Genetic Diversity and Virulence of Wheat and Barley Strains of *Xanthomonas translucens* from the Upper Midwestern United States

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ABSTRACT

Bacterial leaf streak (BLS) of wheat and barley, caused by *Xanthomonas translucens* pv. *undulosa* and *X. translucens* pv. *translucens*, has been of growing concern in small grains production in the Upper Midwestern United States. To optimize disease resistance breeding, a greater awareness is needed of the pathovars and genetic diversity within the pathogens causing BLS in the region. Multilocus sequencing typing (MLST) and analysis (MLSA) of four common housekeeping genes (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) was used to evaluate the genetic diversity of 82 strains of *X. translucens* isolated between 2006 and 2013 from wheat, barley, rye, and

intermediate wheatgrass. In addition, in planta disease assays were conducted on 75 strains to measure relative virulence in wheat and barley. All strains were determined by MLSA to be related to *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens*. Clustering of strains based on Bayesian, network, and minimum spanning trees correlated with relative virulence levels in inoculated wheat and barley. Thus, phylogeny based on *rpoD*, *dnaK*, *fyuA*, and *gyrB* correlated with host of isolation and was an effective means for predicting virulence of strains belonging to *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa*.

Bacterial leaf streak (BLS) and black chaff of wheat and barley has been a recurring disease problem in the Upper Midwestern United States (Adhikari et al. 2012; Kandel et al. 2012). Beginning with an epidemic in 2005, Minnesota wheat growers reported increased occurrences of BLS affecting yield. Infected seeds are considered the primary source of inoculum, but other important sources include crop residues, volunteer cereals, and weeds (Duveiller et al. 1997; Thompson et al. 1989). BLS affects yield by impairing grain fill and/or reducing kernel number (Duveiller et al. 1997). Management of the disease is difficult since the pathogen, *Xanthomonas translucens* (Jones et al. 1917) Vauterin et al. 1995, is seedborne and no chemical (seed or foliar) treatments are effective for control (Jones et al. 1917; Schaad and Forster 1985; Tubajika et al. 1998). Deployment of host plant resistance has the greatest potential for disease management. However, sources of resistance in wheat or other cereals are limited and quantitative (Adhikari et al. 2011; Kandel et al. 2012, 2015). Multiple pathovars of *X. translucens* are known to cause BLS in cereals, but the relative importance of different or individual pathovars or genetic diversity within a pathovar on breeding for BLS resistance is yet to be determined.

Of the 10 currently recognized pathovars of *X. translucens*, *X. translucens* pv. *undulosa* (Smith et al. 1919) and *X. translucens* pv. *translucens* (Jones et al. 1917) Vauterin et al. 1995 are considered economically important in Minnesota, North Dakota, and South Dakota (Adhikari et al. 2012; Kandel et al. 2015; Smith et al. 1919; Vauterin et al. 1995). *X. translucens* pv. *undulosa* causes disease on wheat and barley, while *X. translucens* pv. *translucens* causes disease on barley (Bragard et al. 1995; Smith et al. 1919;

Vauterin et al. 1995). Two other pathovars, *X. translucens* pv. *cerealis* (Hagborg 1942) Vauterin et al. 1995, a pathogen of wheat, oats, barley, and rye, and *X. translucens* pv. *secalis* (Reddy et al. 1924) Vauterin et al. 1995, a pathogen of rye, wheat, and barley also have been reported in the region (Cunfer and Scholari 1982; Hagborg 1942; Reddy et al. 1924; Vauterin et al. 1995).

Pathovar identification and classification is based on host range and symptom profiles (Dye et al. 1980). Obtaining accurate host range information is time consuming and sometimes provides inconsistent results because of the challenges of generating disease development in inoculated plants that simulates the pathogen–host interactions occurring in natural infections. Molecular characteristics, such as biochemical or genetic markers, alone are insufficient for pathovar identification, however they are helpful in hypothesizing pathovar affiliation (Bull and Koike 2015). Specific and unique phenotypic character states for the pathovars of *X. translucens* have not been identified. Pathovars of *X. translucens* are not clearly separated by protein content, methylated fatty acids, 16S rDNA sequencing, or DNA–DNA hybridization (Bragard et al. 1997; Bragard et al. 1995; Hauben et al. 1997; Moore et al. 1997; Stead 1989; Vauterin et al. 1992). While restriction length polymorphisms (RFLP) have indicated diversity and separation among *X. translucens* strains, it is not sufficient to fully differentiate pathovars and specifically has not distinguished *X. translucens* pv. *undulosa* from *X. translucens* pv. *translucens* (Adhikari et al. 2012; Bragard et al. 1995). Combinations of BOX-, ERIC-, and REP-polymerase chain reactions (PCR) can effectively distinguish *X. translucens* from other *Xanthomonas* species, yet do not separate all *X. translucens* pathovars from each other (Rademaker et al. 2005; Rademaker et al. 2006). In REP-PCR, *X. translucens* pv. *cerealis* and *X. translucens* pv. *translucens* (including strains previously designated *X. translucens* pv. *hordei*) are distributed across two clusters, with some strains grouping with *X. translucens* pv. *undulosa* and *X. translucens* pv. *secalis* pathotype strains, and some with the *X. translucens* pv. *translucens* pathotype strain (Rademaker et al. 2005). More recently, phylogenies

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constructed using sequences of *gyrB* have separated *Xanthomonas* species and many *Xanthomonas* pathovars within species, but did not provide pathovar-specific lineages for *X. translucens* (Parkinson et al. 2009).

Multilocus sequence analyses (MLSA) of housekeeping genes can be used to differentiate closely related taxa as well as to provide fine resolution for population structures within taxa (Fayette et al. 2016; Pérez-Losada et al. 2013). MLSA and multilocus sequence typing (MLST) allow direct genetic comparison of strains across studies because results are highly reproducible and allelic sequences can be retrieved from archival databases, such as the Plant Associated and Environmental Microbes Database (PAMDB) (Almeida et al. 2010). Although previous studies have demonstrated that MLSA can differentiate species and sometimes pathovars of *Xanthomonas*, the *X. translucens* pathovars were not included or not well represented in the investigations (Bragard et al. 1995; Fayette et al. 2016; Fischer-Le Saux et al. 2015; Young et al. 2008; Young et al. 2010). A larger scale MLSA study of an international collection of strains that included all *X. translucens* pathovars, and more specifically the closely related *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa*, was published recently (Langlois et al. 2017). While MLSA provided separation of *X. translucens* pathovars affecting cereals from other noncereal pathovars, it did not separate *X. translucens* pv. *undulosa* from *X. translucens* pv. *translucens* (Langlois et al. 2017). In contrast, another recent MLSA study based on four housekeeping genes separated *X. translucens* pv. *undulosa* from *X. translucens* pv. *translucens* (Peng et al. 2016). The aim of this study is to determine if MLSA can provide insights into the genetic diversity of *X. translucens* pathovars associated with BLS epidemics in the United States.

We conducted a study to examine the genetic diversity and plant reactions of *X. translucens* strains associated with recent outbreaks of BLS in wheat and barley production in the Upper Midwest. This in-depth evaluation of genetic diversity and plant reactions can be expected to inform the development and deployment of disease management strategies.

MATERIALS AND METHODS

Bacterial strains and growth conditions. A total of 96 *Xanthomonas* strains from a variety of sources were used in this study (Tables 1 and 2). Sixty-one bacterial strains were obtained from symptomatic wheat and barley samples submitted by growers and crop scouts in Minnesota from 2009 to 2013. Additionally, three strains were collected from intermediate wheatgrass (*Thinopyrum intermedium*) in St. Paul, MN. One strain isolated in 2013 from barley in Wisconsin was also included. Ten strains from wheat were received from South Dakota State University. Three DNA samples from wheat and barley were obtained from North Dakota State University. Six

strains representing Arkansas, Montana and Colorado were obtained from Colorado State University. In addition, the species type strain, *X. translucens* pv. *translucens*, and eight pathotype strains representing *X. translucens* pv. *cerealis*, *X. translucens* pv. *arrhenatheri*, *X. translucens* pv. *secalis*, *X. translucens* pv. *undulosa*, *X. translucens* pv. *phleipratensis*, *X. translucens* pv. *phlei*, *X. translucens* pv. *poae*, and *X. translucens* pv. *graminis* were included. Three type strains of closely related species *X. hyacinthi*, *X. sacchari*, and *X. theicola* were used as outgroups (Table 1).

The Minnesota strain collection, which included 61 *Xanthomonas* strains from wheat and barley and three from intermediate wheatgrass, was obtained by isolating bacteria from leaves with symptoms typical of BLS. Leaves were chopped and a small amount of tissue (~2 cm²) was transferred to 1 ml of 0.85% (wt/vol) NaCl buffer. Samples were rocked for 5 min. Serial dilutions were plated onto Wilbrink's agar (WBA) and incubated for 3 to 5 days at 27°C (Sands et al. 1986). Colonies with characteristic lemon-yellow color and shiny texture were subcultured (one to two per sample) and confirmed as pathogenic representatives of *Xanthomonas translucens* by 16S rDNA sequencing and by virulence assays conducted in a greenhouse. Strains were stored in nutrient broth with yeast extract (NBY) containing 15% glycerol at -80°C.

Greenhouse study of plant virulence. Plants for the greenhouse assays were grown in the University of Minnesota Plant Growth Facility, St. Paul, MN. Seeds were sown in pots (10 × 8 × 12 cm; 1 × w × h) filled with Sunshine MPV soilless media (Sun Gro Horticulture, Quincy, MI) containing vermiculite, sphagnum peat moss, gypsum and dolomitic limestone. Five seeds were planted per pot at a depth of 1.5 to 2.0 cm. Plants were grown in 18 to 22°C and with 16 h of supplemental lighting provided by high-pressure sodium lamps (400 W, emitting ca. 300 μmol photons s⁻¹ m⁻²).

A total of 75 strains were tested for virulence in the greenhouse including 45 strains from wheat and 30 strains from barley. The majority of these 75 strains were collected from Minnesota with some representatives from South Dakota, Wisconsin, Montana, Colorado, and Arkansas (Tables 1 and 2). Three-day-old bacterial cells grown on WBA from freezer stocks were subcultured as bacterial lawns on WBA. A few colonies were suspended in sterile 0.85% NaCl buffer, to obtain approximately 10⁷ CFU/ml (OD₅₄₀ = 0.01). The hard red spring wheat cultivar RB07 and the six-rowed spring barley cultivar Quest, both of which are considered to be moderately susceptible to BLS, were inoculated at the flag leaf stage. Each replicate consisted of five plants per pot. Each strain was applied to five flag leaves per pot by leaf infiltration with a blunt-end syringe. Control plants were inoculated with sterile 0.85% NaCl. Each treatment was replicated three times. Inoculated plants were randomized and incubated in the greenhouse at 27°C for 21 days with a 16 h photoperiod. Lesion lengths (centimeters) were

TABLE 1. List of historic type, pathotype, and other previously characterized strains of *Xanthomonas* species and pathovars used as comparisons in this study^x

Species	Pathovar	Strain designation ^y	Host of origin ^z	Origin	Year of isolation
<i>X. translucens</i>	<i>arrhenatheri</i>	LMG 727 ^{PT}	<i>Arrhenatherum elatius</i>	Switzerland	1978
<i>X. translucens</i>	<i>cerealis</i>	B50 = NCPPB 1943	<i>Hordeum vulgare</i>	United States	1942
<i>X. translucens</i>	<i>cerealis</i>	LMG 679 ^{PT}	<i>Bromus inermis</i>	United States	1941
<i>X. translucens</i>	<i>graminis</i>	LMG 726 ^{PT}	<i>Dactylis glomerata</i>	Switzerland	1973
<i>X. translucens</i>	<i>phlei</i>	LMG 730 ^{PT}	<i>Phleum pratense</i>	Norway	1978
<i>X. translucens</i>	<i>phleipratensis</i>	LMG 843 ^{PT}	<i>Phleum pratense</i>	United States	1942
<i>X. translucens</i>	<i>poae</i>	B86 = LMG 728 ^{PT} = ATCC 33804 ^{PT}	<i>Poa trivialis</i>	Switzerland	1978
<i>X. translucens</i>	<i>poae</i>	LMG 728 ^{PT}	<i>Poa trivialis</i>	Switzerland	1978
<i>X. translucens</i>	<i>secalis</i>	LMG 883 ^{PT}	<i>Secale cereale</i>	Canada	1966
<i>X. translucens</i>	<i>translucens</i>	LMG 876 ^T	<i>Hordeum vulgare</i>	Minnesota	1933
<i>X. translucens</i>	<i>undulosa</i>	LMG 892 ^{PT}	<i>Triticum turgidum</i>	Canada	1966
<i>X. hyacinthi</i>		LMG 739 ^T	<i>Hyacinthus orientalis</i>	Netherlands	1958
<i>X. sacchari</i>		LMG 471 ^T	<i>Saccharum officinale</i>	Guadeloupe	Unknown
<i>X. theicola</i>		LMG 8684 ^T	<i>Camellia sinensis</i>	Japan	1974

^x All strains were obtained from the Belgian Co-Ordinated Collections of Micro-Organisms (BCCM/LMG), except B50 and B86, which were obtained from J. Leach, Colorado State University.

^y Type strains (^T); pathotype strains (^{PT}).

^z Plant species from which the strain originated.

recorded 21 days after infiltration and used as a discrete measurement of virulence. The entire experiment was performed twice.

DNA extraction. Strains from glycerol stocks were streaked for single colonies on WBA grown for 3 days at 27°C, and then subcultured to form bacterial lawns at 3 days. A small amount of the bacterial lawn was suspended in 1 ml of Tris EDTA (pH 8) buffer. DNA was extracted according to the manufacturer's protocol using the IBI Blood and Tissue DNA extraction kit (MidSci, St. Louis, MO). Extracted DNA was stored at -20°C.

Amplification and sequencing. Four housekeeping genes were selected for sequencing: *rpoD*, *dnaK*, *fyuA*, and *gyrB* (Young et al. 2008). Primers for *dnaK* and *fyuA* as reported by Young et al. (2008) did not amplify the *X. translucens* group and were therefore

redesigned using the Primer 3 program and based on draft genome sequences provided by Jan Leach, Colorado State University (Table 3) (Koressaar and Remm 2007; Langlois et al. 2017).

All amplifications were done with a HotStarTaq Master Mix Kit (Qiagen, Germantown, MD) in 50-µl reactions. For *rpoD*, *dnaK*, and *fyuA*, PCR reactions were performed with an initial denaturation at 95°C for 15 min followed by 35 cycles of 94°C for 1 min (denaturation), 60°C for 1 min (annealing), and 72°C for 1 min (extension) and a final extension at 72°C for 10 min. The same cycle was used for *gyrB*, but with an annealing temperature of 54°C for 1 min. Products were visualized via gel electrophoresis on 1% agarose in TBE buffer stained with ethidium bromide. PCR products were purified with ExoSAP-IT (Affymetrix, Santa Clara, CA) and

TABLE 2. Origin, multilocus sequencing analysis (MLSA) group, and sequence types of type and pathotype strains of *Xanthomonas translucens* pv. *translucens*, *X. translucens* pv. *secalis*, *X. translucens* pv. *undulosa*, and 82 strains of *X. translucens* isolated between 2006 and 2013 from wheat, barley, rye, and intermediate wheatgrass

Strain ID ^u	Pathovar designation prior to this study	Host of origin	Geographic origin ^v	Year of isolation	MLSA group ^w	Sequence type ^x
CIX87	n.d. ^y	<i>Hordeum vulgare</i>	MN	2011	Xtt A	15
B1 ^z	n.d.	<i>H. vulgare</i>	ND	unknown	Xtt A	15
LMG876 ^T	<i>X. translucens</i> pv. <i>translucens</i>	<i>H. vulgare</i>	MN	1933	Xtt A	22
CIX43	n.d.	<i>H. vulgare</i>	MN	2009	Xtt A	24
CIX34	n.d.	<i>H. vulgare</i>	MN	2010	Xtt A	25
CIX76, CIX90	n.d.	<i>H. vulgare</i>	MN	2011	Xtt A	31
CIX132	n.d.	<i>H. vulgare</i>	WI	2013	Xtt B	41
CIX26	n.d.	<i>H. vulgare</i>	MN	2010	Xtt B	42
B69	<i>X. translucens</i> pv. <i>translucens</i>	<i>H. vulgare</i>	CO	2010	Xtt B	43
CIX20-22, CIX28-30	n.d.	<i>H. vulgare</i>	MN	2010	Xtt B	43
CIX84, CIX86	n.d.	<i>H. vulgare</i>	MN	2011	Xtt B	43
CIX41, CIX45-46, CIX52	n.d.	<i>H. vulgare</i>	MN	2009	Xtt C	23
CIX78-79, CIX81-82, CIX85, CIX92, CIX94-95	n.d.	<i>H. vulgare</i>	MN	2011	Xtt C	23
CIX32	n.d.	<i>H. vulgare</i>	MN	2010	Xtu	36
B74	<i>X. translucens</i> pv. <i>translucens</i>	<i>H. vulgare</i>	MT	2007	Xtu	36
LMG 883 ^{PT}	<i>X. translucens</i> pv. <i>secalis</i>	<i>Secale cereale</i>	Canada	1966	Xtu	5
B43	<i>X. translucens</i> pv. <i>secalis</i>	<i>S. cereale</i>	Unknown	2006	Xtt A	15
CIX33	n.d.	<i>Triticum aestivum</i>	MN	2010	Xtt B	43
CIX126	n.d.	<i>T. aestivum</i>	MN	2013	Xtt C	16
CIX127	n.d.	<i>T. aestivum</i>	MN	2013	Xtu	4
CIX103	n.d.	<i>T. aestivum</i>	MN	2011	Xtu	10
XctSD-003	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2008	Xtu	19
XctSD-002	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2008	Xtu	21
XctSD-009	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2009	Xtu	28
XctSD-006	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2008	Xtu	29
CIX102	n.d.	<i>T. aestivum</i>	MN	2011	Xtu	29
CIX36, CIX53-54	n.d.	<i>T. aestivum</i>	MN	2010	Xtu	30
CIX35	n.d.	<i>T. aestivum</i>	MN	2010	Xtu	32
XctSD-010	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2009	Xtu	34
CIX49	n.d.	<i>T. aestivum</i>	MN	2009	Xtu	35
CIX38, CIX40	n.d.	<i>T. aestivum</i>	MN	2010	Xtu	36
CIX42, CIX44, CIX48, CIX50-51	n.d.	<i>T. aestivum</i>	MN	2009	Xtu	36
CIX91, CIX104, CIX107, CIX109	n.d.	<i>T. aestivum</i>	MN	2011	Xtu	36
CIX128-130	n.d.	<i>T. aestivum</i>	MN	2013	Xtu	36
CIX31	n.d.	<i>T. aestivum</i>	MT	2010	Xtu	36
LB10 ^z , LW16 ^z	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	ND	Unknown	Xtu	36
XctSD-001, XctSD-004-005, XctSD-017	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2008	Xtu	36
CIX47	n.d.	<i>T. aestivum</i>	MN	2009	Xtu	37
B75	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	AR	2010	Xtu	38
CIX23, CIX37, CIX39	n.d.	<i>T. aestivum</i>	MN	2010	Xtu	38
CIX88, CIX106, CIX125, CIX131	n.d.	<i>T. aestivum</i>	MN	2011	Xtu	38
CIX105	n.d.	<i>T. aestivum</i>	MN	2011	Xtu	39
XctSD-008	<i>X. translucens</i> pv. <i>undulosa</i>	<i>T. aestivum</i>	SD	2009	Xtu	40
CIX134	n.d.	<i>Thinopyrum intermedium</i>	MN	2013	Xtu	33
CIX133	n.d.	<i>T. intermedium</i>	MN	2012	Xtu	36
CIX135	n.d.	<i>T. intermedium</i>	MN	2012	Xtu	39
LMG892 ^{PT}	<i>X. translucens</i> pv. <i>undulosa</i>	<i>Triticum turgidum</i>	Canada	1966	Xtu	36

^u Type strain (T); pathotype strain (PT).

^v MN = Minnesota, ND = North Dakota, WI = Wisconsin, CO = Colorado, MT = Montana, SD = South Dakota, and AR = Arkansas.

^w MLSA groups are based on the phylogeny of the concatenated sequence of *rpoD*, *dnaK*, *fyuA*, and *gyrB* (Young et al. 2008) as depicted in Figure 2.

^x Each unique concatenated sequence, based on *rpoD*, *dnaK*, *fyuA*, and *gyrB*, was assigned a sequence type.

^y For strains isolated in this study, pathovar had not been previously determined; n.d. indicates not determined. All other pathovar designations came from the providers.

^z DNA only.

submitted to ACGT Inc. (Wheeling, IL) for Sanger sequencing. All sequences from this study were deposited into the NCBI (<https://www.ncbi.nlm.nih.gov/>) as nucleotide sequence accession numbers; KY801336 to KY801434, KY814374 to KY814472, KY801435 to KY801533, and KY801534 to KY801632.

MLSA. CLC Main Workbench 7 (Qiagen) was used to assemble, align, and trim sequences to the following fragment lengths according to the work of Zacaroni et al. (2012): *rpoD* (674 nucleotides), *dnaK* (762 nucleotides), *fyuA* (522 nucleotides), and *gyrB* (687 nucleotides). Sequences were concatenated for a final length of 2645 positions.

Model testing was conducted through CLC Main Workbench 7 on individual loci and concatenated loci. The general time reversible (GTR+G+T) model was used in all tree constructions. The program Bayesian Evolutionary Analysis Sampling Trees (BEAST 1.8.2) (Drummond et al. 2012) was used for Bayesian MCMC analyses, assuming a strict molecular clock and 10 million generations. The output was analyzed with Tracer v1.6.0 (Rambaut et al. 2014), and visualized in FigTree v1.4.2 (<http://tree.bio.ed.ac.uk/software/figtree>). Addition of metadata and tree editing were completed in CLC Main Workbench 7. A median-joining network to infer phylogenetic relationships among haplotypes was constructed with Network version 4.2.0.1 (www.fluxus-technology.com).

MLST. Unique alleles were consecutively numbered using a custom Python script. Each unique concatenated sequence was assigned a sequence type (ST) via Poppr (Kamvar et al. 2014). A subset of 85 strains was used to assess diversity within *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa* (Table 2). Sequence variation and diversity statistics were calculated using DnaSP v5 (Rozas et al. 2003). Relatedness of STs was calculated in eBurst v3 (<http://eburst.mlst.net>) using the default settings in which strains are placed in the same group if they have identical alleles at three of the four loci. Clonal complexes within the *X. translucens* clades and inferred evolutionary descent were estimated by generating a minimum spanning tree (Feil et al. 2004).

Statistical analyses of virulence data. Virulence data were transformed by taking the square root of the lesion length (centimeter) to normalize the data. The isolate, host species origin, test host (wheat or barley), and trial were fitted into multiple regression models for analysis of variance (ANOVA). Fisher's least significant difference in the R library 'agricolae' was used for mean separation tests.

RESULTS

Virulence of *X. translucens* strains from wheat and barley. All 75 strains evaluated in the greenhouse caused BLS symptoms on both wheat and barley, though lesion lengths varied significantly (Fig. 1). Lesion development was first observed 4 to 5 days postinoculation (dpi). Lesions progressively turned yellow and eventually brown and necrotic. Through preliminary experiments, we determined that lesion length captured at 14 dpi provided optimal differentiation of symptom development prior to leaf senescence (data not shown). When lesion lengths were compared with lesion area, measured digitally via Fiji (ImageJ), lesion length and lesion

area were highly correlated (data not shown) (Abrahamoff et al. 2004). Therefore, only lesion length was recorded for further experiments. The mean lesion lengths measured in both wheat and barley at 14 dpi ranged from 0.16 to 11.2 cm. Lesions did not develop in wheat or barley plants infiltrated with the NaCl buffer alone.

The length of lesions at 14 dpi was affected by multiple factors. ANOVA identified isolate, inoculated host (wheat or barley), and host of isolation $\times$ inoculated host as highly significant variables (Prob>F = <2.2e-16 for each variable). There were no significant differences between the two greenhouse trials. In general, strains originating from wheat caused longer lesions than those originating from barley (Fig. 1, Table 4). Most strains caused longer lesions on the host from which they were isolated than on the alternative host. In this study, alternative host is defined as a plant species other than the host species from which it was isolated on which a bacterial plant pathogen can cause disease. Three strains were statistically more virulent on the alternative host than on their host of origin (Fig. 1). Strain B74, which came from barley, produced longer lesions in wheat than in barley; B74 produced lesion length (square root cm) values of 1.99 (standard error [SE] = 0.08) and 1.47 (SE = 0.18) in wheat and barley, respectively. In contrast, strains CIX33 and CIX126, which came from wheat, produced longer lesions in barley; lesion length (square root cm) values of 0.72 (SE = 0.29) and 0.87 (SE = 0.21) in wheat and 1.51 (SE = 0.12) and 1.5 (SE = 0.16) in barley, were obtained for CIX33 and CIX126, respectively. CIX32 produced the second longest lesion on wheat of all the barley strains examined; however, the mean lesion length it caused in wheat was not significantly different from the mean lesion length caused in barley.

Virulence data were not obtained for six strains of *X. translucens* from small grains: *X. translucens* pv. *translucens* LMG 876^T, *X. translucens* pv. *secalis* LMG 883^{PT}, *X. translucens* pv. *undulosa* LMG 892^{PT}, *X. translucens* pv. *undulosa* LB10, *X. translucens* pv. *undulosa* LW16, and *X. translucens* pv. *translucens* B1. *X. translucens* pv. *undulosa* LB10 and *X. translucens* pv. *undulosa* LW16 were previously reported to favor wheat as a host, whereas *X. translucens* pv. *translucens* B1 was reported to cause disease on barley but not wheat (Adhikari et al. 2012).

Phylogeny of *X. translucens* strains from wheat and barley. All four loci (*rpoD*, *dnaK*, *fyuA*, and *gyrB*) were successfully amplified from the 96 *X. translucens* strains included in this study (Tables 1 and 2). Bayesian inference of phylogenies, based on concatenated sequences, revealed that all field strains from wheat and barley fell within the *X. translucens* clade (Fig. 2). In addition, the wheat and barley strains from the Midwestern United States were part of an *X. translucens* pv. *undulosa*/*X. translucens* pv. *translucens* clade (posterior probability = 0.86) (Fig. 2). Within this clade were 85 strains that separated into four distinct clades (posterior probabilities of 0.86 to 1.0). The *X. translucens* pv. *undulosa* clade (Xtu) contained the pathotype strains for *X. translucens* pv. *undulosa* (LMG 892^{PT}) and for *X. translucens* pv. *secalis* (LMG 883^{PT}). The *X. translucens* pv. *translucens* clade A (*X. translucens* pv. *translucens* group A = Xtt A) contained the type strain for *X. translucens* pv. *translucens* (LMG 876^T) (Fig. 3). Clades *X. translucens* pv. *translucens* group B (=Xtt B) and group C (=Xtt C) were largely

TABLE 3. Primers for housekeeping genes targeted in this study

Gene	Sequence (5'-3')	Gene product	Tm (°C) ^z	Aligned fragment length (bp)	Reference
<i>rpoD</i>	F: TGGAACAGGGCTATCTGACC R: CATTCTYAGGTTGGTCTGRTT	RNA polymerase sigma factor	54	674	(Young et al. 2008) (Zacaroni et al. 2012)
<i>dnaK</i>	F: TCCTAAGCACCTCAACATCAAG R: CTCTTGTCGTCCTTGACCTC	Chaperone protein	59	762	This study (Zacaroni et al. 2012)
<i>fyuA</i>	F: CTCGCAGAACGGCCTGTA R: GTAGCCGGGCATCTTCAACT	TonB-dependent receptor	61	522	This study (Zacaroni et al. 2012)
<i>gyrB</i>	F: ACGAGTACAACCCGGACAA R: CCCATCARGGTGCTGAAGAT	DNA gyrase, subunit B	54	687	(Young et al. 2008) (Zacaroni et al. 2012)

^z Thermal melting point.

composed of strains from barley with neutral or greater virulence on barley versus wheat (Figs. 1 and 3). The phylogenetic branch lengths indicated a high degree of genetic similarity between these four clades (<0.5 substitutions per 100 nucleotide sites; Figs. 2 and 3). The taxa most closely related to the *X. translucens* pv. *undulosa* *X. translucens* pv. *translucens* clade were *X. translucens* pv. *poae*, *X. translucens* pv. *phleipratensis*, *X. translucens* pv. *arrhenatheri*, *X. translucens* pv. *phlei*, and *X. translucens* pv. *cerealis*, all of which cause disease on monocotyledonous hosts (Fig. 2). Posterior probabilities associated with these branches were >0.6, even though the rate of genetic change predicted between the different pathovar lineages was low (<0.05).

Most (86.5%) of the strains within Xtu originated from wheat. The two barley strains (CIX32 and B74) that produced long lesions on wheat also clustered within Xtu. The Xtu clade also included strains CIX133, CIX134, and CIX135 from intermediate wheat-grass. One wheat isolate, CIX127, was the most genetically distant of strains in the Xtu clade. Although the pathotype strain for *X. translucens* pv. *secalis* (LMG 883^{PT}) grouped with Xtu strains, B43, also from rye (*Secale cereale*), grouped within Xtt A.

With the exception of CIX32 and CIXB74, all strains from barley belonged to Xtt A, Xtt B, or Xtt C (Fig. 3). The two wheat strains, CIX33 and CIX126, which were more virulent on barley than wheat, were assigned to Xtt B and Xtt C, respectively (Figs. 1 and 3). Based on this MLSA schema, group Xtt A was more closely related to the Xtu group than to Xtt groups B and C (Fig. 2). Group Xtt A was also more genetically diverse than Xtt B and C (Fig. 3).

The median-joining network analysis, which depicts evolutionary pathways among organisms based on mutational rate, also produced four lineages of *X. translucens* strains (Fig. 4). The most divergent of the lineages were Xtu and Xtt B. Xtt A was most closely related to Xtu, while Xtt C is intermediate between Xtt A and Xtt B. There is a consistent association between host of isolation and phylogeny as represented by the subclades (Fig. 4). In addition, the reticulations observed in the network tree (Fig. 4) provide evidence that bifurcated phylogenetic trees, like those presented in Figures 2

TABLE 4. Mean separation of the average square root-transformed lesion lengths (centimeters) measured in flag leaves of wheat and barley 14 days postinoculation with 75 *Xanthomonas translucens* strains originating from either wheat or barley^y

Host of origin	Inoculated host	Mean lesion length (square root cm) ^z	Standard deviation	Standard error
Wheat	Wheat	1.78 a	0.53	0.03
Barley	Barley	1.37 b	0.44	0.03
Wheat	Barley	1.11 c	0.50	0.03
Barley	Wheat	1.07 c	0.46	0.03

^y Forty *Xanthomonas translucens* strains came from wheat and 35 strains came from barley.

^z Two means were designated as different based on Fisher's least significant difference. Analysis of variance identified isolate, inoculated host (wheat or barley), and host of isolation × inoculated host as highly significant variables (Prob > F = <2.2e-16 for each variable).

Fig. 1. Square root-transformed mean lesion length measurements in infiltrated leaves 14 days postinoculation; **A**, 45 strains of *Xanthomonas translucens* isolated from wheat inoculated into wheat (cultivar RB07) and barley (cultivar Quest) and **B**, 30 strains of *X. translucens* isolated from barley inoculated into wheat (cultivar RB07) and barley (cultivar Quest). Error bars represent standard error across two trials with three replications per trial. Strain B75 and all strains beginning with “XctSD” were designated in other collections as *X. translucens* pv. *undulosa*. Strains B69 and B74 were designated in other collections as *X. translucens* pv. *translucens*. All strains beginning with “CIX” are from this study and had not been assigned previously to a pathovar.

and 3, do not capture the full evolutionary history of *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens*.

MLST and minimum spanning trees (MST). The 85 strains from the *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* clades were analyzed by MLST to determine genetic diversity within these closely related clades. The genetic diversity of these clades was low, with only 51 total polymorphic sites in the total 2,645 base pairs of the concatenated sequence (Table 5). For *dnaK*, *fyuA*, and *gyrB*, the percentage of polymorphic sites was between 1.1 and 1.9. The most diverse gene fragment was *rpoD*, with 4.2% polymorphic sites. Twenty-seven STs, each based on a unique concatenated sequence, were identified within the 85 strains evaluated (Table 6).

The minimum spanning tree (MST) generated in eBurst identified three clonal complexes, defined as groups of strains that are connected by single locus variants and predicted to have descended from the same founding genotype (Fig. 5) (Feil et al. 2004). All strains from Xtu grouped within the largest clonal complex. ST 36 was predicted to be the founding ST of the Xtu clade, with ST 37 as a subgroup founder ST. Xtt A was contained within one clonal complex with ST 24 as its predicted founder ST. Xtt B and Xtt C formed a clonal complex with founder ST 43.

Geographic and phylogenetic distribution of STs of pathotypes and Midwest strains. With few exceptions, STs within a clonal complex, as depicted in the minimum spanning tree, were not associated with specific geographic origins or year of isolation (Tables 2 and 6; Fig. 5). The founder ST 36 was the most common and widespread in the Xtu clonal complex. ST 36 included strains from Minnesota, Montana, North Dakota, and South Dakota and the pathotype strain *X. translucens* pv. *undulosa* LMG 892^{PT}, isolated in Canada in 1966. The majority of strains in ST 36 were from wheat, with the exception of two barley strains, CIX32 from Minnesota and

B74 from Montana. The ST of the pathotype strain *X. translucens* pv. *secalis* LMG 883^{PT}, which originated from Canada, fell within the Xtu clade but was distinct from other strains (ST 5). Founder ST 24 of Xtt A included an isolate from Minnesota and an isolate originally identified as *X. translucens* pv. *secalis*, based on its origin from rye. While the pathotype strain of *X. translucens* pv. *translucens*, LMG 876^{PT}, which is also the type strain for the species, grouped with Xtt A, its ST differed from the Xtt A founder ST. No pathotype strains were associated with clonal complexes Xtt B or Xtt C. The founder ST for Xtt B, ST 43, included barley strains from multiple states and two wheat strains. All strains related to ST 23 of Xtt C were collected from barley in Minnesota. ST 16 of Xtt C was comprised of a single wheat isolate from Minnesota (Fig. 5).

DISCUSSION

The present study provides evidence that bacterial strains genetically related to *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* are the major pathogens associated with recent BLS outbreaks in the Upper Midwest of the United States. All strains within our collection from Minnesota, North Dakota, and South Dakota were classified as part of an *X. translucens* pv. *undulosa*/*X. translucens* pv. *translucens* clade. Multilocus sequence alignment based on four loci (*rpoD*, *dnaK*, *fyuA*, and *gyrB*), used previously in genetic diversity studies of *Xanthomonas* (Young et al. 2008), proved to be an efficient means of distinguishing *X. translucens* strains collected from barley and wheat within the region. Strains closely related to the pathotype strain of *X. translucens* pv. *undulosa* clustered in one monophyletic clade. Strains isolated from barley from the Upper Midwest were distributed across three clades of *X. translucens* pv. *translucens*: Xtt A, Xtt B, and Xtt C. Xtt A contained the type

Fig. 2. Bayesian tree of *Xanthomonas translucens* pathovars and closely related *Xanthomonas* species constructed from concatenated sequences of fragments of the genes *rpoD*, *dnaK*, *fyuA*, and *gyrB*. Species type and pathotype strains are indicated by the superscript letters T and PT, respectively. The four nodes encapsulated by the dashed box are collapsed and contain the strains collected for this study that are described in detail in Figure 3. Posterior probabilities of >0.5 are shown at the corresponding node. Branch lengths are drawn to scale and represent sequence changes since the common ancestor.

strain and is nested within the Xtu clade whereas the other two, Xtt B and Xtt C, are more closely related to each other than to either Xtu or Xtt A. Plant reactions of strains in Xtt A, Xtt B, and Xtt C were similar to each other but were different from those of strains in Xtu. Because Xtt A, Xtt B, and Xtt C strains produce similar plant reactions in the same hosts and are phylogenetically related, we designated them as *X. translucens* pv. *translucens*. Multiple clades representing genetic diversity within *X. translucens* pv. *translucens* have been reported previously based on AFLP and combined BOX-, ERIC-, and REP-PCR genomic fingerprints (Bragard et al. 1997; Rademaker et al.

2006). However, this is the first report of two clades of barley-adapted strains of *X. translucens* that have diverged phylogenetically from an *X. translucens* pv. *undulosa*/*X. translucens* pv. *translucens* clade.

Strains with similar relative virulence on inoculated wheat and barley plants consistently clustered together in Bayesian, network and minimum spanning trees. We found two strains originating from wheat (CIX126 and CIX33) and two strains originating from barley (CIX32 and B74) that grouped via MLSA with strains with similar or greater virulence in the alternative host. In fact, CIX32 from barley has the same ST (ST 36) as the pathotype strain for

Fig. 3. Circular cladogram showing the branch order of nodes obtained from Bayesian analysis of the concatenated sequences of fragments from the genes *rpoD*, *dnaK*, *fyuA*, and *gyrB* for the 85 *Xanthomonas translucens* strains used in this study. The cladogram provides detail for the four *X. translucens* clades (Xtu, Xtt group A, Xtt group B, and Xtt group C) identified in the dashed box presented in Figure 2. Posterior probabilities of >0.5 are displayed at nodes. Branch lengths are not drawn to scale. LMG 876^T is the species type strain for *X. translucens* and the pathotype strain for *X. translucens* pv. *translucens*. LMG 883^{PT} is the pathotype strain for *X. translucens* pv. *secalis*. LMG892^{PT} is the pathotype strain for *X. translucens* pv. *undulosa*. Strains B75, LB10, LW16, and all strains beginning with “XctSD” were received as *X. translucens* pv. *undulosa*. Strains B1, B69, and B74 were received as *X. translucens* pv. *translucens*. Strain B43 was received as *X. translucens* pv. *secalis*. All strains beginning with “CIX” are from this study and had not been assigned to a pathovar. Relative virulence refers to which host, wheat (cultivar RB07) or barley (cultivar Quest), exhibited significantly longer lesion lengths for each strain in greenhouse tests (light blue = *Triticum aestivum* (RB07), orange = *Hordeum vulgare* (Quest), gray = no significant difference between virulence in wheat and virulence in barley, white = not tested). Fisher’s least significant difference in the R library ‘agricolae’ was used for mean separation tests. Host origin indicates the host from which the strain was initially isolated (light blue = *Triticum aestivum*, dark blue = *Triticum turgidum*, red = *Thinopyrum intermedium*, green = *Secale cereale*, and orange = *Hordeum vulgare*).

X. translucens pv. *undulosa*, LMG 892^{PT}, further supporting identification of CIX32 as *X. translucens* pv. *undulosa*. Such interloper strains might be commonly associated with disease on another host crop, but are also able to cause disease on more than one host species, in this case wheat and barley. Thus, for Upper Midwestern strains in our collection, clades generated by MLSA were highly correlated to virulence profiles on wheat and barley. Similar conclusions can be drawn from an MLSA study based on *dnaK*, *gyrB*, *groEL*, and *recA* that included a smaller number of wheat and barley isolates and used presence of water-soaking and lesion length to determine host specificity and virulence, respectively (Peng et al. 2016). MLSA of a small number of housekeeping genes effectively predicts the assignment of strains to *X. translucens* pv. *translucens* and *X. translucens* pv. *undulosa*. It also has potential for predicting a strain's ability to incite disease on a given host. Critically, this approach will facilitate the rapid identification of strains associated with new BLS outbreaks.

The median-joining haplotype network and minimum spanning trees place Xtt A in a group between Xtu and Xtt B/C. The reticulations in the median-joining haplotype network tree suggest there is more complexity in the evolutionary pathway of the *X. translucens* pv. *undulosa*/*X. translucens* pv. *translucens* clade than can be seen in bifurcated phylogenetic trees. The reticulations in the median-joining haplotype network might further indicate that some of the genetic differences among strains within these clades arose from recombination, as has been observed for other *Xanthomonas* species (Timilsina et al. 2015). Xtt B and Xtt C strains might represent STs of *X. translucens* pv. *translucens* that have become prevalent in the Upper Midwest. Alternatively, Xtt B and Xtt C might indicate the population of *X. translucens* in the region contains an as yet to be described pathovar of *X. translucens*. Future studies on host range and virulence would be necessary to test this possibility. For example, in order for Xtt B or Xtt C to be considered a new pathovar, strains in these clades would have to be shown to cause different types of symptoms on the same known hosts for *X. translucens* pv. *translucens* or cause disease in different ranges of hosts. We propose it is more likely that clades Xtt B and Xtt C reflect recent genetic divergence within *X. translucens* pv. *translucens*, as has been suggested previously (Bragard et al. 1997; Rademaker et al. 2006). In the future, whole genomic comparisons of select

strains from this study could provide evidence to support the congruence or incongruence of Xtt A, Xtt B, and Xtt C (Baltrus et al. 2014).

One explanation for recent outbreaks of BLS in the Upper Midwest is that the pathogen has evolved to become better adapted to the region or to some of the current varieties. In Minnesota, typically 10 to 12 wheat varieties accounted for about 90% of the acreage planted, with another 40-plus varieties filling in the remaining 10%. The finding that some STs, such as ST 36 of Xtu, were found in several states over multiple years suggests that segments of the pathogen population have remained the same over the years and region. In contrast, the majority of strains and STs in the Xtt B and Xtt C clonal complexes come from recent BLS outbreaks in 2010 to 2013 from Minnesota, Wisconsin, and Colorado. This finding suggests additional STs have become established in the region more recently. Another explanation for the association of STs in the Xtt B and Xtt C clonal complexes with recent epidemics is that the sampling process used to obtain strains created an inherent bias in the STs obtained. Isolates were obtained from symptomatic plants collected nonrandomly by growers and collaborators. Thus, the strain collection used in the study does not represent a strategic sampling of infected wheat and barley acreage, and thus likely does not represent the total genetic diversity of *X. translucens* within the Upper Midwest or United States. More

TABLE 5. Sequence variation of 85 strains in the *Xanthomonas translucens* pv. *undulosa* group and *X. translucens* pv. *translucens* groups A, B, and C

Locus	Number of sites	S ^u	%S ^v	Hap ^w	Hd ^x	π ^y
<i>rpoD</i>	674	28	4.2	8	0.634	0.00312
<i>dnaK</i>	762	8	1.1	7	0.736	0.00305
<i>fyuA</i>	522	7	1.3	5	0.537	0.00332
<i>gyrB</i>	687	8	1.2	5	0.550	0.00100
Concat ^z	2645	51	1.9	27	0.875	0.00259

^u S = number of polymorphic sites.

^v %S = percentage of polymorphic sites.

^w Hap = number of haplotypes.

^x Hd = haplotype diversity.

^y π = nucleotide diversity.

^z Concat denotes the concatenated sequence data of all four loci.

Fig. 4. Median-joining haplotype network tree derived from analysis of the *rpoD*, *dnaK*, *fyuA*, and *gyrB* concatenated sequences of 85 *Xanthomonas translucens* strains from clades *X. translucens* pv. *undulosa* (Xtu) and *X. translucens* pv. *translucens* (Xtt group A, Xtt group B, and Xtt group C). Branch lengths are proportional to mutational distance (numeric values not shown). Circle size is proportional to the number of strains at each node. Circle shading is indicative of the host of origin of the strains (dark gray = *Triticum aestivum*, medium gray = *Secale cereale*, light gray = *Hordeum vulgare*, and no shading = *Thinopyrum intermedium*).

TABLE 6. Geographic and temporal distribution of multilocus sequencing typing clonal complexes and sequence types of *Xanthomonas translucens* pv. *undulosa* (Xtu) and three groups (A, B, and C) of *X. translucens* pv. *translucens* (Xtt)^r

Clonal complex	Sequence types	Number of isolates	Geographic distribution ^s	Year of isolation
Xtt A	15	3	MN, ND	2006, 2011
	22 ^t	1	MN	1933
	24 ^u	1	MN	2009
	25	1	MN	2010
	31	2	MN	2011
Xtt B	41	1	WI	2013
	42	1	MN	2010
	43 ^v	10	CO, MN, WI	2010, 2011, 2013
Xtt C	16	1	MN	2013
	23 ^w	12	MN	2009, 2011
Xtu	4	1	MN	2013
	5 ^x	1	Canada	1966
	10	1	MN	2011
	19	1	SD	2008
	21	1	SD	2008
	28	1	SD	2009
	29	2	MN, SD	2008, 2011
	30	3	MN	2010
	32	1	MN	2010
	33	1	MN	2013
	34	1	SD	2009
	35	1	MN	2009
	36 ^{y,z}	25	MN, MT, ND, SD	2007–2013
	37	1	MN	2009
	38	8	AR, MN	2010, 2011, 2013
	39	2	MN	2011, 2012
	40	1	SD	2009

^r Includes all 85 strains listed in Table 2.

^s MN = Minnesota, ND = North Dakota, WI = Wisconsin, CO = Colorado, MT = Montana, SD = South Dakota, and AR = Arkansas.

^t Includes *X. translucens* pv. *translucens* type strain LMG 876.

^u Founder sequence type for clonal complex Xtt A.

^v Founder sequence type for clonal complex Xtt B.

^w Founder sequence type for clonal complex Xtt C.

^x Includes *X. translucens* pv. *secalis* pathotype strain LMG 883.

^y Includes *X. translucens* pv. *undulosa* pathotype strain LMG 892.

^z Founder sequence type for clonal complex Xtu.

studies would be necessary to determine if region-specific STs and clades identified in this study are better adapted than others from the region. Nonetheless, this study provides evidence that the Upper Midwest population of *X. translucens* from wheat and barley contains STs not reported previously in association with BLS epidemics.

Pathovar is an infra-subspecific level of classification used to designate bacterial strains within a species that differ in plant reactions, usually measured by host range (Dye et al. 1980). In the case of pathovars of *X. translucens*, the overlap in host range of some pathovars makes it difficult to conclusively assign an isolate to a specific pathovar simply based on the host from which it was isolated. Two character states, presence or absence of water soaking and length of lesions in inoculated plants have been used to identify pathovars of *X. translucens*. It is also important to consider that results obtained from infiltrated leaves grown under greenhouse conditions might not adequately represent the host–pathogen interactions occurring under field conditions. For example, artificial leaf infiltrations might allow a weaker pathogen to overcome host infection barriers to appear more virulent than it would be in the field. However, our findings obtained with quantitative measurements of virulence in wheat and barley support the pathovar classifications *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens*, in that the majority of strains producing longer lesions in wheat than barley grouped in Xtu, while the majority of strains producing greater or similar lesion lengths in barley than wheat grouped in Xtt A, Xtt B, or Xtt C.

Phylogenies generated by this and other MLSA studies support *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* as being closely related but distinct taxa (Langlois et al. 2017; Peng et al. 2016). Despite these results, distinct lineages for *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* were not obvious in recent phylogenies created by MLSA of 12 loci or by comparisons of coding sequences common across multiple *X. translucens* pathovars (Langlois et al. 2017; Peng et al. 2016). The differences between results can be explained partially by the fact that an MLSA scheme based on a small number of loci provides only a limited view of the evolutionary history of very closely related organisms, such as *X. translucens* pv.

Fig. 5. Minimum spanning tree depicting clonal complexes for the 85 *Xanthomonas translucens* strains from clades *X. translucens* pv. *undulosa*, *X. translucens* pv. *translucens*: Xtt group A, Xtt group B, and Xtt group C. A clonal complex is defined as a group of strains that are connected by single locus variants and predicted to have descended from the same founding genotype. Numbers indicate sequence type (ST). Each unique concatenated sequence obtained from the amplified regions of *rpoD*, *dnaK*, *fyuA*, and *gyrB* was assigned an ST number. Lines connect STs that vary at a single locus. Large dots (ST 36, 43, and 24) indicate the founder of each clonal complex; ST 37, indicated with a medium-sized dot, is a subgroup founder. All other STs are represented with a small dot.

undulosa and *X. translucens* pv. *translucens*. Analyses including many more loci are likely to reveal additional genetic relationships and evolutionary patterns, which may or may not support phylogenetic predictions obtained with fewer loci (Baltrus et al. 2014). The molecular basis for the host adaptation of *X. translucens* pathovars associated with cereals and grasses is not yet known, but valuable insights into its complexities have been gained through whole genomic comparisons (Hersemann et al. 2017; Langlois et al. 2017; Peng et al. 2016). Nonetheless, our MLSA study provides biologically relevant correlations between allelic profiles and relative virulence on wheat and barley. It also offers evidence for additional diversity within *X. translucens* strains isolated from barley, e.g., Xtt B and Xtt C. The Upper Mid-western isolate collection with its distinct genetic lineages and cataloged plant reactions serves as a foundation for future studies on host adaptation and virulence in *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens*. Knowledge of the pathovars and genetic diversity within pathovars present in the region will ultimately assist breeders in selecting plants that are resistant to the common STs and most virulent strains.

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