Bacteriology

e-**X**tra*

Genetic and Phenotypic Characterization of *Xanthomonas* Species Pathogenic in Wheat in Uruguay

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ABSTRACT

Bacterial diseases affecting wheat production in Uruguay are an issue of growing concern yet remain largely uninvestigated in the region. Surveys of 61 wheat fields carried out from 2017 to 2019 yielded a regional collection of 63 strains identified by 16S rRNA gene analysis as *Xanthomonas* spp. A real-time PCR protocol with species-specific primers previously reported allowed the identification of 44 strains as *X. translucens*, the causal agent of bacterial leaf streak (BLS) in wheat and other cereal crops. Multilocus sequence analysis of four housekeeping genes (*dnaK*, *fyuA*, *gyrB*, and *rpoD*) revealed that these strains were most closely related to *X. translucens* pv. *undulosa*, the pathovar that is most commonly associated with BLS of wheat. Multilocus sequence typing was applied to examine the genetic diversity of *X. translucens* strains. Strains were assigned to four different sequence types, three of which were previously reported globally. Additionally, 17 *Xanthomonas* strains not belonging to *X. translucens* were obtained from diseased wheat leaves. Phylogenetic analysis showed that these strains are closely related to *X. prunicola* and clustered together with previously uncharacterized *Xanthomonas* strains isolated from wheat in Minnesota. In planta pathogenicity assays carried out on a BLS-susceptible wheat cultivar showed that *X. translucens* pv. *undulosa* strains caused brown necrosis symptoms typical of BLS, whereas non-*translucens Xanthomonas* sp. strains elicited an atypical symptom of dry necrosis. These findings suggest that local wheat fields are affected by *X. translucens* pv. *undulosa* and by a new wheat pathogen within the *Xanthomonas* genus.

Keywords: bacterial leaf streak, bacterial pathogens, multilocus sequence analysis and typing, population biology, Uruguay, wheat, *Xanthomonas*

Wheat (*Triticum aestivum* L.) is the main winter crop in Uruguay. In the last 10 years, an average of 348,210 hectares of wheat has been planted (Font et al. 2019, 2020). Plant diseases in this crop are of great relevance because they regularly constrain the ability to achieve high grain yields and good-quality grain in Uruguay (Altier 1996; Pereyra et al. 2011).

Fungal diseases, including leaf spots caused by fungi of the genera *Drechslera* and *Zymoseptoria*, powdery mildew, rusts, and Fusarium head blight, have been major factors affecting grain yield and wheat production in Uruguay (Pereyra 2013). Knowledge of the epidemiology and management of fungal diseases has been generated nationally in the last 20 years, resulting in broader availability of resistant cultivars and the regular use of fungicides to achieve effective control (Pereyra 2013). However, this has led to more healthy leaf tissue being available and the elimination of potentially beneficial mycoflora from the phyllosphere, which may partially explain increased infection and colonization of leaves by pathogenic bacteria (Gu et al. 2010; Pereyra 2013). Although bacterial diseases of wheat have traditionally been considered to be of secondary importance, they have been increasingly reported in recent decades (Adhikari et al. 2011; Curland et al. 2018; Duveiller et al. 1997; Sapkota et al. 2020).

Bacterial leaf streak (BLS), a foliar disease of small grains caused by pathovars of *Xanthomonas translucens* (Jones et al. 1917; Vauterin et al. 1995) has been reported to cause significant crop losses, representing the most limiting bacterial disease to wheat production (Duveiller et al. 1997). BLS has a worldwide distribution, including South and North America, Africa, Australia, Asia, and Eastern Europe (Duveiller et al. 1997; Egorova et al. 2014; Kandel et al. 2012; Khojasteh et al. 2019; Murray and Brennan 2009; Raja et al. 2010; Sands and Fourrest 1989). It was first reported in barley in 1917 and later in wheat in 1919 (Jones et al. 1917; Smith et al. 1919). Its prevalence has recently increased worldwide (Adhikari et al. 2011; Curland et al. 2018; Kandel et al. 2012; Khojasteh et al. 2019). Although BLS may reduce grain yield by 10% on average, decreases of ≤40% have been estimated under high disease pressure (Forster and Schaad 1988). Symptoms of BLS on wheat include expanding water-soaked lesions that eventually become chlorotic or necrotic. Infections reduce the active photosynthetic area, decreasing the number of kernels per spike or grain weight (Kandel et al. 2012). In advanced BLS infections, there may be infection of the glumes, accompanied by distinctive dark stripes and banding of the awns, called black chaff. Severe BLS infections can cause sterile spikes, leading to a complete loss of yield (Sapkota et al. 2020).

At present, *X. translucens* pathovars reported as potential causal agents of BLS in small-grain cereals (Sapkota et al. 2020) include *X. translucens* pv. *cerealis*, *X. translucens* pv. *secalis*, *X. translucens* pv. *translucens*, and *X. translucens* pv. *undulosa* (Hagborg 1942; Jones et al. 1917; Reddy et al. 1924; Smith et al. 1919). *X. translucens* pv. *undulosa* and *X. translucens* pv. *translucens* are the most economically important pathovars, with *X. translucens* pv. *undulosa* being the most common pathovar affecting wheat. In contrast, *X. translucens* pv. *translucens* is specific to barley (Bragard et al. 1997; Curland et al. 2018; Khojasteh et al. 2019). This pathogen was first reported in Uruguay in 1986 (Frommel 1986).

Despite its impact, few rapid and specific molecular diagnostic and identification methods are available for this pathogen. A molecular detection method for all pathovars within *X. translucens* has been developed, based on conventional PCR with species-specific primers directed to the spacer region between 16S and 23S rRNA genes. However, this method involves two consecutive amplification reactions and requires visualization of results on agarose gels, which may be cumbersome and time-consuming (Maes et al. 1996). More recently, loop-mediated isothermal amplification primers were developed for *X. translucens* diagnosis, which are able to distinguish between strains that cause BLS on cereals (translucens group) and those that cause bacterial wilt on forage grasses (graminis group) (Langlois et al. 2017).

Although host range assays are necessary for true pathovar assignation, they are time consuming, and the results obtained are not always reproducible, making identification of strains with different pathogenic profiles or isolated from new hosts a complex process (Rademaker et al. 2006). Discrimination of pathovars within *X. translucens* is also challenging, largely because of the overlap in host range between different pathovars and variation of host range within a pathovar (Maes et al. 1996; Sapkota et al. 2020). Multilocus sequence analysis (MLSA) and multilocus sequence typing (MLST) have become common sequence-based approaches to determining genetic diversity and population structure among closely related strains, in large part because of the high reproducibility of results, high resolution, and efficiency, and may also be useful in helping to predict pathovars (Croce et al. 2016; Maiden et al. 1998; Pérez-Losada et al. 2013). An MLSA/MLST scheme using the partial sequences of four housekeeping genes (*dnaK*, *fyuA*, *gyrB*, and *rpoD*) has been developed for the *Xanthomonas* genus (Young et al. 2008), and it was successfully adapted and conducted on multiple *X. translucens* strains, resulting in the best method so far for assessing *X. translucens* diversity (Curland et al. 2018, 2020; Khojasteh et al. 2019). Although this approach has been successful in elucidating the diversity of *X. translucens* populations in North America and Iran, South American populations of this pathogen remain largely uninvestigated.

Although bacterial diseases affecting local wheat crops in Uruguay have been frequently reported, no regional collection of small grain-pathogenic xanthomonads exists, and no in-depth studies of pathogen diversity has been conducted. Hence, the aim of this work was to develop a collection of *Xanthomonas* strains from Uruguay with a survey of wheat fields; identify isolates by using diagnostic primers, MLSA, and in planta studies; and examine the diversity within strains through MLST.

MATERIALS AND METHODS

Bacterial strain collection and isolation. A total of 61 wheat fields were surveyed in October and November 2017, 2018, and 2019, in southwestern and northwestern Uruguay (Fig. 1). Samples of symptomatic leaf tissue were taken at 30 different points within each field, in a circular pattern with approximately 20 m between each collection spot. The survey covered 15 different commercial cultivars commonly grown by farmers in the region (Table 1). Samples were labeled and placed in envelopes, left to dry at room temperature for 48 h, and stored at 4°C until they were processed.

Two to four typically symptomatic leaves were selected from each field. Leaf segments (5 cm long) were superficially disinfected with 1% sodium hypochlorite for 1 min and subsequently rinsed with distilled water. The leaf tissue was chopped into pieces of approximately 1 cm in length, placed in 1 ml of 0.9% NaCl, and gently shaken for 15 min to allow bacterial cells to disperse into the solution. Serial dilutions of this suspension were plated onto Wilbrink's agar (WBA) and incubated for 72 to 96 h at 28°C (Sands et al. 1986). Characteristic pale yellow mucoidal colonies with a shiny texture were transferred onto fresh WBA plates and incubated for 48 to 72 h at 28°C. Single colonies were subcultured onto fresh WBA plates to obtain pure cultures, which were subsequently grown on nutrient broth with yeast extract for 24 h at 28°C and stored with 10% glycerol at −70°C.

Real-time PCR-based identification of strains. Genomic DNA of all strains was extracted according to Sambrook and Russell (2001). First, 2 ml of bacterial culture in nutrient broth with yeast extract grown for 24 h was used for DNA extraction. Cetrimonium bromide–NaCl was used as the extraction buffer, and 24:1 chloroform/isoamyl alcohol solution was used to remove proteins. DNA pellets were suspended in 50 µl of Tris–EDTA buffer and stored at −20°C. DNA extracts were visualized by electrophoresis with 1% (wt/vol) agarose gel in 0.5× Tris–borate–EDTA (TBE) stained with GoodView nucleic acid stain.

Preliminary identification of strains was performed by real-time PCR with the T1/T2 pair of *X. translucens* species-specific primers targeting a 139-bp fragment within the 16S–23S rDNA spacer region (Maes et al. 1996) (Table 2). A real-time PCR protocol was adapted with Rotor-Gene-Q equipment (QIAGEN, Germany). Amplifications were performed in 20-µl reaction mixtures containing 1× Applied BioSystems' Power SYBR Green PCR Master Mix, 0.4 µM of each primer, and 20 ng of genomic DNA as the template. The amplification program consisted of an initial polymerase activation step of 10 min at 95°C and 30 two-step cycles of denaturation for 15 s at 95°C followed by combined annealing and extension for 1 min at 60°C. A melt curve was generated for each reaction immediately after the amplification program was finished.

We calculated amplification efficiency by performing a calibration curve with 10-fold serial dilutions of genomic DNA of strain MAI5067, from 10 ng/µl to 1 pg/µl. Reactions were prepared as described previously. Three replicates of each dilution were performed, and a control without DNA template was included.

16S rRNA gene sequencing-based identification of strains. Strains that did not amplify with the species-specific primers T1/T2 were further subjected to a broader identification approach with the universal primer pair 27F/1492R targeting the 16S rRNA gene (Table 2) (Lane 1991). DNA amplifications were performed in 25-µl reactions containing 1× standard Taq reaction buffer, 200 µM of each dNTP, 0.2 µM of each primer, 0.625 units of Taq DNA polymerase (New England BioLabs), and 20 ng of DNA template. The PCRs consisted of an initial denaturation step for 30 s at 95°C, 30 three-step cycles of denaturation for 30 s at 94°C, annealing for 1 min at 55°C and extension for 1 min at 68°C, and a final extension step for 5 min at 68°C. PCR products were visualized in 1% (wt/vol) agarose gels in 0.5× TBE stained with GoodView nucleic acid stain. The PCR products were purified by desalting and sequenced with both forward and reverse primers by Macrogen Inc. (Seoul, South Korea). Sequences were assembled and edited in Geneious Pro 8.0.5 software (Biomatters, New Zealand) and compared with existing sequences in the GenBank database with the nucleotide BLAST tool. The sequences were deposited into the National Center for Biotechnology Information (NCBI; https://www.ncbi.nlm.nih.gov/) as nucleotide sequence accession numbers MZ196391 to MZ196409.

Multilocus sequence analysis. Strains identified as *X. translucens* by real-time PCR were subjected to MLSA with partial sequences of four housekeeping genes (*dnaK*, *fyuA*, *gyrB*, and *rpoD*) previously demonstrated to show consistent differentiation within the genus *Xanthomonas* and between highly similar *X. translucens* strains (Curland et al. 2018; Young et al. 2008) (Table 2). Sequences of type and pathotype reference strains LMG 679[PT], LMG 876[T], LMG 883[PT], and LMG 892[PT] and representative *X. translucens* pv. *undulosa* strains CIX88, CIX102, CIX128, and XctSD-0100, reported by Curland et al. (2018) were used to compare the phylogenetic relationships of the strains isolated in Uruguay. LMG 726[PT], and the pathotype strain for *X. translucens* pv. *graminis* was used as an outgroup to root the tree (Table 3).

Strains identified as *Xanthomonas* spp. by 16S rRNA gene analysis were also included in MLSA phylogenies to further elucidate species identification. Because no successful amplification occurred when *fyuA* primers were used for these strains, a separate analysis

was performed based on the concatenated partial sequences of *dnaK*, *gyrB*, and *rpoD* genes. In order to determine the phylogenetic position of these strains within the genus, sequences from type strains of all currently reported species within the *Xanthomonas* genus were retrieved from the NCBI database and included in the analysis (Supplementary Table S1). Previously unreported *Xanthomonas* spp. strains CIX89, CIX97, W2.22, and W2.24, isolated from wheat in the Upper Midwest of the United States as part of previous surveys, were also included in this analysis (Table 3) (Curland et al. 2018, 2020). *Stenotrophomonas maltophilia* served as an outgroup to root the tree (Supplementary Table S1).

Amplification of all four genes was performed in 50-μl reactions containing 1× standard Taq reaction buffer, 200 μM of each dNTP, 0.2 μM of each primer, 1.25 units of Taq DNA polymerase (New England BioLabs), and 20 ng of DNA template. Reactions for *dnaK*, *fyuA*, and *rpoD* consisted of an initial denaturation step for 30 s at 95°C; 35 three-step cycles of denaturation for 30 s at 94°C, annealing for 1 min at 60°C, and extension for one minute at 68°C; and a final extension step for 5 min at 68°C. The same cycle was used for *gyrB* but with an annealing temperature of 54°C for 1 min (Curland et al. 2018). PCR products were visualized, purified, and sequenced in 1% (wt/vol) agarose gel in 0.5× TBE stained with

GoodView nucleic acid stain, purified by desalting, and sequenced with both forward and reverse primers by Macrogen Inc. (Seoul, South Korea).

Geneious Pro 8.0.5 software (Biomatters, New Zealand) was used to assemble, align, edit, trim, and concatenate the sequences. Sequences were concatenated in alphabetical order of the genes, for a final length of 2645 bp. Individual gene fragment lengths were used based on previous studies: 762 bp for *dnaK*, 522 bp for *fyuA*, 687 bp for *gyrB*, and 674 bp for *rpoD* (Curland et al. 2018, 2020; Zacaroni et al. 2012).

The sequences were deposited into NCBI (https://www.ncbi.nlm.nih.gov/) as nucleotide sequence accession numbers MZ201068 to MZ201301.jModelTest 2.1.10 (Darriba et al. 2012; Guindon and Gascuel 2003) was used to perform model testing on the aligned and concatenated sequences, which assigned the highest likelihood score to the general time reversible with gamma distribution with invariant sites (GTR G+I) substitution model.

MEGA X 10.1.0 software was used to carry out phylogenetic analyses (Kumar et al. 2018). The maximum likelihood method was applied with GTR G+I as the nucleotide substitution model. The tree topology was assessed by bootstrap resampling of 1,000 replicates. Phylogenetic trees were also reconstructed in Bayesian Evolutionary Analysis Sampling Trees (BEAST) 1.10.4 (Suchard et al. 2018),
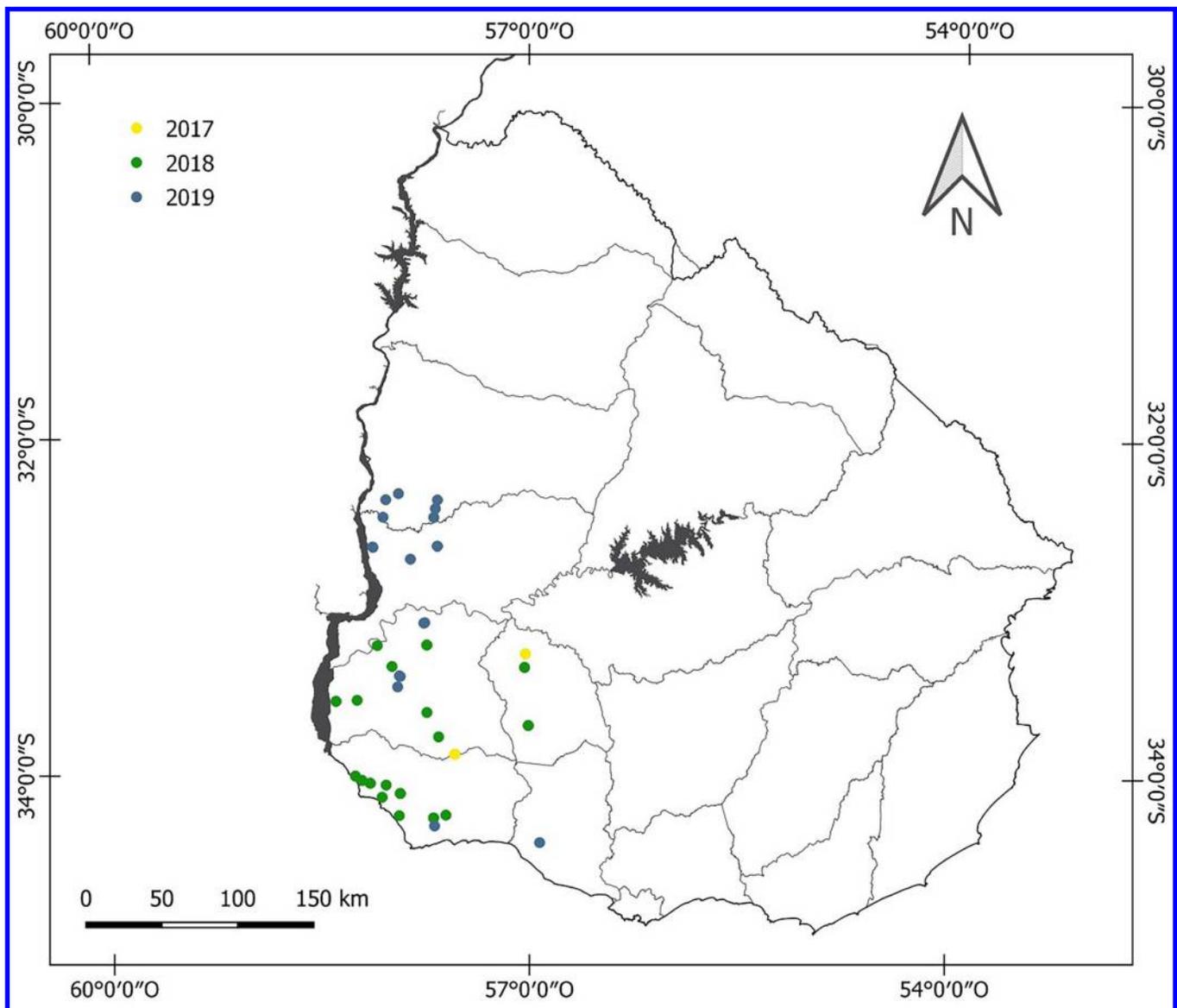

Fig. 1. Map of Uruguay showing the geographic distribution of wheat fields (dots) sampled in October and November 2017, 2018, and 2019, from which *Xanthomonas* spp. strains were isolated.

assuming a strict molecular clock, Yule process speciation as a tree prior, and 1,000,000 generations. Tracer 1.7.1 (Rambaut et al. 2018) was used to analyze the output, and burn-in was set at 100,000 states. The resulting trees were visualized and annotated in MEGA X 10.1.0 software.

Multilocus sequence typing. MLST was performed for a subset of 39 strains identified as *X. translucens*. Haplotype data were generated in DnaSP version 6 (Librado and Rozas 2009). Each unique sequence of a locus was assigned an allele number, and the sequence type of the strain was defined as the combination of these allele numbers. Statistics and diversity estimates for these strains were calculated in DnaSp version 6 (Librado and Rozas 2009). Patterns of evolutionary descent and relatedness among sequence types of *X. translucens* strains identified in this work were explored and displayed with a global optimal eBURST analysis (Francisco et al. 2009), which is an implementation of the eBURST algorithm rules proposed by Feil et al. (2004). The linear correlation between strains' place of origin, collection date, wheat cultivar, and assigned sequence type was evaluated by Pearson correlation coefficient, with a level of significance of $P < 0.05$.

TABLE 1. Origin, pathogenicity evaluation, and identification via real-time PCR, closest taxon in multilocus sequence analysis, and multilocus sequence typing of 63 *Xanthomonas* spp. strains isolated from wheat in Uruguay

| Strain | Origin | | | Symptom[a] | T1/T2[b] | MLSA[c] | ST[d] |
	Location	Year	Cultivar				
MAI5003	Arroyo Malo, Flores	2017	Fuste	BN	+	Xtu	1
MAI5004, MAI5006	Arroyo Malo, Flores	2017	Genesis 2366	BN	+	Xtu	*
MAI5008	Arroyo Malo, Flores	2017	Genesis 2366	BN	+	Xtu	2
MAI5009-5012	Arroyo Malo, Flores	2017	Fuste	BN	+	Xtu	2
MAI5030-5031	Trinidad, Flores	2018	Algarrobo	BN	+	Xtu	1
MAI5034	Dolores, Soriano	2018	Algarrobo	BN	+	Xtu	3
MAI5035	Dolores, Soriano	2018	Algarrobo	BN	+	Xtu	2
MAI5040	La Casilla, Flores	2018	Algarrobo	BN	+	Xtu	2
MAI5041	La Casilla, Flores	2018	Algarrobo	BN	+	Xtu	4
MAI5042	La Casilla, Flores	2018	Algarrobo	BN	+	Xtu	3
MAI5043	Minas de Narancio, Colonia	2018	Genesis 6.28	BN	+	Xtu	4
MAI5045-5046	Minas de Narancio, Colonia	2018	Genesis 6.28	BN	+	Xtu	2
MAI5053	Buenahora, Colonia	2018	Genesis 2375	BN	+	Xtu	*
MAI5055	Buenahora, Colonia	2018	Genesis 6.28	BN	+	Xtu	4
MAI5056	Buenahora, Colonia	2018	Genesis 6.28	BN	+	Xtu	*
MAI5057	Egaña, Soriano	2018	n.d.	BN	+	Xtu	1
MAI5058-5059	Mercedes, Soriano	2018	Genesis 2375	BN	+	Xtu	2
MAI5061	Guerrero, Soriano	2018	Genesis 2375	BN	+	Xtu	2
MAI5062	Semillero, Colonia	2018	n.d.	BN	+	Xtu	4
MAI5064	Semillero, Colonia	2018	n.d.	BN	+	Xtu	*
MAI5067	Conchillas, Colonia	2018	Fuste	BN	+	Xtu	2
MAI5071	Dolores, Soriano	2018	Curupay	BN	+	Xtu	2
MAI5074	Mercedes, Soriano	2018	Algarrobo	BN	+	Xtu	4
MAI5044	Minas de Narancio, Colonia	2018	Genesis 6.28	n.d.	+	Xtu	4
MAI5054	Buenahora, Colonia	2018	Genesis 6.28	n.d.	+	Xtu	2
MAI5076-5077	Young, Río Negro	2019	Audaz	n.d.	+	Xtu	2
MAI5079	La Paraguaya, Soriano	2019	Ceibo	n.d.	+	Xtu	2
MAI5080	San Dios, Soriano	2019	Syngenta 300	n.d.	+	Xtu	1
MAI5085	Porvenir, Paysandú	2019	Genesis 2375	n.d.	+	Xtu	4
MAI5086	Esperanza, Paysandú	2019	n.d.	n.d.	+	Xtu	2
MAI5090	Piedras Coloradas, Paysandú	2019	n.d.	n.d.	+	Xtu	1
MAI5091	Palmitas, Soriano	2019	Syngenta 300	n.d.	+	Xtu	4
MAI5093	Young, Río Negro	2019	ACA 20	n.d.	+	Xtu	2
MAI5094	Young, Río Negro	2019	Basilio	n.d.	+	Xtu	4
MAI5095	Semillero, Colonia	2019	Tarariras 1974	n.d.	+	Xtu	4
MAI5096	La Boyada, San José	2019	n.d.	n.d.	+	Xtu	4
MAI5014	San Dios, Soriano	2017	Genesis 2375	NS	−	Xarb	n.d.
MAI5032	Lomas de Carmelo, Colonia	2018	Genesis 2375	NS	−	Xarb	n.d.
MAI5001	Arroyo Malo, Flores	2017	Genesis 2366	DN	−	Xpru	n.d.
MAI5027	San Pedro, Colonia	2018	Baguette 11	DN	−	Xpru	n.d.
MAI5037, MAI5039	Mercedes, Soriano	2018	Genesis 2375	DN	−	Xpru	n.d.
MAI5050	Buenahora, Colonia	2018	Genesis 2375	DN	−	Xpru	n.d.
MAI5069-5070	Conchillas, Colonia	2018	Fuste	DN	−	Xpru	n.d.
MAI5075	Tarariras, Colonia	2018	Genesis 6.28	DN	−	Xpru	n.d.
MAI5081	San Dios, Soriano	2019	Syngenta 200	n.d.	−	Xpru	n.d.
MAI5082	San Javier, Río Negro	2019	Genesis 2375	n.d.	−	Xpru	n.d.
MAI5083	Bellaco, Río Negro	2019	Genesis 4.33	n.d.	−	Xpru	n.d.
MAI5084	Porvenir, Paysandú	2019	Genesis 2375	n.d.	−	Xpru	n.d.
MAI5087	La Tentación, Paysandú	2019	Genesis 4.33	n.d.	−	Xpru	n.d.
MAI5088	La Tentación, Paysandú	2019	n.d.	n.d.	−	Xpru	n.d.
MAI5089	Palmitas, Soriano	2019	Curupay	n.d.	−	Xpru	n.d.
MAI5092	San Javier, Río Negro	2019	Genesis 2375	n.d.	−	Xpru	n.d.
MAI5023	Arroyo Malo, Flores	2017	Fuste	n.d.	−	Xpru	n.d.

[a] Symptoms on the susceptible wheat cultivar INIA Tijereta as described in Fig. 2. BN, brown necrosis; DN, dry necrosis; n.d., not determined; NS, no symptoms.

[b] Results of real-time PCR with T1/T2 primers.

[c] Taxon of the most closely related reference strain based on phylogenetic grouping via multilocus sequence analysis (MLSA) group. Xarb, *X. arboricola* pv. *juglandis* ICMP 35[T]; Xpru, strains grouped together, *X. prunicola* CFBP 8353[T]; Xtu, *X. translucens* pv. *undulosa* LMG 892[PT].

[d] Sequence type assigned. *, using concatenated sequences of three genes (*dnaK*, *fyuA*, and *rpoD*); n.d., not determined.

In planta pathogenicity assays. A subset of 30 *X. translucens* and 10 *Xanthomonas* spp. strains, representative of the diversity across the geographic origin, was selected for in planta pathogenicity assays. Strains from freezer stocks were plated onto WBA medium and incubated for 72 h at 28°C. Strains were then plated on WBA as bacterial lawns and incubated for 24 to 48 h at 28°C. Inoculum was prepared by suspending cells in sterile 0.9% NaCl (wt/vol) solution and adjusted to an optical density at 600 nm of 0.01, equivalent to 10^7 CFU/ml.

Pathogenicity tests were carried out under controlled conditions in growth chambers. Three to six seeds of the wheat cultivar INIA Tijereta, susceptible to BLS (Castro et al. 2020), were sown in 10-cm-diameter plastic pots containing a 1:1:1 mixture of sand/soil/commercial substrate (Bioterra, Juanicó, Uruguay). Each pot was thinned to one plant per pot when the seedlings were at the first leaf stage (ZGS 1.1; Zadoks et al. 1974). Seedlings were maintained in greenhouse conditions at 24°C and fertilized every week with soluble NPK (12-8-5, NPK + micronutrients, foliar fertilizer; ISUSA, San José, Uruguay) applied at 10 ml per pot with a solution of 400 ml fertilizer per liter of water.

We inoculated wheat seedlings at the third leaf stage (ZGS 1.3; Zadoks et al. 1974) by infiltrating the most recently emerged leaf with approximately 100 µl of bacterial suspension with a needleless syringe. *X. translucens* pv. *undulosa* strain CIX88 was used as a positive control, and sterile 0.9% NaCl solution was included as a mock-inoculated control. Treatments were replicated six times within the experiment. Inoculated plants were kept in growth chambers at 27°C with a relative humidity of 75% and a 12-h photoperiod. Traits associated with pathogenicity including water soaking, dry necrosis, and no visible symptoms were recorded 12 days postinoculation.

Koch's postulates were accomplished by isolating the infiltrated strains from symptomatic leaves on WBA medium. The identity of the recovered strains was confirmed by morphology assessment and with the real-time PCR protocol. The pathogenicity assays, including recovery of the strains, was performed twice.

RESULTS

Preliminary identification and pathogenic evaluation of *Xanthomonas* strains affecting wheat crops in Uruguay. We obtained the first regional collection of strains of the genus *Xanthomonas* affecting wheat crops by consecutive annual surveys of the cereal-growing regions of Uruguay (Table 1). Although no colonies with the expected morphologies were obtained from samples proceeding from eight of the 61 fields surveyed, 63 xanthomonad-like bacterial strains forming yellow mucoidal colonies on Wilbrink's agar were isolated from symptomatic leaf tissue samples obtained from 53 different fields.

A real-time PCR protocol was implemented for primary identification of *X. translucens* strains (Supplementary Fig. S1). A positive result was obtained for 44 strains, yielding a single amplification product with a melting temperature of 87°C. The amplification was negative for the remaining 19 strains, all of them isolated from symptomatic wheat leaves. Therefore, the 16S rRNA gene region of these non-*translucens* *Xanthomonas* strains was sequenced. According to nucleotide BLAST analysis, the 16S rRNA gene sequences of these 19 strains showed >99% identity with multiple strains of the genus *Xanthomonas*, and thus no species-level identification could be achieved.

Included in the greenhouse pathogenicity assays were 30 strains identified as *X. translucens* with real-time PCR, and 10 non*translucens* strains only identified as *Xanthomonas* spp. with 16S rRNA gene sequencing. All 30 *X. translucens* strains produced expanding water-soaking symptoms that turned dark brown when assessed at

TABLE 2. Pairs of primers used in this study

Primer	Primer sequence	PCR product	Annealing temperature (°C)	Reference
27F	AGAGTTTGATCCTGGCTCAG	16S rRNA	55	Lane 1991
1492R	TACGGYTACCTTGTTACGACTT			
T1	CCGCCATAGGGCGGAGCACCCCGAT	16S-23S rRNA spacer fragment	60	Maes et al. 1996
T2	GCAGGTGCGACGTTTGCAGAGGG ATCTTCTGCAAA			
dnaK-F	TCCTAAGCACCTCAACATCAAG	Chaperone protein	60	Curland et al. 2018; Young et al. 2008
dnaK-R	CTTCTTGTCGTCCTTGACCTC			
fyuA-F	CTCGCAGAACGGCCTGTA	TonB-dependent receptor	60	
fyuA-R	GTAGCCGGGCATCTTCAACT			
gyrB-F	ACGAGTACAACCCGGACAA	DNA gyrase subunit B16S-263	54	
gyrB-R	CCCATCARGGTGCTGAAGAT			
rpoD-F	TGGAACAGGGCTATCTGACC	RNA polymerase sigma factor	60	
rpoD-R	CATTCYAGGTTGGTCTGRTT			

TABLE 3. Accession information including the host, place of origin, and reference for the type, pathotype, and other previously characterized strains of *Xanthomonas* species and pathovars used for comparisons in this study

Species	Pathovar	Strain ID[a]	Host of origin[b]	Place of origin	Reference
X. translucens	*cerealis*	LMG 679[PT]	*Bromus inermis*	United States	Hagborg 1942
X. translucens	*graminis*	LMG 726[PT]	*Dactylis glomerata*	Switzerland	Egli et al. 1975
X. translucens	*secalis*	LMG 883[PT]	*Secale cereale*	Canada	Reddy et al. 1924
X. translucens	*translucens*	LMG 876[T]	*Hordeum vulgare*	United States	Jones et al. 1917
X. translucens	*undulosa*	LMG 892[PT]	*Triticum turgidum*	Canada	Smith et al. 1919
X. translucens	*undulosa*	CIX88	*T. aestivum*	United States	Curland et al. 2018
X. translucens	*undulosa*	XctSD-010	*T. aestivum*	United States	Curland et al. 2018
X. translucens	*undulosa*	CIX128	*T. aestivum*	Minnesota	Curland et al. 2018
X. translucens	*undulosa*	CIX102	*T. aestivum*	Minnesota	Curland et al. 2018
Xanthomonas sp.		CIX89	*T. aestivum*	Minnesota	Curland et al. 2018
Xanthomonas sp.		CIX97	*T. aestivum*	Minnesota	Curland et al. 2018
Xanthomonas sp.		W2.22	*T. aestivum*	Minnesota	Curland et al. 2020
Xanthomonas sp.		W2.24	*T. aestivum*	Minnesota	Curland et al. 2020

[a] PT, pathotype strain; T, type strain.
[b] Plant species from which strain was isolated.

12 days postinoculation. These symptoms were similar to the lesions registered in seedlings inoculated by the *X. translucens* pv. *undulosa* reference strain CIX88 (Fig. 2A and Table 1). Eight non-*translucens* *Xanthomonas* spp. strains caused an expanding dry necrosis on infiltrated leaves, which is distinct from typical BLS symptoms caused by *X. translucens* pv. *undulosa* (Fig. 2B and Table 1). Two non-*translucens* *Xanthomonas* spp. strains, MAI5014 and MAI5032, produced no symptoms on wheat. Likewise, no symptoms developed in the control plants, infiltrated with 0.9% NaCl, in either trial.

MLSA and typing of *X. translucens* strains causing BLS in Uruguay. Of the 44 strains identified as *X. translucens* with species-specific primers, amplification of *gyrB* could not be accomplished for five strains (MAI5004, MAI5006, MAI5053, MAI5056, and MAI5064), and they were therefore excluded from the analysis. MLSA and MLST were performed on the remaining 39 strains. The phylogenetic trees, constructed via maximum likelihood method and Bayesian analysis, had remarkably similar topologies, so only the maximum likelihood trees are shown in this work. All 39 strains isolated from wheat in Uruguay grouped together, along with pathotype strains for *X. translucens* pv. *secalis* LMG883[PT], *X. translucens* pv. *undulosa* LMG892[PT], and *X. translucens* pv. *undulosa* reference strains CIX88, CIX102, CIX128, and XctSD-010 from the United States (Fig. 3A).

Four sequence types (STs) were assigned to the 39 *X. translucens* pv. *undulosa* strains identified (Table 1 and Fig. 3), indicating a low level of polymorphism in the concatenated sequence within the strains in the collection. Only four polymorphic sites were observed: two from *dnaK*, one each from *gyrB* and *rpoD*, and none for *fyuA*. Out of the four STs assigned, three have been previously reported (Supplementary Table S2) (Curland et al. 2018; Khojasteh et al. 2019). The most prevalent ST identified was ST 2 (51.3%), followed by ST 4 (28.2%) and ST 1 (15.4%) (Fig. 3). In this study, a previously unreported ST designated as ST3 was assigned to isolates MAI5034 and MAI5042. This ST is a single-locus variant of ST2 with one sequence variation on *rpoD*. Both strains were isolated in 2018 from fields in different locations (Table 1). Sequence type 4 has one sequence variation on *gyrB*, and ST 1 is also a single-locus variant of ST 2 but with two sequence variations in *dnaK*. The goeBURST analysis performed predicted ST 2 to be the founding genotype, and because all sequence types assigned in this work are single-locus variants of ST 2, strains form only one clonal complex (Fig. 3B).

Phylogenetic identification of non-*translucens* *Xanthomonas* strains isolated from wheat crops. Non-*translucens* *Xanthomonas* spp. strains were also subjected to MLSA. Amplification of *dnaK*, *gyrB*, and *rpoD* was successful, but for *fyuA* it resulted in multiple PCR products of varied sizes, so this gene was excluded from the

analysis (Table 1). Both nonpathogenic strains (MAI5014 and MAI5032) grouped together and most closely to *X. arboricola* pv. *juglandis* ICMP35[T] and *X. arboricola* pv. *pruni* ICMP51[PT] (Fig. 4).

The remaining 17 strains (Fig. 4, green highlight), including all eight strains that caused dry necrosis on wheat, grouped together and with four strains (CIX89, CIX97, W2.22, and W2.24) isolated from wheat in Minnesota (Curland et al. 2018, 2020). These strains grouped most closely with the recently described nectarine pathogen *X. prunicola* CFBP 8353[T] (López et al. 2018). *X. translucens* pv. *translucens* reference strain LMG 876[T] (Fig. 4, purple highlight) did not group with any strains included in this analysis and branched outside the *X. translucens* pv. *undulosa* clades.

DISCUSSION

Although BLS of wheat has been an issue of increasing relevance in Uruguay in the last decade, this study presents the first report in South America of the genetic diversity of *X. translucens*. This work also resulted in the establishment of the first collection of strains of the genus *Xanthomonas* affecting wheat crops in Uruguay. We also described the phylogenetic relation of these strains to reference strains within the genus, specifically within the *X. translucens* group.

The MLSA scheme based on *dnaK*, *fyuA*, *gyrB*, and *rpoD* has previously been used to assess the genetic diversity of the genus *Xanthomonas* (Young et al. 2008) and of the *X. translucens* group. It was applied effectively to distinguish *X. translucens* strains isolated from wheat and barley in the Midwest of the United States and in Iran, as well as successfully predicting the pathovar of strains as *X. translucens* pv *translucens*, *X. translucens* pv. *undulosa*, and *X. translucens* pv. *cerealis* (Curland et al. 2018, 2020; Khojasteh et al. 2019). The phylogenies produced by this MLSA scheme have proven to be consistent with the previously defined pathovars, although it does not allow clear differentiation between *X. translucens* pv. *undulosa* and *X. translucens* pv. *secalis*. In this study, this scheme was used to characterize 39 strains identified as *X. translucens*. Combined with the groupings of the MLSA phylogenies, all strains producing brown necrosis on wheat were identified as *X. translucens* pv. *undulosa*, which is in accordance with most reports indicating that *X. translucens* pv. *undulosa* is the prevalent pathovar affecting wheat crops (Adhikari et al. 2011; Curland et al. 2018; Khojasteh et al. 2019). In Uruguay, *X. translucens* pv. *translucens* was not detected in wheat, although it has been isolated from barley (data not shown).

MLST allows a more in-depth look at the genetic diversity within the local population of *X. translucens* strains. The diversity estimates for each locus and for the concatenated sequences of the strains examined in this study revealed a low genetic diversity and high
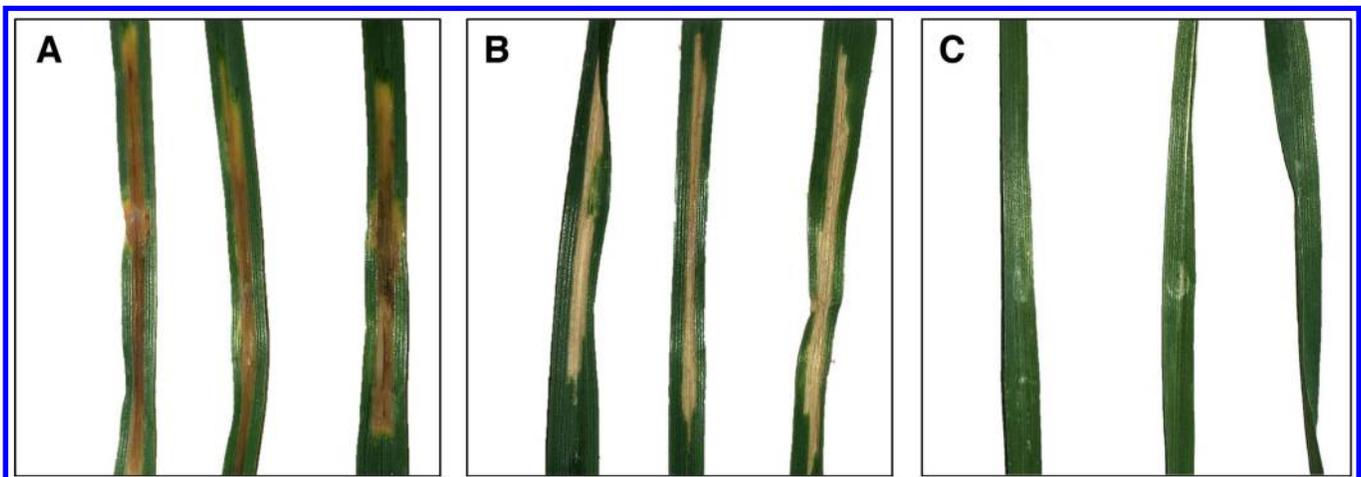

Fig. 2. Types of symptoms elicited by representative *Xanthomonas* pathogenic strains on wheat (cultivar Tijereta) 12 days after leaf infiltration. **A,** Brown necrosis (*X. translucens* strain MAI5067). **B,** Dry necrosis (*Xanthomonas* sp. MAI5069). **C,** No symptoms (mock-inoculated control, infiltrated with sterile saline solution).

homogeneity among the Uruguayan *X. translucens* strains. This was made evident by the low amount of polymorphism in the analyzed loci: only four polymorphic sites in 2,645 bp of the concatenated sequence. In contrast, there were 14 polymorphic sites in 2,463 bp among 58 Iranian *X. translucens* pv. *undulosa* strains and 15 polymorphic sites among 75 non-Iranian *X. translucens* pv. *undulosa* strains (Khojasteh et al. 2019). Although *X. translucens* pv. *undulosa* has been demonstrated to be a group with low genetic diversity compared with *X. translucens* pv. *translucens*, the percentage of polymorphic sites for all four loci evaluated was even lower for Uruguayan *X. translucens* pv. *undulosa* strains than for those reported in the Midwest of the United States and Iran. Factors such as the main wheat cultivars used, the homogeneity of the weather conditions across the wheat production region in Uruguay, and the smaller surface area destined for wheat production in this region than those of both Iran and the Upper Midwest of the United States may partially explain these differences. Furthermore, the strains present in Uruguay were assigned to only four different STs. Most of these isolates (37/39) were assigned to STs previously reported in other

regions, which suggests that they may have been introduced through the importation of infected seeds. The most prevalent ST among the strains isolated from wheat in Uruguay is ST2, which is coincidentally the most prevalent ST reported in Iran and North America, and it is also the ST of pathotype strain *X. translucens* pv. *undulosa* LMG 892[PT]. ST2 was predicted to be the founding genotype of *X. translucens* pv. *undulosa*, as has been previously reported (Curland et al. 2018, 2020; Khojasteh et al. 2019).

There was no significant correlation between the *X. translucens* pv. *undulosa* strains' assigned STs and their place of origin ($P = 0.138$), their collection date ($P = 0.161$), or the wheat cultivars from which they were isolated ($P = 0.361$). Diversity of STs of *X. translucens* pv. *undulosa* within fields has been previously reported (Curland et al. 2020). This may imply that if seeds were the main source of inoculum for *X. translucens* pv. *undulosa*, there could be multiple strains present in infested seed lots. Additionally, weedy grass species in and around wheat fields have been shown to host a diverse range of STs of *X. translucens* pv. *undulosa*, indicating there may be multiple sources of inoculum contributing to BLS
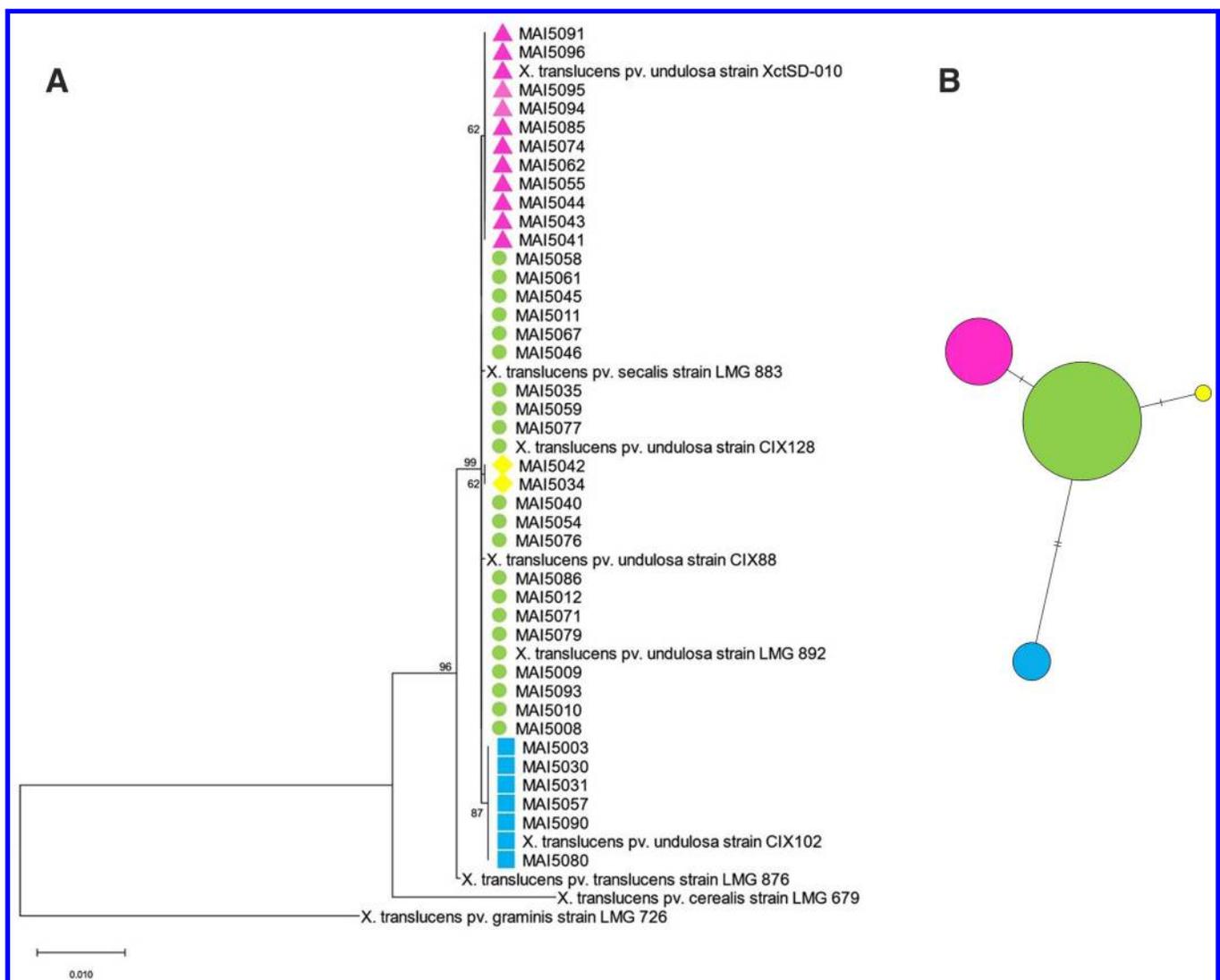

Fig. 3. A, Maximum likelihood tree based on concatenation of partial sequences of *dnaK*, *fyuA*, *gyrB*, and *rpoD* genes for *Xanthomonas translucens* strains isolated from wheat crops in Uruguay. Only bootstrap values >50% are displayed at each node. Sequence types assigned to each strain are represented by colored shapes (ST 1, blue square; ST 2, green circle; ST 3, yellow diamond; ST 4, pink triangle). Type and pathotype strains for pathovars within *X. translucens* species causing cereal leaf streak (LMG 679 PT, LMG 876 T, LMG 883 PT, and LMG 892 PT) are included. Strains reported by Curland et al. (2018) as *X. translucens* pv. *undulosa* showing the same sequence type as local strains (XctSD-010, CIX128, CIX102) and the reference strain used in pathogenicity assays (CIX88) are included. LMG 726 PT, the pathotype strain for *X. translucens* pv. *graminis*, was used as an outgroup to root the tree. The bar presents numbers of substitutions per site (0.010). B, Minimum spanning tree depicting the single clonal complex for the 39 *X. translucens* pv. *undulosa* strains isolated in Uruguay. The sequence types assigned are represented as colored circles (ST 1, blue; ST 2, green; ST 3, yellow; ST 4, pink). The size of the circles represents the relative frequency of the particular ST. The hatch marks along the branches connecting STs represents the number of mutations.
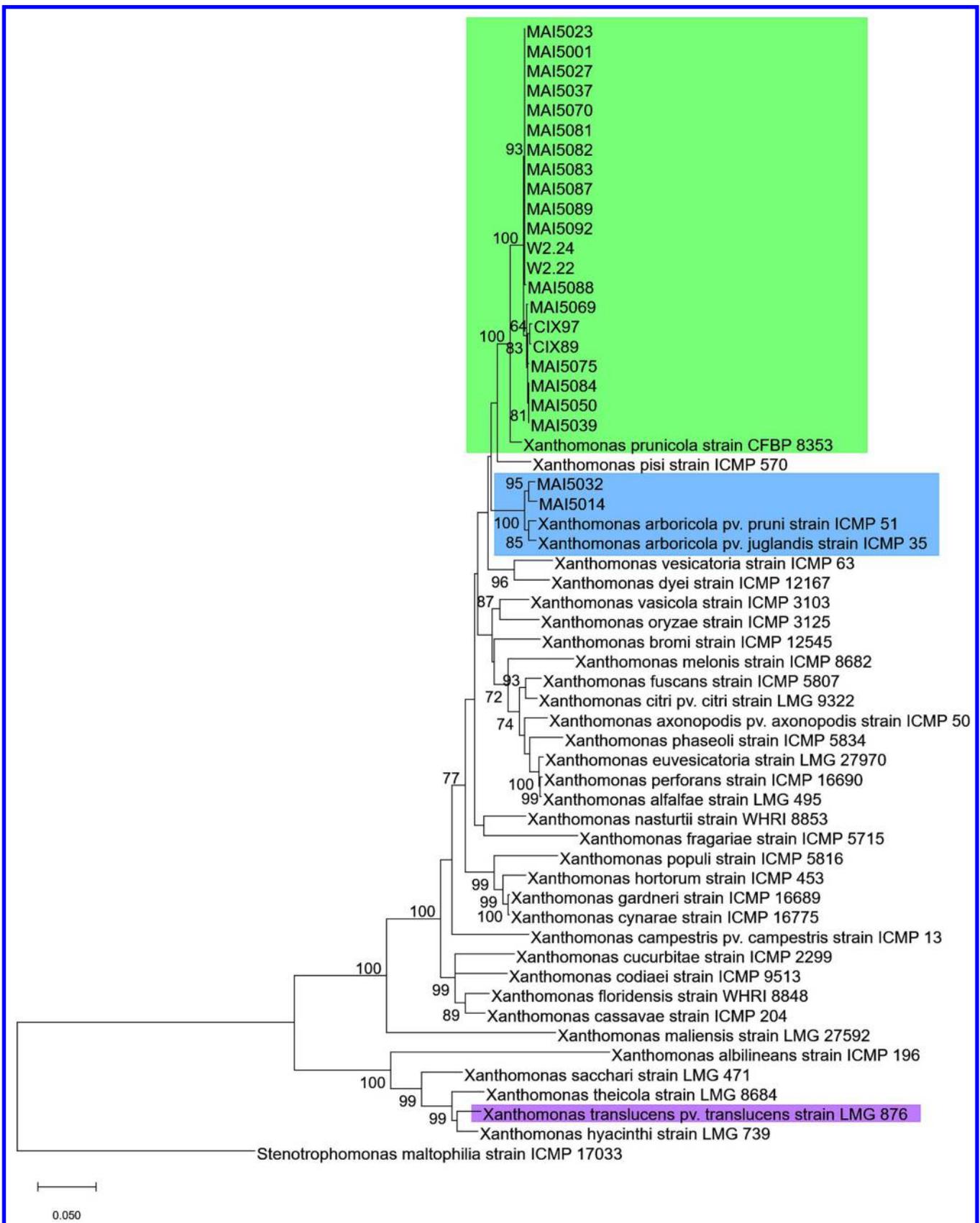
Fig. 4. Maximum likelihood tree based on concatenation of partial sequences of *dnaK*, *gyrB*, and *rpoD* genes of pathogenic (green) and nonpathogenic (blue) non-translucens *Xanthomonas* spp. strains isolated from wheat in Uruguay. Only bootstrap values >70% are displayed at each node. Type and pathotype strains for all currently reported species within the *Xanthomonas* genus were included, with *X. translucens* pv. *translucens* reference strain highlighted in purple. Strains CIX89, CIX 97, W2.22, and W2.24, isolated from wheat in Minnesota, grouped with the pathogenic *Xanthomonas* sp. strains identified and were likewise included. *Stenotrophomonas maltophilia* was added as an outgroup to root the tree. The bar presents numbers of substitutions per site.

outbreaks (Ledman et al. 2021). Weedy or cultivated grasses and residues of wheat crops could potentially serve as pathogen reservoirs, although this possibility has not been thoroughly investigated in Uruguay.

Nineteen *Xanthomonas* spp. strains that do not belong to the *X. translucens* species were isolated from wheat fields in Uruguay. The adapted MLSA scheme produced a phylogenetic analysis based on three genes that showed that two of these strains, which generated no symptoms on wheat, grouped most closely with the type strains for pathovars of *X. arboricola*. Pathovars *celebensis*, *fragariae*, and *populi*, as well as other non-pathovar-assigned strains within that species, have been considered to be nonpathogenic or opportunistic pathogens, and some strains have been found coinhabiting the same host plant with pathogenic strains (Garita-Cambronero et al. 2018). Nonpathogenic or low-virulence *X. arboricola* strains have been isolated from a wide range of host plants, including barley, but there are no published reports of *X. arboricola* being isolated from wheat (Garita-Cambronero et al. 2017, 2018). However, a study has shown that a pathogenic strain of *X. arboricola* pv. *pruni* is able to grow epiphytically on wheat and other nonhost plants when inoculated (Zarei et al. 2018).

Remarkably, the 17 remaining non-*translucens Xanthomonas* sp. strains that caused atypical dry necrosis symptoms clustered together in a single group along with four previously unreported strains from the United States (Fig. 4). In greenhouse pathogenicity assays, the four U.S. strains caused dry necrosis symptoms that were similar to those observed with the 17 strains from Uruguay (data not shown). The closest species to this group is *X. prunicola*, a nectarine pathogen recently reported in Spain (López et al. 2018). The symptoms caused by these *Xanthomonas* sp. strains on wheat are clearly different from BLS symptoms caused by *X. translucens* pathovars, and they are phylogenetically distinct from the *X. translucens* group. The isolation of similar strains occurring across such large distances suggests that this yet-to-be defined group of strains may be distributed in association with wheat worldwide. Locally, farmers and agronomists have regularly reported symptoms related to drying of leaves in wheat fields, with frequent exudates, which could be attributed to these strains. Further research is needed to establish the prevalence, severity, and yield losses associated with these strains.

This study shows that strains identified as *X. translucens* are clearly associated with BLS outbreaks in wheat fields in Uruguay, with all regional strains identified as *X. translucens* grouping together in the *X. translucens* pv. *undulosa* clade. Generating information and awareness on the genetic diversity and structure of local populations of this pathogen is essential to determine main sources of inoculum and to manage this disease. This new evidence, along with the newly characterized collection of strains from Uruguay, can be useful to incorporate genetic resistance to BLS in lines of the national wheat breeding program, because this strategy remains the most effective approach to mitigate BLS. Additionally, there is evidence that another potential pathogen is widely associated with wheat in Uruguay and is also present in the United States. Further studies may reveal the role of this pathogen in wheat production, both locally and globally.

Data availability. Nucleotide sequence data reported are available in GenBank database under accession numbers MZ196391 to MZ196409 and MZ201068 to MZ201301.

LITERATURE CITED

Adhikari, T. B., Hansen, J. M., Gurung, S., and Bonman, J. M. 2011. Identification of new sources of resistance in winter wheat to multiple strains of *Xanthomonas translucens* pv. *undulosa*. Plant Dis. 95:582-588.

Altier, N. 1996. Desarrollo de epidemias: Análisis de sus componentes como base para un controlracional. Pages 1-9 in: Manejo de Enfermedades en Cereales de Invierno y Pasturas. M. Díaz de Ackerman, ed. INIA La Estanzuela, Uruguay.

Bragard, C., Singer, E., Alizadeh, A., Vauterin, L., Maraite, H., and Swings, J. 1997. *Xanthomonas translucens* from small grains: Diversity and phytopathological relevance. Phytopathology 87:1111-1117.

Castro, M., Germán, S., and Pereyra, S. 2020. Caracterización sanitaria de cultivares de trigo y cebada. http://www.inia.uy/Documentos/P%C3%BAblicos/INIA%20La%20Estanzuela/Actividades%202020/caracterizaci%C3%B3n%20sanitaria%20trigo%20y%20cebada%202020.pdf

Croce, V., Pianzzola, M. J., Durand, K., González-Arcos, M., Jacques, M. A., and Siri, M. I. 2016. Multilocus sequence typing reveals high variability among *Clavibacter michiganensis* subsp. *michiganensis* strains affecting tomato crops in Uruguay. Eur. J. Plant Pathol. 144:1-13.

Curland, R. D., Gao, L., Bull, C. T., Vinatzer, B. A., Dill-Macky, R., Van Eck, L., and Ishimaru, C. A. 2018. Genetic diversity and virulence of wheat and barley strains of *Xanthomonas translucens* from the upper midwestern United States. Phytopathology 108:443-453.

Curland, R. D., Gao, L., Hirsch, C. D., and Ishimaru, C. A. 2020. Localized genetic and phenotypic diversity of *Xanthomonas translucens* associated with bacterial leaf streak on wheat and barley in Minnesota. Phytopathology 110:257-266.

Darriba, D., Taboada, G. L., Doallo, R., and Posada, D. 2012. jModelTest 2: More models, new heuristics and parallel computing. Nat. Methods 9:772.

Duveiller, E., Fucikovsky, L., and Rudolph, K. 1997. The Bacterial Diseases of Wheat: Concepts and Methods of Disease Management. CIMMYT, Mexico. http://hdl.handle.net/10883/1227

Egli, T. T., Goto, M., and Schmidt, D. 1975. Bacterial wilt, a new forage grass disease. J. Phytopathol. 82:111-121.

Egorova, M. S., Mazurin, E. S., Polityko, V. A., and Ignatov, A. N. 2014. Diversity of phytopathogenic bacteria of genus *Xanthomonas* isolated from Poaceae plants in Russia. RUDN J. Agron. Anim. Ind. 4:47-53.

Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P., and Spratt, B. G. 2004. eBURST: Inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing data. J. Bacteriol. 186:1518-1530.

Font, V., Fern, J., Bianchi, M., De Le, F., and Ortiz, A. 2019. Anuario Estadístico Agropecuario 2019. https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2019/Anuario2019.pdf

Font, V., Fern, J., Bianchi, M., De Le, F., and Ortiz, A. 2020. Anuario Estadístico Agropecuario 2020. https://descargas.mgap.gub.uy/DIEA/Anuarios/Anuario2020/ANUARIO2020.pdf

Forster, R. L., and Schaad, N. W. 1988. Control of black chaff of wheat with seed treatment and a foundation seed health program. Plant Dis. 72:935-938.

Francisco, A. P., Bugalho, M., Ramirez, M., and Carriço, J. A. 2009. Global optimal eBURST analysis of multilocus typing data using a graphic matroid approach. BMC Bioinformatics 10:152.

Frommel, M. I. 1986. *Xanthomonas campestris* pv. *translucens* (J.J. y R.) DYE, agente causal de la estría bacteriana del trigo (*T. aestivum* L.) en el Uruguay. Dirección de Sanidad Vegetal, Montevideo, Uruguay.

Garita-Cambronero, J., Palacio-Bielsa, A., and Cubero, J. 2018. *Xanthomonas arboricola* pv. pruni, causal agent of bacterial spot of stone fruits and almond: Its genomic and phenotypic characteristics in the *X. arboricola* species context. Mol. Plant Pathol. 19:2053-2065.

Garita-Cambronero, J., Palacio-Bielsa, A., López, M. M., and Cubero, J. 2017. Pan-genomic analysis permits differentiation of virulent and nonvirulent strains of *Xanthomonas arboricola* that cohabit *Prunus* spp. and elucidate bacterial virulence factors. Front. Microbiol. 8:1-17.

Gu, L., Bai, Z., Jin, B., Hu, Q., Wang, H., Zhuang, G., and Zhang, H. 2010. Assessing the impact of fungicide enostroburin application on bacterial community in wheat phyllosphere. J. Environ. Sci. (China) 22:134-141.

Guindon, S., and Gascuel, O. 2003. A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol. 52:696-704.

Hagborg, W. A. F. 1942. Classification revision in *Xanthomonas translucens*. Can. J. Res. 20c:312-326.

Jones, L. R., Johnson, A. G., and Reddy, C. S. 1917. Bacterial blight of barley. J. Agric. Res. 11:625-643.

Kandel, Y. R., Glover, K. D., Tande, C. A., and Osborne, L. E. 2012. Evaluation of spring wheat germplasm for resistance to bacterial leaf streak caused by *Xanthomonas campestris* pv. *translucens*. Plant Dis. 96:1743-1748.

Khojasteh, M., Taghavi, S. M., Khodaygan, P., Hamzehzarghani, H., Chen, G., Bragard, C., Koebnik, R., and Osdaghi, E. 2019. Molecular typing reveals high genetic diversity of *Xanthomonas translucens* strains infecting small-grain cereals in Iran. Appl. Environ. Microbiol. 85:1-15.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Mol. Biol. Evol. 35:1547-1549.

Lane, D. J. 1991. 16S/23S rRNA sequencing. Pages 115-175 in: Nucleic Acid Techniques in Bacterial Systematics. E. Stackebrandt and M. Goodfellow, eds. Wiley, New York. https://books.google.com.uy/books?id=89PwAAAAMAAJ

Langlois, P. A., Snelling, J., Hamilton, J. P., Bragard, C., Koebnik, R., Triplett, L. R., Blom, J., Tisserat, N. A., Leach, J. E., Verdier, V., Triplett, L. R., Blom, J., Tisserat, N. A., and Leach, J. E. 2017. Characterization of the *Xanthomonas translucens* complex using draft genomes, comparative

genomics, phylogenetic analysis, and diagnostic LAMP assays. Phytopathology 107:519-527.

Ledman, K. E., Curland, R. D., Ishimaru, C. A., and Dill-Macky, R. 2021. *Xanthomonas translucens* pv. *undulosa* identified on common weedy grasses in naturally infected wheat fields in Minnesota. Phytopathology 111:1114-1121.

Librado, P., and Rozas, J. 2009. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25:1451-1452.

López, M. M., Lopez-Soriano, P., Garita-Cambronero, J., Beltrán, C., Taghouti, G., Portier, P., Cubero, J., Le Saux, M. F., and Marco-Noales, E. 2018. *Xanthomonas prunicola* sp. nov., a novel pathogen that affects nectarine (*Prunus persica* var. nectarina) trees. Int. J. Syst. Evol. Microbiol. 68:1857-1866.

Maes, M., Garveva, P., and Kamoen, O. 1996. Recognition and detection in seed of the *Xanthomonas* pathogens that cause cereal leaf streak using rDNA spacer sequences and polymerase chain reaction. Phytopathology 86:63.

Maiden, M. C. J., Bygraves, J. A., Feil, E., Morelli, G., Russell, J. E., Urwin, R., Zhang, Q., Zhou, J., Zurth, K., Caugant, D. A., Feavers, I. M., Achtman, M., and Spratt, B. G. 1998. Multilocus sequence typing: A portable approach to the identification of clones within populations of pathogenic microorganisms. Proc. Natl. Acad. Sci. 95:3140-3145.

Murray, G. M., and Brennan, J. P. 2009. Estimating disease losses to the Australian wheat industry. Australas. Plant Pathol. 38:558-570.

Pereyra, S. 2013. Nuevos desafíos para el manejo de enfermedades en cereales de invierno. In 3rd National Agriculture Symposium (Facultad D). E. Hoffmann, A. Ribeiro, O. Ernst, and F. García, eds. Hemisferio Sur, Buenos Aires, Argentina.

Pereyra, S., Germán, S., and Díaz, M. 2011. Del patógeno al cultivo: Sus interacciones y alternativas de manejo en la producción de trigo y cebada. Pages 89–110 in: 2nd National Agriculture Symposium. E. Hoffman, A. Ribeiro, O. Ernst, and F. García, eds, Hemisferio Sur, Buenos Aires, Argentina.

Pérez-Losada, M., Cabezas, P., Castro-Nallar, E., and Crandall, K. A. 2013. Pathogen typing in the genomics era: MLST and the future of molecular epidemiology. Infect. Genet. Evol. 16:38-53.

Rademaker, J. L. W. W., Norman, D. J., Forster, R. L., Louws, F. J., Schultz, M. H., De Bruijn, F. J., and De Bruijn, F. J. 2006. Classification and identification of *Xanthomonas translucens* isolates, including those pathogenic to ornamental asparagus. Phytopathology 96:876-884.

Raja, N. I., Rashid, H., Khan, M. H., Chaudhry, Z., Shah, M., and Bano, A. 2010. Screening of local wheat varieties against bacterial leaf streak caused by different strains. Pak. J. Bot. 42:1601-1612.

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., and Suchard, M. A. 2018. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. Syst. Biol. 67:901-904.

Reddy, C. S., Godkin, J., and Johnson, A. G. 1924. Bacterial blight of rye. J. Agric. Res. 28:1039. https://naldc.nal.usda.gov/download/IND43966840/PDF

Sambrook, J. F., and Russell, D. 2001. Molecular Cloning: A Laboratory Manual. Vol. 3. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sands, D. C., and Fourrest, E. 1989. *Xanthomonas campestris* pv. *translucens* in North and South America and in the Middle East. EPPO Bull. 19:127-130.

Sands, D. C., Mizrak, G., Hall, V. N., Kim, H. K., Bockelman, H. E., and Golden, M. J. 1986. Seed transmitted bacterial diseases of cereals: Epidemiology and control. Arab J. Plant Prot. 4:127-125.

Sapkota, S., Mergoum, M., & Liu, Z. 2020. The translucens group of *Xanthomonas translucens*: Complicated and important pathogens causing bacterial leaf streak on cereals. Mol. Plant Pathol. 21:1-12.

Smith, E. F., Jones, L. R., and Reddy, C. S. 1919. The black chaff of wheat. Science 50:48. https://pubmed.ncbi.nlm.nih.gov/17801665/

Suchard, M. A., Lemey, P., Baele, G., Ayres, D. L., Drummond, A. J., and Rambaut, A. 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. Virus Evol. 4:vey016.

Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45:472-489.

Young, J. M., Park, D. C., Shearman, H. M., and Fargier, E. 2008. A multilocus sequence analysis of the genus *Xanthomonas*. Syst. Appl. Microbiol. 31:366-377.

Zacaroni, A. B., Koike, S. T., de Souza, R. M., and Bull, C. T. 2012. Bacterial leaf spot of radicchio (*Cichorium intybus*) is caused by *Xanthomonas hortorum*. Plant Dis. 96:1820.

Zadoks, J. C., Chang, T. T., and Konzak, C. F. 1974. A decimal code for the growth stages of cereals. Weed Res. 14:415-421.

Zarei, S., Taghavi, S. M., Hamzehzarghani, H., Osdaghi, E., and Lamichhane, J. R. 2018. Epiphytic growth of *Xanthomonas arboricola* and *Xanthomonas citri* on non-host plants. Plant Pathol. 67:660-670.