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Isolation of atypical wheat-associated xanthomonads in Algeria

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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.

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>
<thead>
<tr>
<th>Isolate</th>
<th>Gene</th>
<th>GenBank Accession number</th>
<th>Sample</th>
<th>Symptoms*</th>
<th>Wheat cultivar</th>
<th>Location</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>X1</td>
<td>gyrB</td>
<td>MF142045</td>
<td>Leaf</td>
<td>BLS</td>
<td>‘Simeto’</td>
<td>El Goléa</td>
<td>2016</td>
</tr>
<tr>
<td>X2</td>
<td>gyrB</td>
<td>MF142046</td>
<td>Leaf</td>
<td>BLS</td>
<td>‘Simeto’</td>
<td>El Goléa</td>
<td>2016</td>
</tr>
<tr>
<td>X3</td>
<td>gyrB</td>
<td>MF142048</td>
<td>Leaf</td>
<td>BLS</td>
<td>‘Simeto’</td>
<td>El Goléa</td>
<td>2016</td>
</tr>
<tr>
<td>X4</td>
<td>gyrB</td>
<td>MF142049</td>
<td>Leaf</td>
<td>BLS</td>
<td>‘Simeto’</td>
<td>El Goléa</td>
<td>2016</td>
</tr>
<tr>
<td>X5</td>
<td>gyrB</td>
<td>MF142050</td>
<td>Crop residue</td>
<td>No visible symptoms ‘Hiddab’ (HD1220)</td>
<td>Algiers experimental station</td>
<td>2016</td>
<td></td>
</tr>
<tr>
<td>X8</td>
<td>gyrB</td>
<td>MF142051</td>
<td>Spike</td>
<td>BC</td>
<td>‘Simeto’</td>
<td>El Goléa</td>
<td>2016</td>
</tr>
<tr>
<td></td>
<td>rpoD</td>
<td>MF142052</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X12</td>
<td>gyrB</td>
<td>MF142053</td>
<td>Crop residue</td>
<td>No visible symptoms ‘Hiddab’ (HD1220)</td>
<td>Algiers experimental station</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rpoD</td>
<td>MF142054</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X13</td>
<td>gyrB</td>
<td>MF142055</td>
<td>Crop residue</td>
<td>No visible symptoms ‘Hiddab’ (HD1220)</td>
<td>Algiers experimental station</td>
<td>2010</td>
<td></td>
</tr>
<tr>
<td></td>
<td>rpoD</td>
<td>MF142056</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X16</td>
<td>gyrB</td>
<td>MF142057</td>
<td>Spike</td>
<td>BC</td>
<td>Breeding line</td>
<td>Algiers experimental station</td>
<td>2016</td>
</tr>
<tr>
<td>X17</td>
<td>gyrB</td>
<td>MF142058</td>
<td>Spike</td>
<td>BC</td>
<td>Breeding line</td>
<td>Algiers experimental station</td>
<td>2016</td>
</tr>
</tbody>
</table>

*BLS = bacterial leaf streak, BC = black chaff.
In light of recent scientific endeavors, various methods have been employed for the isolation and characterization of atypical wheat-associated xanthomonads in Algeria. Samples collected from wheat crops in different regions of Algeria were subject to a battery of diagnostic tests aimed at identifying the bacterial strains. These tests included conventional methods such as the KOH test, oxidase test using tetramethyl-p-phenylenediamine dihydrochloride reagent, liquefaction of gelatine, H₂S production, catalase reaction, and production of β-galactosidase, among others. Additionally, DNA manipulations were carried out to confirm the identity of the isolated strains.

**Phenotypic characterization**

Established tests were utilized to identify the bacterial strains (Bradbury, 1986; Van den Mooter and Swings, 1990; Schaad et al., 2001). These included the 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, yeast extract-calcium carbonate agar (GYCA) tubes for short-term conservation and at -80°C in 20% glycerol for long-term storage.

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 (Manceau, 2007), with modifications. The reaction mixture was prepared in a total volume of 25 μL comprising 5 μL of 5 × PCR buffer, 2.5 μL MgCl₂ (25 mM), 0.75 μL dNTP mixture (25 mM), 0.5 μL of each PCR primer at 10 μM (T1, 5'-CCGC-CATAGGGCGGAGCACCCCGAT; T2, 5'-GCAGGT-GCGACGTTTGCAGGGATCTGCAAATC), 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 × 10⁸ CFU mL⁻¹ for hypersensitive reaction tests on tobacco plants of the variety Xanthi and to 1 × 10⁷ 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 ‘Ascad 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,
Isolation of atypical wheat-associated xanthomonads in Algeria

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, gelatin 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).

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. thecila 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. pseudalbilin-eans” were included (Pieretti et al., 2015; Hersemann

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)).

<table>
<thead>
<tr>
<th>Test</th>
<th>Algerian isolates</th>
<th>Reference strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mucoid and yellow colonies on GYCA medium</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram staining</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Oxidase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Metabolism of glucose</td>
<td>+</td>
<td>oxidative</td>
</tr>
<tr>
<td>Tween 80 hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Aesculin hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Levane sucrase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Liquefaction of gelatine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H2S production from cysteine</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction to nitrite</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Growth at 35 °C</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in 2% NaCl</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Growth in 5% NaCl</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Hypersensitive reaction on tobacco</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pathogenicity on wheat cv. Acsad 885</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Diagnostic PCR (Maes et al., 1996)</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

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.
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 inocula-
Isolation of atypical wheat-associated xanthomonads in Algeria

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 Maraire, 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 codiae, 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. codiae 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. vasicala), 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 whether-

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.
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 NCP-PB 2983, which was deposited as Xanthomonas camp- estris pv. phormicola 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. campes- tris pv. phormicola, are able to colonize plants in the Aspara- gales, and future work will evaluate whether the Alger- ian 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, phytohor- mones 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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