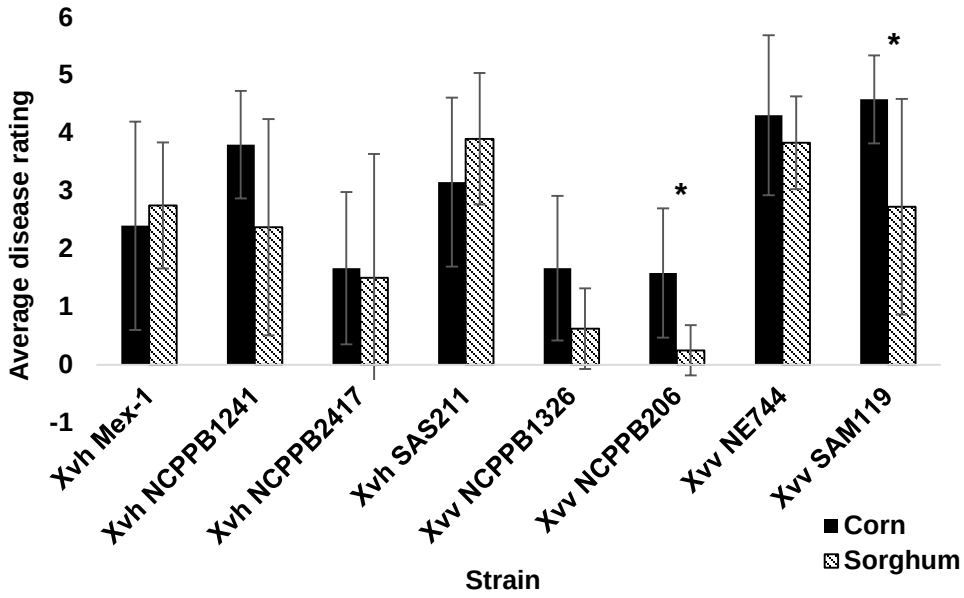


Supplementary Fig. 1. Phylogeny based on partial *gyrB* sequence alignment. Bootstrap values are shown at nodes generated from 1000 replicates. Branch lengths measured in the number of substitutions per site. There were a total of 540 bp in the final dataset.



Supplementary Fig. 2. Disease caused by *X. vasicola* pv. *vasculorum* (Xvv) and *X. v.* pv. *holcicola* (Xvh) on corn (cv. hybrid DKC 61-88) and sorghum (cv. Mycogen IG588). Stems of four week old plants were injected with 10^8 CFU ml^{-1} of each strain, and disease was assessed at 7 days post inoculation (dpi). Disease was rated according a scale of 1 to 5, with 1 demonstrating minimal disease and 5 meaning extensive lesions (Coutinho, 1988). The entire experiment was replicated four times and combined data from all replications is shown here. Bars represent standard error and asterisks designate significance at $P < 0.0021$ and $P < 0.0040$ for Xvv NCPPB206 and SAM119, respectively.

Xvh Mex-1 **Xvv NE744**

Supplementary Fig. 3. Qualitative disease phenotyping of *X. v.* pv. *vasculorum* (Xvv) and *X. v.* pv. *holcicola* (Xvh) on sorghum (cv.Mycogen IG588). Four week old plants were stabbed and infiltrated with 10^8 CFU ml^{-1} of each strain. Reactions were recorded 7dpi.

Supplementary Fig. 4. *X. v.* pv. *vasculorum* NE744 bacterial exudate on corn (cv. hybrid DKC 61-88) 7dpi.

Supplementary Table 1. Quantitative disease response of sugarcane to infection with diverse strains

Strain	Host[a]	Mean Lesion Length (cm)[bc]
Xanthomonas vasicola* pv. *vasculorum		
NCPPB1326	Sugarcane	1.21 ± 0.67 c
ZCP611	Sugarcane	5.26 ± 5.01 ab
UVZ411	Sugarcane	4.47 ± 4.87 abc
CO-5	Corn	4.54 ± 4.07 c
NE744	Corn	3.91 ± 2.98 bc
SAM119	Corn	6.18 ± 4.82 ab
X. v.* pv. *holcicola		
Mex-1	Sorghum	3.32 ± 2.63 bc
NCPPB2417	Sorghum	6.01 ± 4.57 ab
NCPPB1241	Sorghum	7.65 ± 5.45 a
SAS211	Sorghum	6.34 ± 5.40 ab
X. campestris* pv. *musacearum		
NCPPB4381	Banana	0 ± 0 d

[a]Original host bacterial strain was isolated from

[b]Leaves were infiltrated with 10^8 CFU/ml of bacteria suspended in sterile water in two spots per leaf, on either side of the main vein.

[c]Mean lesion length ± standard deviation. Lesion lengths averaged over two independent replications. Means followed by the same letter are not significantly different according to Tukey's honestly significant differences test ($P < 0.05$).