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Research Paper

Isolation of atypical wheat-associated xanthomonads in Algeria

Bahria KHENFOUS-DJEBARI[1,*], Mohamed KERKOUD[2], Marion FISCHER-LE SAUX[3], Traki BENHASSINE[4], Zouaoui BOUZNAD[1], Ralf KOEBNIK[5], Claude BRAGARD[6]

[1] *Laboratoire de Phytopathologie et de Biologie Moléculaire, Département de Botanique, ENSA El Harrach, Alger, Algérie*
[2] *DiagGene, 8, Rue le Nôtre 49066 Angers, France*
[3] *IRHS, INRA, Agrocampus-Ouest, Université d'Angers, SFR 4207 QuaSaV, 49071, Beaucouzé, France*
[4] *Département de Biologie Cellulaire et de Biologie Moléculaire, USTHB, Bab Ezzouar, Alger, Algerie*
[5] *IRD, Cirad, Université de Montpellier, IPME, Montpellier, France*
[6] *Applied Microbiology-Phytopathology, Earth & Life Institute, UC Louvain, Louvain-la-Neuve, Belgium*
*Corresponding author: b.djebari@ensa.dz

Summary. Bacterial leaf streak and black chaff are important bacterial diseases of wheat, which have been reported to be caused by *Xanthomonas translucens*. In 2016, symptoms of bacterial leaf streak and black chaff were observed in Algeria, at experimental wheat breeding stations and in farmers' fields under sprinkler irrigation on two wheat cultivars, 'Hiddab' and 'Simeto'. Yellow *Xanthomonas*-like bacterial colonies were isolated from plant material, including leaves, spikes and post-harvest crop residues. Initial characterisation using biochemical, physiological and pathogenicity tests identified the bacteria as *Xanthomonas*. Diagnostic PCR targeting the 16S-23S rRNA intergenic region indicated that the strains were *X. translucens*, a clade-1 xanthomonad. However, partial DNA sequences of the housekeeping genes *gyrB* and *rpoD* revealed that the strains belong to clade 1, but likely represent a new *Xanthomonas* species that has not been previously described on wheat or other *Gramineae*. The most closely related strain, NCPPB 2654, was isolated from a bean plant in the United Kingdom in 1974. Further characterization is required to clarify the taxonomic status of the Algerian *Xanthomonas* isolates from wheat, and to determine their host ranges and impacts on plant cultivation.

Keywords. *Xanthomonas*, bacterial leaf streak, black chaff, wheat.

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INTRODUCTION

Wheat is a major crop worldwide, affected by some bacterial diseases, among which bacterial leaf streak (BLS) is the most important. BLS is caused by *Xanthomonas translucens* (ex Jones *et al.* 1917) (Vauterin *et al.*, 1995). When symptoms occur on the wheat plant glumes, the disease caused by

X. translucens is called black chaff (BC) (Duveiller *et al*., 1997). BLS is widely distributed in the world and the disease is prevalent in most regions where small-grain cereals are cultivated (Paul and Smith, 1989; Duveiller and Maraite, 1994). However, these diseases have not been extensively studied in North Africa, despite sporadic reports of occurrence in countries close to Algeria, including Libya (Bragard *et al*., 1995), Morocco and Tunisia (Sands and Fourest, 1989).

Yield losses caused by BLS are generally not considered problematic. However, losses of up to 40% have been recorded under conditions that are conducive for the pathogen (Duveiller *et al*., 1997). Algeria cultivates cereals on an area of 3.3 million ha, 1.3 million of which are irrigated and thus likely to be vulnerable to BLS. Furthermore, more than 20% of yield may be lost if 50% of flag leaf area is affected by the disease (Duveiller and Maraite, 1993). BLS generally appears late in the growing season in regions with temperate climate or in warmer environments characterized by cool nights and frequent temperature variations (Duveiller *et al*., 2002). The symptoms are usually more obvious after heading (Wiese, 1987). All aerial parts of host plants can be affected, but the leaves and glumes are more often affected than the other parts. In cases of severe damage, the seeds can be blackened and wrinkled (Zillinsky, 1983). BC is characterized by many black longitudinal stripes on the upper portions of the glumes (Smith, 1917), that can be identified by greasy appearance. Yellow bacterial droplets exude along the lesions, particularly in wet weather.

Xanthomonas species are known for their ability to adhere to and colonize host leaf surfaces as epiphytes before invading the intercellular spaces (Boulanger *et al*., 2014; Dutta *et al*., 2014; Zarei *et al.,* 2018). Xanthomonads have evolved several strategies for successful infection, including mechanisms to suppress host plant resistance and access nutrients from host cells (Büttner *et al.,* 2010; Fatima and Senthil-Kumar, 2015; Jacques *et al*., 2016). However, much less is known for *X. translucens*, which belongs to xanthomonad clade 1, while most functional research has been carried out with clade-2 xanthomonads (Parkinson *et al*., 2007). The importance of the bacterial type III secretion system and TAL effectors for pathogenicity has been demonstrated (Wichmann *et al*., 2013; Peng *et al*., 2016; Falahi Charkhabi *et al*., 2017; Pesce *et al.,* 2017).

Since no data are available on bacterial diseases of small grain cereals in Algeria, the objective of this study was to assess the presence of wheat-associated xanthomonads in this country, to verify their ability to cause BLS on cereals and to determine their identity. This research was based on morphological, biochemical and physiological characterization of bacterial isolates, complemented by pathogenicity tests on host plants and DNA-based molecular diagnostics.

MATERIALS AND METHODS

Bacterial strains

Bacteria were isolated from symptomatic wheat leaf and spike samples and from post-harvest plant residues originating from fields that had shown symptoms of BLS and BC in the previous growing season (Table 1) (Kar-

Table 1. Details of Algerian bacterial isolates obtained from wheat.

Isolate	Gene	GenBank Accession number	Sample	Symptoms[a]	Wheat cultivar	Location	Year
X1	*gyrB* *rpoD*	MF142045 MF142046	Leaf	BLS	'Simeto'	El Goléa	2016
X2	*gyrB*	MF142047	Leaf	BLS	'Simeto'	El Goléa	2016
X3	*gyrB*	MF142048	Leaf	BLS	'Simeto'	El Goléa	2016
X4	*gyrB*	MF142049	Leaf	BLS	'Simeto'	El Goléa	2016
X5	*gyrB*	MF142050	Crop residue	No visible symptoms	'Hiddab' (HD1220)	Algiers experimental station	2016
X8	*gyrB* *rpoD*	MF142051 MF142052	Spike	BC	'Simeto'	El Goléa	2016
X12	*gyrB* *rpoD*	MF142053 MF142054	Crop residue	No visible symptoms	'Hiddab' (HD1220)	Algiers experimental station	2010
X13	*gyrB* *rpoD*	MF142055 MF142056	Crop residue	No visible symptoms	'Hiddab' (HD1220)	Algiers experimental station	2010
X16	*gyrB*	MF142057	Spike	BC	Breeding line	Algiers experimental station	2016
X17	*gyrB*	MF142058	Spike	BC	Breeding line	Algiers experimental station	2016

[a]BLS = bacterial leaf streak, BC = black chaff.

avina *et al.*, 2008). Samples (10 g each) of plant organs or post-harvest stubble residues were each ground to a fine powder and mixed with 50 mL phosphate-buffered saline (PBS). The resulting cell suspension was shaken for 20 min and allowed to settle for 3 h. Series of ten-fold dilutions were prepared from the suspension supernatants and appropriate amounts of each dilution were plated on standard nutrient agar medium and on semi-selective Wilbrink's medium supplemented with 0.75 g L^{-1} of boric acid, 10 mg L^{-1} of cephalexin and 75 mg L^{-1} of cycloheximide (WBC medium) to reduce fungal growth (Duveiller, 1990). For comparison, *X. translucens* pv. *undulosa* strain UPB753, which had been isolated from wheat in Brazil (Bragard *et al.*, 1995), was used as a reference strain. Pure cultures were obtained upon cultivation at 30°C. Based on morphological characteristics, *Xanthomonas*-like bacterial strains were assigned a designation number and kept at 4°C on glucose, yeast extract-calcium carbonate agar (GYCA) tubes for short-term conservation and at -80°C in 20% glycerol for long-term storage.

Phenotypic characterization

Established tests were used to identify the bacteria (Bradbury, 1986; Van den Mooter and Swings, 1990; Schaad *et al.*, 2001). These included formation of mucoid colonies on GYCA medium, Gram reaction using the KOH test, oxidase test using tetramethyl-*p*-phenylenediamine dihydrochloride reagent, oxidative and fermentative metabolism of glucose, hydrolysis of Tween 80, aesculin and starch, production of levane sucrase, production of catalase, liquefaction of gelatine, H_2S production from cysteine, nitrate reduction, growth at 35°C, and growth on 2% and 5% NaCl media.

DNA manipulations

For rapid tests, bacterial cells were lysed by brief boiling and then directly used for PCR, as described previously (Maes *et al.*, 1996). For PCR amplification of housekeeping genes, DNA was isolated using the Wizard® Genomic DNA Purification Kit (Promega Corp.), following the manufacturer's instructions.

PCR amplification of the alanine-specific tRNA gene in the 16S-23S rRNA intergenic region was performed as described previously (Maes *et al.*, 1996), with modifications. The reaction mixture was prepared in a total volume of 25 µL comprising 5 µL of 5 × PCR buffer, 2.5 µL $MgCl_2$ (25 mM), 0.75 µL dNTP mixture (25 mM), 0.5 µL of each PCR primer at 10 µM (T1, 5'-CCGCCATAGGGCGGAGCACCCCGAT; T2, 5'-GCAGGTGCGACGTTTGCAGAGGGATCTGCAAATC), 2.5 µL DNA sample (50 ng μL^{-1}), 0.2 µL Taq polymerase (Promega), and 13.05 µL distilled water. PCR was performed with the following conditions: 90°C for 2 min, 29 cycles of 93°C for 30 s, 50°C for 30 s, 72°C for 30 s, and a final extension for 10 min at 72°C. The PCR products were separated by electrophoresis on 2% agarose gel in TAE buffer, stained with Midori green (Nippon Genetics Europe) and visualized under UV light.

Previously published MLSA primers were used for PCR amplification and partial DNA sequencing of two housekeeping genes, *gyrB* and *rpoD* (Fargier and Manceau, 2007). PCR amplifications were performed as recommended (Mhedbi-Hajri *et al.*, 2013) in a 50 µL reaction mixture containing 1 × GoTaq® buffer, 200 µM dNTP, 0.5 µM of each primer, 0.4 U of GoTaq® DNA polymerase (Promega), and 3 ng of genomic DNA, with an initial denaturation at 94°C for 2 min, 30 cycles of denaturation for 1 min at 94°C, annealing for 1.5 min at 60°C, extension for 1.5 min at 72°C, and a final extension for 10 min at 72°C. 8 µL of PCR products reaction mixtures were analysed by electrophoresis on 1.5% agarose gel in TAE buffer, stained with Midori green direct and visualized under UV light. The remaining amplified PCR products were purified with the Wizard® PCR clean-up kit (Promega) and sequenced with reverse and forward primers using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems). Both forward and reverse sequences were aligned and manually edited before deposition in the GenBank database (Table 1).

Pathogenicity tests

Bacterial cells were suspended in sterile solution of 0.90% (w/v) of NaCl and the concentration of cells was adjusted to 1×10^9 CFU mL^{-1} for hypersensitive reaction tests on tobacco plants of the variety Xanthi and to 1×10^7 CFU mL^{-1} for pathogenicity tests. Sterile saline solution served as negative controls in the pathogenicity assays.

All strains were tested for pathogenicity, by inoculation on the sensitive wheat cultivar 'Acsad 885', using three different assays. First, after injection of sterile water into plant leaf sheath at 2.5 cm above soil level, three-leaf stage seedlings were puncture inoculated with a sterile needle that had been passed through a bacterial colony ("pricking inoculation") (Bragard and Maraite, 1992). Second, using a needle-less plastic syringe, bacterial suspensions were infiltrated through the upper leaf surfaces until appearance of liquid-soaked areas of about 2 cm length ("leaf infiltration") (Bragard and Maraite, 1992). Third, whole leaves were immersed into bacterial

solutions for 20 sec ("dip inoculation") (Darsonval *et al.*, 2009). Plants were incubated at 28°C and 95% relative humidity with a photoperiod of 16h/8h (day/night), and symptoms were scored over time.

To re-isolate bacteria from infected plant material, symptomatic leaf segments were cut into small pieces in sterile physiological saline and plated on standard and semi-selective media.

Bioinformatic analyses

To link the different strains with their respective taxa among the species of *Xanthomonas*, corresponding *gyrB* and *rpoD* gene portions were retrieved from GenBank and PAMDB databases (https://www.ncbi.nlm.nih.gov, http://www.pamdb.org) (Almeida *et al.*, 2010; Sayers *et al.*, 2019). For recently described species and pathovars that are not represented in PAMDB, such as "*Xanthomonas pseudalbilineans*", *X. maliensis*, *X. floridensis*, *X. nasturtii*, and *X. prunicola*, corresponding sequences were extracted from the genome sequences (Supplementary Table 1) (Pieretti *et al.*, 2015; Triplett *et al.*, 2015; Hersemann *et al.*, 2016b; Vicente *et al.*, 2017; López *et al.*, 2018).

Multiple sequence alignments were performed using the MUSCLE algorithm (https://www.ebi.ac.uk/Tools/msa/muscle/) (Edgar, 2004). Phylogenetic trees were generated using the phylogeny.fr pipeline, with default parameters (http://www.phylogeny.fr) (Dereeper *et al.*, 2008). Newick files were generated and the tree was manipulated using the iTOL website (https://itol.embl.de) (Letunic and Bork, 2011) to improve visualization.

RESULTS

Isolation of bacteria from wheat

BLS symptoms were observed in various plots of the Algiers experimental station, at the tillering and at the heading crop growth stages (Figure 1A). Additionally, severe disease symptoms of BC were observed at the heading stage (Figure 1B). Affected varieties in the Algiers area included wheat 'Hiddab' (HD1220), and also several breeding lines and cultivars grown at the Algiers experimental station for studies under the pedoclimatic conditions of the region, to verify their performance, efficiency, specific features and disease resistance. Symptoms were also observed on the cultivar 'Simeto', grown under the sprinkler irrigation at El Ménéa (El Goléa), South Algeria. This is an area characterized by cool nights and high day temperatures. Furthermore, during the 2016 agricultural campaign, the disease was sporadically present in plots of the Algiers experimental station, and more widespread in plots under pivot irrigation, where 'Simeto' was sown. Yellow colonies on media, resembling *Xanthomonas*, were easily isolated from the plant symptoms. In order to evaluate if post-harvest material could serve as reservoirs for infections, crop residues from plots at the Algiers multiplication station that had shown symptoms, were analysed as well, as described previously (Karavina *et al.*, 2008). *Xanthomonas*-like bacteria were also isolated from this material.

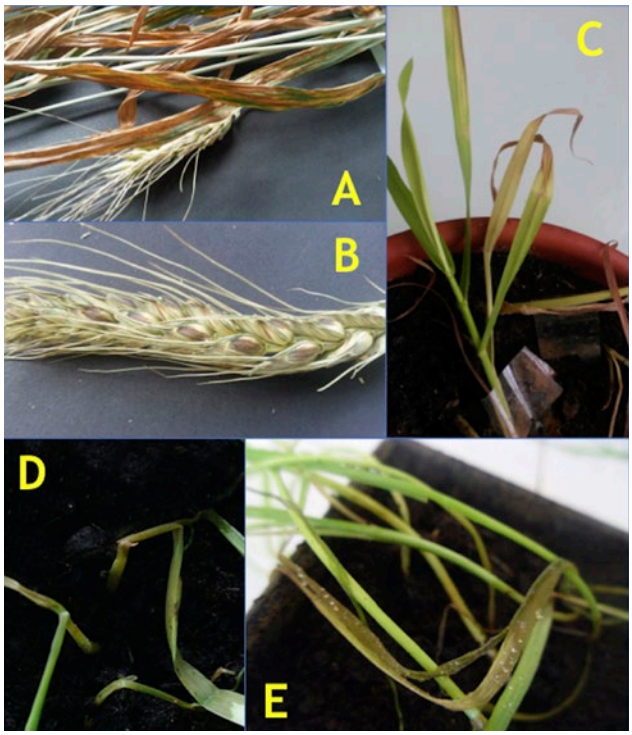

Figure 1. Disease symptoms on wheat. (A) Symptoms of bacterial leaf streak at the heading stage, (B) symptoms of black chaff at the heading stage, (C) chlorotic to necrotic lesions from leaf infiltration 7 d after inoculation, (D) disease symptoms 8 d after pricking inoculation of wheat seedlings at the three-leaf stage, and (E) water-soaked spots associated with bacterial exudates 8 d after dip inoculation of leaves.

Phenotypical characterization of bacteria isolated from wheat

Among all the strains obtained from the different origins, 30 were retained (Table 2) with consistent biochemical and physiological test responses corresponding to those obtained with the *X. translucens* reference strain (Bradbury, 1986; Van den Mooter and Swings,

Table 2. Biochemical and physiological tests used to identify bacterial isolates from wheat, and a reference strain (*Xanthomonas* translucens pv. *undulosa* strain UPB753 (Bragard *et al.*, 1995)).

Test	Algerian isolates	Reference strain
Mucoid and yellow colonies on GYCA medium	+	+
Gram staining	-	-
Oxidase	-	-
Metabolism of glucose	oxidative	oxidative
Tween 80 hydrolysis	+	+
Aesculin hydrolysis	+	+
Starch hydrolysis	+	+
Levane sucrase	+	+
Catalase	+	+
Liquefaction of gelatine	+	+
H_2S production from cysteine	+	+
Nitrate reduction to nitrite	-	-
Growth at 35 °C	+	+
Growth in 2% NaCl	+	+
Growth in 5% NaCl	-	-
Hypersensitive reaction on tobacco	+	+
Pathogenicity on wheat cv. Acsad 885	+	+
Diagnostic PCR (Maes *et al.*, 1996)	+	+

1990; Schaad *et al.*, 2001). All these strains grew as mucoid and yellow pigmented colonies, and they were negative for Gram staining, oxidase activity and nitrate reduction. The strains could hydrolyse aesculin, gelatine and starch and produced catalase, levane sucrase, lipase and hydrogen sulphide from cysteine. Bacteria grew at 35°C and in nutrient broth supplemented with 2% sodium chloride, but not when supplemented with 5% sodium chloride. All strains triggered hypersensitive reactions on tobacco. These analyses indicated that the wheat-associated bacteria belonged to the genus *Xanthomonas* and may be related to *X. translucens*.

Pathogenicity assays with xanthomonads isolated from wheat

Three inoculations methods were applied to evaluate the pathogenicity of the bacterial strains. Upon leaf infiltration of a susceptible cultivar, chlorotic to necrotic lesions developed within 7 d after infection (Figure 1C). When pricking three-leaf stage wheat seedlings, similar symptoms were observed 8 d after inoculation (Figure 1D). Dip inoculation of wheat leaves resulted in symptoms that were clearly visible after 8 d, including typical water-soaked spots associated with bacterial exudates from the plant organs (Figure 1E).

Bacteria were re-isolated from infected plant material. Morphological, biochemical and physiological characterization confirmed the identity of the re-isolated bacteria with the inoculum, thus fulfilling the Koch's postulates.

DNA-based diagnostics of xanthomonads isolated from wheat

In order to evaluate whether the strains belong to *X. translucens*, they were subjected to a protocol that had been developed for specific detection of this species, based on a discriminatory region in the 16S-23S intergenic region, which encodes two tRNAs (Maes *et al.*, 1996). A DNA fragment with a size of 139 bp was amplified with PCR primers T1 and T2 for all 30 strains.

To further characterize ten representative strains from wheat, a portion of the *gyrB* gene that was previously used in multiple locus sequence analysis (MLSA) was amplified by PCR and sequenced, using previously developed primers (Fargier and Manceau, 2007; Young *et al.*, 2008). All sequences were identical. Homologous sequences of type, pathotype or other *Xanthomonas* strains were retrieved from the PAMDB database (Almeida *et al.*, 2010). In addition, sequences for those of *Xanthomonas* species that were not available at PAMDB, but had been included in a previous *gyrB*-based phylogenetic study, including sequences for undescribed species ("slc" species-level clades) (Parkinson *et al.*, 2009), were used for comparison. Sequences were aligned using MUSCLE and manually trimmed to 528 bp. A phylogenetic tree was calculated using the Phylogeny.fr pipeline (Figure 2). This analysis revealed that the Algerian sequences clustered with other sequences from clade-1 xanthomonads, including *Xanthomonas hyacinthi*, *X. theicola* and *X. translucens*. The closest sequence corresponded to strain NCPPB 2654 from species-level clade 5. This strain was isolated in 1974 by F. W. Catton, from navy bean (*Phaseolus vulgaris*) in the United Kingdom. No other sequence information is available for this species-level clade.

This result prompted us to partially sequence another housekeeping gene *rpoD*, that was previously used in MLSA studies (Young *et al.*, 2008). Multiple sequence alignment of four 870-bp sequences from representative Algerian strains revealed their identity with each other. A phylogenetic tree was generated including representative haplotypes for clade-1 xanthomonads from PAMDB. In addition, sequences from three additional *X. translucens* pathotype strains and two sequences belonging to the recently suggested clade-1 species "*X. pseudalbilineans*" were included (Pieretti *et al.*, 2015; Hersemann

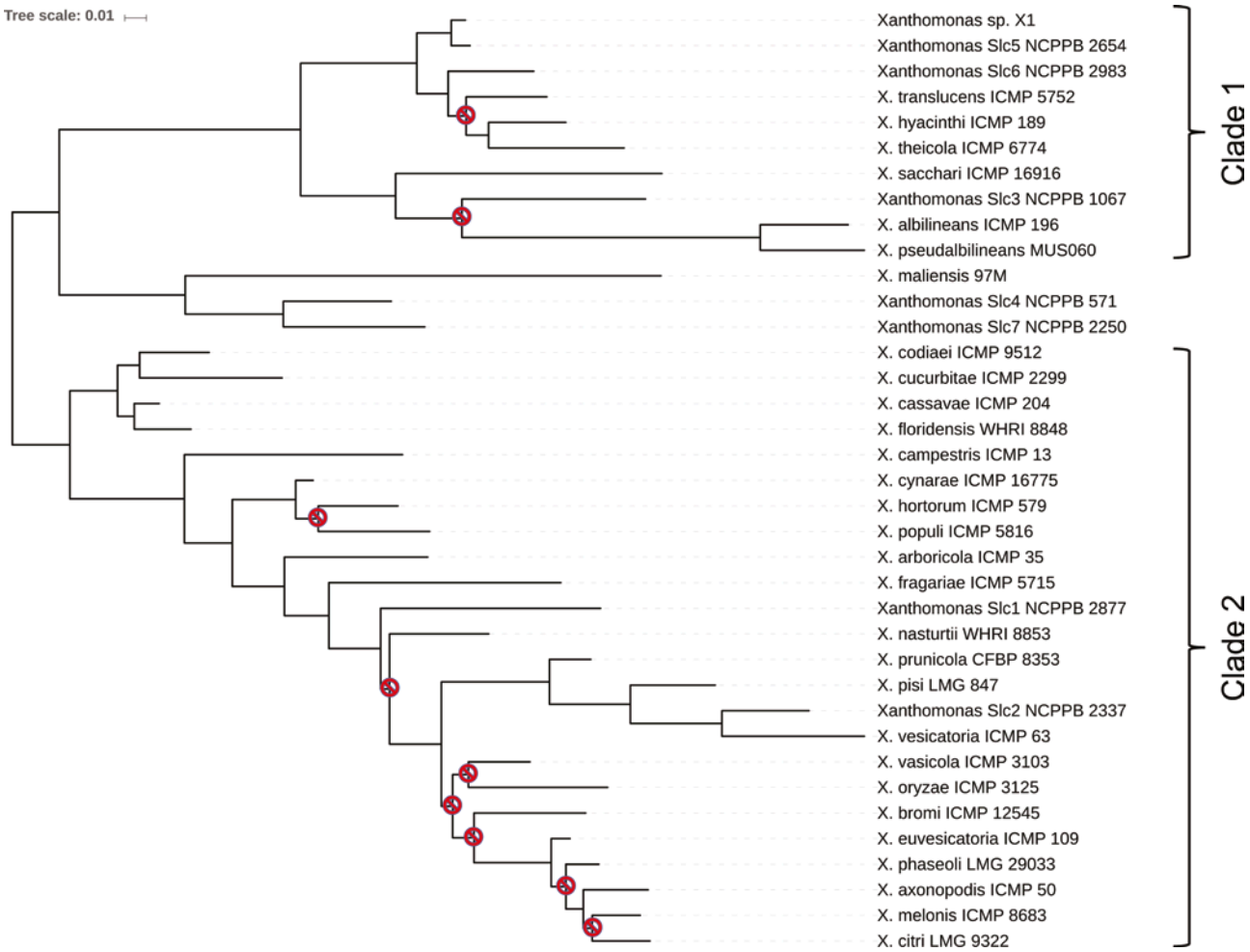

Figure 2. *gyrB*-based phylogenetic classification of the Algerian wheat-associated xanthomonads. Representative strains of all species following taxonomic revisions of *Xanthomonas gardneri* and *X. axonopodis* species complex are included (Constantin *et al.*, 2016; Timilsina *et al.*, 2019). Taxonomically unassigned species-level clades (Slc 1–7) are indicated (Parkinson *et al.*, 2009). All sequences were trimmed to the portion that is available in GenBank for the *Xanthomonas phaseoli* type strain (528 bp, Accession number KT585789). The tree was constructed with the Phylogeny.fr pipeline, using default parameters, and graphically edited using the iTOL suite. All nodes were supported by bootstrap values greater than 0.75, except for those marked with a stop symbol. The scale of branch lengths is indicated at the top left.

et al., 2016b). This analysis confirmed that the Algerian strains belong to clade-1, but did not cluster with any of the six described species. This indicates that the Algeria strains from wheat belong to another species, with strain NCPPB 2654 as the likely founder (Figure 3).

DISCUSSION

Diseased wheat plants were reported from several plots in Algeria, with symptoms on the leaves and spikes as well as melanotic areas on the glumes, that were similar to those described for leaf streak and black chaff (Duveiller *et al.*, 2002). These diseases result from bacterial infections, often in high temperature and high humidity conditions, and the observed symptoms have long been described for these diseases (Smith, 1917; Johnson and Hagborg, 1944). The strains characterized in the present study were mostly obtained from sprinkler-irrigated fields or from breeding stations where genotype behaviour was being assessed.

All strains obtained from affected wheat samples, including leaves, spikes and crop residues, had phenotypic characteristics that corresponded to those described in the literature for *X. translucens*. Moreover, inoculation of wheat seedlings at the three-leaf stage caused water-soaked lesions within 8 d after inoculation, accompanied by signs of necrosis around the inoculat-

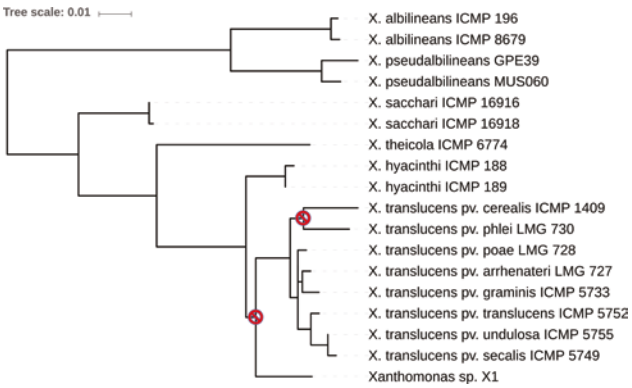

Figure 3. *rpoD*-based phylogenetic classification of the Algerian wheat-associated xanthomonads. Sequences were trimmed to the size of the sequences that were retrieved from PAMDB (855 bp for *X. translucens* and the Algerian strain). The phylogenetic tree was constructed with the Phylogeny.fr pipeline, using default parameters, and graphically edited using the iTOL suite. All nodes were supported by bootstrap values greater than 0.75, except for those marked with a stop symbol. The scale of branch lengths is indicated at the top left.

ed areas and ultimately causing the death of the leaves. When the pricking inoculation method was used, initial local necrosis expanded with time and affected whole leaf blades, concomitantly showing typical exudate droplets. The inoculation techniques mimicked temperature and moisture conditions conducive for the disease (Duveiller and Maraite, 1993; Duveiller *et al*, 1997). These results confirmed that the Algerian *Xanthomonas*-like strains were pathogenic on wheat cultivar 'Acsad 885'. Bacteria could also be isolated from crop residues 3 to 4 weeks after harvest, indicating that the pathogen can survive on plant material. This may serve as reservoirs for new infections in the next cropping cycle. Similar observations have been made for *X. translucens*, which was found to survive on crop debris for more than 30 months under laboratory conditions, and for less than 8 months under field conditions (Malavolta Jr. *et al*., 2000). Occurrence on, and isolation from, wheat plants and the symptoms observed in the fields and upon artificial inoculation strongly indicated that the strains were *X. translucens*.

Initial molecular characterization of the strains using a PCR assay that was developed for detection of *X. translucens*, including pathovars infecting small-grain cereals (pvs. *cerealis*, *hordei*, *secalis*, *translucens* and *undulosa*) and those infecting forage grasses (pvs. *arrhenatheri*, *graminis*, *phlei*, *phleipratensis* and *poae*), further supported that the Algerian strains were *X. translucens* (Maes *et al*., 1996). This assay targets the 16S-23S intergenic region, which encodes two tRNAs in all xanthomonads, one for alanine (UGC anticodon) and one for isoleucine (GAU anticodon). The diagnostic primers T1 and T2 anneal immediately upstream and downstream of the tRNA (Ala) gene. Most xanthomonads have short regions of 14 to 19 bp between the transcribed sequences for the tRNA (Ala) and the tRNA (Ile) (Gonçalves and Rosato, 2002), and therefore lack the target region for the T2 primer. However, *X. translucens*, two other clade-1 species (*X. hyacinthi*, *X. theicola*) and two clade-2 species (*Xanthomonas codiaei*, *Xanthomonas melonis*) have longer regions of 75 to 79 bp, that are fairly conserved and might allow annealing of the T2 primer under less stringent conditions (data not shown). Since the region corresponding to the T1 primer is less similar for *X. codiaei* and *X. melonis*, the PCR should not amplify the diagnostic DNA fragment of 139 bp, as had been confirmed for *X. melonis* (Maes *et al*., 1996). However, that the PCR could amplify the diagnostic DNA fragment for bacteria of the *X. hyacinthi*-*X. theicola*-*X. translucens* subclade cannot be excluded. When the assay was developed by Maes and co-workers, all xanthomonads that were known as pathogens on *Gramineae* (i.e. *X. albilineans*, *X. axonopodis*, *X. bromi*, *X. oryzae* and *X. vasicola*), and representative strains of most described species of *Xanthomonas*, were included, although some species of clade-1 were not tested (e.g. *X. hyacinthi* and *X.theicola*). Yellow disease of hyacinth, a monocot of the *Asparagaceae*, was the first disease described to be caused by *Xanthomonas* (Van Doorn and Roebroeck, 1993). Infection of asparagus by *X. translucens* has been reported (Rademaker *et al*., 2006). Therefore, the taxonomic status of the Algerian strains remained uncertain based on the diagnostic PCR.

Since we felt that the diagnostic PCR is not able to unambiguously identify strains of *X.translucens*, two housekeeping genes that are included in MLSA schemes were analysed (Young *et al*., 2008). Partial sequences of both genes, *gyrB* and *rpoD*, clustered with sequences from other clade-1 xanthomonads, such as *X. hyacinthi*, *X. theicola* and *X. translucens*, but were distant enough to question whether they belonged to any of the described species. The *gyrB* gene has been used for exhaustive phylogenetic analyses of *Xanthomonas*, including strains from species-level clades that still await precise taxonomic assignment (Parkinson *et al*., 2007, 2009). The partial *gyrB* gene sequences from the Algerian isolates were very similar to that of strain NCPPB 2654, which was isolated from a navy bean plant in the United Kingdom (https://www.fera.co.uk/ncppb). According to the NCPPB website, this strain has a fatty acid profile typical of *Xanthomonas*, but is not pathogenic on bean pods. It would be interesting to know wheth-

er this strain was isolated near a wheat field, and to test this strain for pathogenicity on wheat plants. Likewise, the next similar sequence corresponded to strain NCPPB 2983, which was deposited as *Xanthomonas campestris* pv. *phormiicola* and belongs to species-level clade 6. This strain was isolated in Japan from New Zealand flax (*Phormium tenax*), a member of the *Asparagales* (*Asphodelaceae*). Three species related to the Algerian strains, *X. hyacinthi*, *X. translucens* and *X. campestris* pv. *phormiicola*, are able to colonize plants in the *Asparagales*, and future work will evaluate whether the Algerian strains from wheat can infect these plants as well.

In conclusion, this is the first description of wheat-pathogenic xanthomonads from Algeria, which were atypical in that they most likely do not belong to *X. translucens*. Further characterization, ideally including whole-genome sequencing, will clarify their taxonomic status and their host range (Peng *et al*., 2016; Langlois *et al*., 2017). It will also be important to compare these strains with other bacterial pathogens of cereals, and to elucidate whether candidate type III effectors, phytohormones and/or toxins are involved in pathogenicity and host adaptation (Royer *et al*., 2013; Gardiner *et al*., 2014; Hersemann *et al*., 2016a, 2016b, 2017; Triplett *et al*., 2016; Nagel and Peters, 2017).

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