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Pathovar-Specific PCR Method for Detection and Identification of Xanthomonas translucens pv. undulosa

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1 **Heading title:** Detection of *Xanthomonas translucens* pv. *undulosa*

2

3 **Pathovar-Specific PCR Method for Detection and Identification of *Xanthomonas translucens* pv.**
4 ***undulosa***

5

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Abstract

Bacterial leaf streak disease caused by *Xanthomonas translucens* pv. *undulosa* is an economically important disease threatening wheat and barley crops around the globe. So far, specific PCR-based detection and identification tests for *X. translucens* pathovars are not available. In this study, we used comparative genomics approach to design a pathovar-specific primer pair for detection of *X. translucens* pv. *undulosa* in naturally infected seeds and its differentiation from other pathovars of the species. For this aim, complete genome sequences of strains of different *X. translucens* pathovars were compared and the specific PCR primer pair XtuF/XtuR was designed. These primers were strictly specific to *X. translucens* pv. *undulosa* as the expected 229 bp DNA fragment was not amplified in the closely-related pathovars nor in other xanthomonads, wheat pathogenic bacteria, and other plant pathogenic bacteria. High sensitivity of the primer pair XtuF/XtuR allowed detection of pure DNA of the pathogen in a concentration as low as 4.5 pg/µl. The pathogen was also detected in water suspension at a concentration of 8.6×10^2 cfu/ml. The PCR test was capable of detecting the pathogen in extracts of naturally infected wheat seeds at a concentration of 3.5×10^4 cfu/g while culture plate method was able to detect the pathogen at a concentration of 50×10^5 cfu/g of the same seeds. The PCR test developed in this study is a step forward for precise detection and identification of *X. translucens* pv. *undulosa* to prevent outbreaks of the bacterial leaf streak disease.

Keywords: Bacterial leaf streak, Quarantine pathogen, Seed borne bacteria, Small grain cereals, *Xanthomonas translucens*

47 **Introduction**

48 *Xanthomonas translucens* is a Gram-negative bacterium infecting small grain cereals and forage
49 grasses in the family *Poaceae*. The species includes nine pathovars, among which, *X. translucens*
50 pv. *undulosa*, *X. translucens* pv. *translucens* and *X. translucens* pv. *cerealis* cause bacterial leaf
51 streak disease, and have wide geographic distribution leading to economic yield losses on wheat
52 (*Triticum aestivum*) and barley (*Hordeum vulgare*) crops (Sapkota et al., 2020). The disease
53 occurs in many countries on winter wheat and barley with a particular importance in regions
54 characterized by rainy and humid spring, and areas where sprinkler irrigation is used (CABI 2022).
55 Members of *X. translucens* that cause leaf streak on small grain cereals belong to the
56 "translucens" group, while those pathovars that cause bacterial wilt on forage grasses are
57 clustered in the "graminis" group (Vauterin et al. 1992, 1995; Peng et al., 2016; Langlois et al.,
58 2017; Hersemann et al., 2017). The translucens group contains three economically important
59 pathovars *i.e. X. translucens* pv. *undulosa* (pathogenic on barley and wheat), *X. translucens* pv.
60 *translucens* (pathogenic only on barley) and *X. translucens* pv. *cerealis* (pathogenic on barley,
61 wheat, oat, and bromegrass). Diseases caused by the latter three pathovars also known as
62 bacterial leaf stripe and black chaff (Sapkota et al., 2020; Shah et al. 2022). Among these
63 pathovars, *X. translucens* pv. *translucens* is on the A2 EPPO list of pathogens recommended for
64 regulation, even if it is not classified as quarantine in Europe (EPPO 2022).

65 Despite their similarities in morphological features and biochemical characteristics, the three
66 pathovars in translucens group are considered independent quarantine pathogens around the
67 world (CABI 2022; EPPO 2022; Osdaghi et al. 2022). During the past decade, the highest
68 economic losses recorded due to *X. translucens* pv. *undulosa* outbreaks, while destructive
69 epidemics have rarely been reported from the two other pathovars in translucens group
70 (Khojasteh et al. 2019). This could be due in part to the differences in their host range as *X.*
71 *translucens* pv. *undulosa* infects both wheat and barley while *X. translucens* pv. *translucens* is
72 restricted to barley under natural conditions. Because of the continuous taxonomic complexities
73 within the species *X. translucens*, the exact distribution of each pathovar has not yet been
74 determined. The information provided in the literature before the reclassification of the species

75 in 1995 (Vauterin et al., 1995) might have referred to bacterial leaf streak as a disease complex
76 instead of determining the pathovar status of the pathogen. Even after the reclassification of the
77 species, most descriptions of the pathogens in areas with no history of the disease did not
78 accurately identify the pathogen at the pathovar level (Shah et al. 2021; CABI 2022).

79 No effective chemicals against the bacterial leaf streak pathogens are known. Thus, the
80 availability of a specific and sensitive diagnostic test for rapid detection of *X. translucens* on plant
81 materials, possibly at the port of entry and into the fields, is a pivotal step forward towards the
82 successful prevention of disease outbreaks (Curland et al. 2018). Traditionally, detection and
83 isolation of the pathogen could be achieved by plating on general or semi-selective culture media
84 (Duveiller and Bragard, 2017). Strains of *X. translucens* pv. *undulosa* is likely to be confused with
85 strains of other *Xanthomonas* strains, other pathovars of the translucens group, or other yellow-
86 pigmented saprophytic bacteria commonly associated with plants and seeds (Harveson et al.
87 2015; Osdaghi et al 2020). Differences in host range and aggressiveness have clearly been
88 demonstrated among the three pathovars of the translucens group (Alizadeh et al., 1995;
89 Bragard et al., 1997; Cunfer & Scolari, 1982; Milus & Mirlohi, 1994). However, they could not be
90 differentiated by any bacteriological assays (Alizadeh et al., 1995; Dye, 1962), DNA hybridization
91 (Vauterin et al., 1992), serological methods (Azad & Schaad, 1988; Bragard & Verhoyen, 1993),
92 and fatty acid profiling (Stead, 1989; Yang et al., 1993). PCR-based detection of the pathogens
93 using genus-specific primer pairs is possible to identify Gram-negative, yellow-pigmented
94 bacterial strains isolated from small grain cereals. Adriko et al. (2014) developed a set of genus-
95 specific PCR primers for the detection and identification of xanthomonads. Furthermore, Maes et
96 al. (1996) developed a PCR test for detection and discrimination of cereal-pathogenic
97 xanthomonads in seed lots. The latter PCR primers were designed to amplify DNA sequences
98 located in the spacer fragment between the 16S and 23S rRNA genes and flanking an alanine-
99 tRNA gene. The primers amplify a 139-bp fragment from all strains of leaf streak pathogens. The
100 assays proved to be fast and relatively sensitive (28×10^3 cfu/g of seed) indicating that the
101 technique might be useful for detecting pathogens in seed (Duveiller and Bragard, 2017).
102 However, using the latter PCR test, assignation to a pathovar is not possible. Indeed, no

103 pathovar-specific PCR primer has yet been developed for the detection and identification of the
104 various bacterial leaf streak pathogens.

105 Identification of the pathogens down to the pathovar level can only be achieved by pathogenicity
106 tests on a set of small grain cereals including, but not limited to, wheat and barley (Sapkota et al.,
107 2020). Multilocus sequence analyses (MLSA) and typing (MLST) using partial sequences of four
108 housekeeping genes (*i.e. dnaK*, *fyuA*, *gyrB* and *rpoD*) also discriminate *X. translucens* strains
109 isolated from gramineous crops, and the resulting scheme is in congruence with their
110 experimental host range, host of isolation, as well as draft genome sequence-based phylogeny
111 (Curland et al., 2018; Khojasteh et al., 2019; Shah et al. 2021). Langlois et al. (2017) developed a
112 loop-mediated isothermal amplification (LAMP) protocol, which can specifically detect *X.*
113 *translucens* pv. *undulosa*, *X. translucens* pv. *translucens* and *X. translucens* pv. *cerealis* but was
114 unable to differentiate the three pathovars. Recently, Sarkes et al. (2022) also reported detection
115 of *X. translucens* pv. *undulosa* from wheat by quantitative PCR. The latter qPCR can detect the
116 bacteria from both infected seed and leaf tissues but it cannot differentiate *X. translucens* pv.
117 *undulosa* and *X. translucens* pv. *translucens* (Sarkes et al., 2022).

118 The purpose of the present study was to develop a sensitive and pathovar-specific PCR assay for
119 detection and identification of *X. translucens* pv. *undulosa* in pure culture and plant materials.
120 PCR primers were developed based on the sequence of a specific DNA fragment in *X. translucens*
121 pv. *undulosa* genome sequence. The developed PCR test was highly sensitive and specific to the
122 pathogen allowing differentiation of *X. translucens* pv. *undulosa* from the other pathovars of the
123 species, other xanthomonads, as well as several bacterial plant pathogens.

124

125 **MATERIALS AND METHODS**

126 **Bacterial strains and culture media**

127 A set of 79 *X. translucens* or *Xanthomonas* sp. strains isolated from *Poaceae* plants representing
128 type strains of all validly described *X. translucens* pathovars, as well as several

129 reference/standard strains of the species from all five continents were used in this study (Table
130 1). Furthermore, a number of bacterial species pathogenic on small grain cereals, bacterial
131 epiphytes isolated from grasses, as well as several pathogenic species on vegetables and annual
132 crops were included in our screening assays. The bacterial strains were received from either
133 CIRM-CFBP culture collection (France) or Department of Plant Protection at Shiraz University
134 (Iran). The bacterial strains were streaked onto nutrient agar (NA) or yeast-extract peptone
135 glucose agar (YPGA) media and incubated at 27°C for 3-4 days. They were re-suspended in sterile
136 distilled water and stored at 4 °C for further use, while maintained in 15% glycerol at -70 °C for
137 the long-term use. Colony color and pigmentation of the bacterial strains were assessed on YPGA
138 and yeast extract-dextrose-calcium carbonate (YDC) agar media as described previously (Osdaghi
139 et al. 2018).

140

141 **Targeting specific sequences and designing PCR primers**

142 Complete genome sequences of *X. translucens* pv. *undulosa* (CFBP 8904, CFBP 8905, CFBP 8908,
143 CFBP 8909, ICMP 11055, LW16, P3, and Xtu 4699), *X. translucens* pv. *translucens* (CFBP 8907,
144 CFBP 8911, DSM 18974^{T}, and km9), and *X. translucens* pv. *cerealis* (CFBP 2541 and NXtc01) were
145 retrieved from the NCBI GenBank and subjected to comparative genomics (Table S1).
146 Furthermore, whole genome sequences of 46 *X. translucens* strains were retrieved from the
147 GenBank for further validation of the analyses (data not shown). A combination of three genome
148 comparison methods was used to target pathovar-specific nucleotide sequences in *X. translucens*
149 pv. *undulosa*. First, pairwise genome collinearity alignment of the strains was performed and
150 visualized with BRIG 0.95 using pairs of genomes representing various pathovars. Then, all
151 genomes were annotated using prokka (Seemann 2014) and pan-core genome was constructed
152 using Roary (Page et al, 2015) on the usegalaxy.eu public server (Afgan et al. 2018). Roary and
153 prokka output files were utilized to extract unique gene sequences using seqinr, tidyr, stringr,
154 dplyr, ggplot2 packages in R and Rstudio. Finally, the targeted pathovar-specific sequences were
155 subjected to BLASTn analyses where the query sequences were used to search against either
156 whole database of nucleotide sequences or the complete genome sequences of *X. translucens*

157 strains as listed in Table S1. To ensure the specificity of the selected sequences, similarity
158 parameters in all the three software were adjusted in a way that they could target the lowest
159 possible similarity between the pairs of sequences being compared. For instance, in BLASTn
160 search, the program was adjusted for detection of "somewhat similar sequences". *Xanthomonas*
161 *translucens* pv. *undulosa*-specific nucleotide sequences retrieved from the corresponding
162 genomes were aligned using MEGA 7 software (Kumar et al. 2016) and the identical set of
163 nucleotides were visually detected. PCR primers were designed using the combination of visual
164 nucleotide selection and Vector NT software (Lu and Moriyama 2004). The resulted primers were
165 checked for physical parameters using Primer-BLAST (Ye et al. 2012), and Primer3Plus online
166 services (Untergasser et al. 2007).

167

168 **Validation of the primers for specificity and sensitivity**

169 Designed primer pairs were subjected to PCR assays to evaluate their specificity and sensitivity in
170 detection of *X. translucens* pv. *undulosa*. Genomic DNA of the representative bacterial strains
171 (CFBP 8904, CFBP 8905, CFBP 8908, and CFBP 8909) was extracted using both Cetyltrimethyl
172 ammonium bromide (CTAB) method and Wizard genomic DNA purification kit (Promega,
173 Madison WI, USA) as detailed previously (Osdaghi et al 2021). The quality and quantity of the
174 DNAs were confirmed by 1.0% agarose gel electrophoresis, then spectrophotometrically
175 evaluated and adjusted to 500 ng/µl using the NanoDrop ND-100 (NanoDrop Technologies,
176 Waltham, MA, USA). For PCR reactions, Universal PCR Kit − Ampliqon® Taq DNA Polymerase
177 Master Mix Red (Ampliqon A/S, Odense, Denmark) − was used according to the manufacturer's
178 recommendations in 50 µl PCR reaction, including bacterial DNA and 2 µl of each primer (10
179 pmol/µl). The best annealing temperature for the primer pairs was determined using a gradient
180 PCR program on BIO-RAD T100 thermal cycler (Hercules, California, USA). The PCR program
181 consisted of an initial denaturation for five min at 95 °C followed by 35 cycles of DNA
182 amplification where each cycle included 60 seconds at 94 °C for template DNA denaturation, 45
183 seconds at 61.5 °C for primer annealing, and 60 seconds at 72 °C for primer extension. Final
184 extension included five min at 72 °C. While pure bacterial DNAs obtained from CTAB and DNA

185 purification kit were used for PCR setup, initial checks, and sensitivity validation, the subsequent
186 PCR tests for validation of specificity of the PCR method were conducted using bacterial whole
187 cells via boiling method. This was due to the fact that using boiling method for PCR reactions
188 would save time and effort for large scale screenings in quarantine ports.

189 In order to confirm the specificity of the primers, 19 known *X. translucens* pv. *undulosa* strains
190 along with several *X. translucens* pv. *translucens* (n=9), *X. translucens* pv. *cerealis* (n=3), as well as
191 members of the graminis group of *X. translucens* strains (n=23) were tested (Table 1). Besides the
192 known *X. translucens* collection, a set of non-*X. translucens* members within the clade I of
193 *Xanthomonas* were also included in the assays (Mafakheri et al. 2022). Non-xanthomonad
194 bacterial pathogens of small grains cereals *i.e. Clavibacter tessellarius*, *C. zhangzhiyongii*,
195 *Rathayibacter tritici* as well as the bacterial strains that were epiphytically isolated from wheat
196 and barley were also tested using the PCR assay (Table 1). To ensure the accuracy of the
197 procedure, cross-laboratory validation of the PCR primers was conducted in two independent
198 institutions i.e. Shiraz University (Iran) and CIRM-CFBP culture collection (France) using different
199 thermal cycler machines and PCR reagents. However, template DNA preparation method
200 (boiling), PCR program, and annealing temperature were the same in both laboratories.

201 To test the sensitivity of the primers, pure genomic DNA of the *X. translucens* pv. *undulosa*
202 strains CFBP 8904, CFBP 8905, CFBP 8908, and CFBP 8909 were prepared in concentrations
203 ranging between 1.8×10^4 pg/µl and 3×10^{-2} pg/µl. From each of the diluted DNA sample, 1 µl
204 was used in PCR reaction using the procedure described above. The experiment was
205 independently repeated three times. A similar procedure was used to evaluate the sensitivity of
206 the primer pair for detection of whole cells of *X. translucens* pv. *undulosa* in water suspension
207 (DNA extracted via boiling method). For this aim, a fresh bacterial suspension was spread-plated
208 on YPGA medium after 1:10 serial dilutions. One µl from each of the bacterial suspensions was
209 used to test the capability of the PCR method to detect the pathogen. The experiments were
210 independently repeated three times.

211

212 **Detection of the pathogen in infected seeds**

213 The selected primer pair was evaluated for its ability to detect *X. translucens* pv. *undulosa* in
214 naturally infected wheat seeds. For this aim, naturally infected seed lots were collected from
215 wheat farms in southern Iran using the procedure described previously (Khojasteh et al. 2019). A
216 seed wash test was conducted using the procedure described by Duveiller and Bragard (2017).
217 First, 25 g of naturally infected wheat seeds were rinsed in 50 ml of distilled water and shaken for
218 15 min at 110 rpm. The resulted suspension was diluted 1:1 (up to 10th dilution) using distilled
219 water, and the concentration of culturable *X. translucens* pv. *undulosa* cells was counted on
220 YPGA medium using the procedure described above. One µl of the same suspensions was used in
221 direct PCR using the criteria described above. Eight naturally infected wheat seed samples were
222 tested independently.

223

224 **RESULTS**

225 **Genome comparison and target selection**

226 Using a combination of BRIG and RStudio, we obtained nucleotide sequences specific to
227 *X. translucens* pv. *undulosa*. Specificity of each nucleotide sequence was further evaluated using
228 BLASTn search among all *X. translucens* and *Xanthomonas* spp. genomic resources in the NCBI
229 GenBank. Two DNA fragments were evaluated using BLASTn while one of these fragments was
230 selected to be used for PCR primers design. The selected DNA target was a 708 bp fragment
231 located between nucleotides 2,731,378 and 2,732,085 in the complete genome sequence of *X.*
232 *translucens* pv. *undulosa* strain ICMP 11055 (GenBank: CP009750.1; locus tag: NZ30_11335).
233 According to the NCBI Prokaryotic Genome Annotation Pipeline, this DNA fragment is a
234 hypothetical protein. BLASTn search revealed no sequence similarity between the selected DNA
235 fragment and the whole genome resources of *X. translucens*, nor other xanthomonads. Hence,
236 the primer pair XtuF: GCCTAAAAATTGCATCAGC / XtuR: GGACATGCTCGGCTCGCTGCTCAG was
237 capable of amplifying a 229 bp DNA fragment in all *X. translucens* pv. *undulosa* strains (Figure
238 S1A).

239

240 **Sensitivity and specificity tests**

241 Sensitivity test for the primer pair XtuF/XtuR using DNA concentrations of 1.8×10^4 pg/µl to $3 \times$
242 10^{-2} pg/µl showed that the expected DNA fragment was amplified in 4.5 pg/µl of the *X.*
243 *translucens* pv. *undulosa* pure DNA (Figure S1B). Hence, the primer pair XtuF/XtuR could
244 successfully detect the pathogen with high sensitivity and efficiency. Specificity of the primer pair
245 XtuF/XtuR was evaluated directly on colonies of the target bacterium *X. translucens* pv. *undulosa*,
246 as well as non-target bacteria *i.e.* other pathovars of *X. translucens,* bacterial pathogens of small
247 grain cereals, and bacterial pathogens that are not pathogenic on grasses. The PCR primers
248 directed the amplification of the expected 229 bp DNA fragment in all *X. translucens* pv. *undulosa*
249 strains while no amplification was observed in the closely-related pathovars *i.e. X. translucens* pv.
250 *translucens* and *X. translucens* pv. *cerealis* (Figure S1A). No amplification was also observed in the
251 strains of the graminis group of *X. translucens* or in the other small grain cereals-associated
252 bacteria *e.g. Clavibacter tessellarius* and *Rathayibacter tritici*. Furthermore, no amplification was
253 observed in non-*X. translucens* plant pathogenic bacteria including *Xanthomonas*, *Pseudomonas*,
254 *Clavibacter* and *Agrobacterium* strains pathogenic on vegetables, annual crops and fruit trees as
255 listed in Table 1. Cross-laboratory validation of the primer pair XtuF/XtuR at two independent
256 institutions produced similar results indicating reliability of the procedure using differed thermal
257 cycler machines and PCR reagents.

258 The PCR primers XtuF/XtuR were also applied to evaluate *Xanthomonas* sp. strains isolated from
259 gramineous plants and deposited in CIRM-CFBP culture collection without pathovar designation
260 (Table 1). Among 26 taxonomically undetermined *Xanthomonas* sp. strains, the expected 229 bp
261 DNA fragment was amplified in 12 strains *i.e.* CFBP 3740, CFBP 3745, CFBP 3746, CFBP 3747,
262 CFBP 3748, CFBP 3749, CFBP 3751, CFBP 3752, CFBP 3753, CFBP 3760, CFBP 3761, and CFBP
263 3762. We traced the 26 *Xanthomonas* sp. strains in the literature to find any recommendations
264 for pathovar designation based on their host range and source of isolation. Using a
265 comprehensive pathogenicity test and DNA fingerprinting assay the strains CFBP 3740, CFBP
266 3749, CFBP 3751, CFBP 3752, CFBP 3753, and CFBP 3761 were assigned into the *X. translucens*

267 pv. *undulosa* clade (Bragard et al. 1997), which is in congruence with the PCR results obtained in
268 this study.

269

270 **Detection of the pathogen in water suspension and infected seeds**

271 Sensitivity of the primer pair XtuF/XtuR was also evaluated on water suspension of *X. translucens*
272 pv. *undulosa* ranging from 1.1×10^5 cfu/ml to 1.3×10^{-1} cfu/ml. The pathogen was detected
273 down to the concentrations of 8.6×10^2 cfu/ml (Figure 1A: lane 8) using the PCR primers
274 XtuF/XtuR, while detection threshold for culture-based method was 1.8×10^3 cfu/ml (Figure 1B).
275 Seed wash test using 25 g of naturally infected wheat seeds showed that the primer pair
276 XtuF/XtuR could detect the pathogen in concentration as low as 3.5×10^4 cfu/g (Figure 1C) while
277 no *X. translucens* pv. *undulosa* colony was observed on YPGA culture plate in this ratio of the
278 seed wash. Culture plate method was able to detect the pathogen in concentration of 50×10^5
279 cfu/g of the infected seeds (Figure 1D).

280

281 **Discussion**

282 In this study, the pathovar-specific PCR primer pair XtuF/XtuR was developed for detection and
283 identification of *X. translucens* pv. *undulosa* the bacterial leaf streak pathogen of small grain
284 cereals. The primer pair showed high specificity when used against closely related pathovars *i.e.*
285 *X. translucens* pv. *translucens*, *X. translucens* pv. *cerealis* as well as a collection of pathovars of
286 the species named as graminis group. High sensitivity of the PCR test was also observed as the
287 primer pair detect up to 4.5 pg/µl of *X. translucens* pv. *undulosa* pure DNA. The primer pair
288 XtuF/XtuR was also capable of detecting the pathogen in the extract of naturally infected wheat
289 seeds in concentration of 3.5×10^4 cfu/g while plating was able to detect the pathogen in
290 concentration of 50×10^5 cfu/g of the same seeds.

291 PCR results using the primer pair XtuF/XtuR was in congruence with the pathovar designation
292 and taxonomic status of almost all *Xanthomonas* sp. strains obtained from CIRM-CFBP culture

293 collection. Exceptions were the strains *X. translucens* pv. *cerealis* CFBP 4166 = UPB 928 isolated
294 from *Bromus* sp. in Iran in 1990, and the pathotype strain of *X. translucens* pv. *secalis* CFBP
295 2539^{PT} isolated from *Secale cereal* in Canada in 1966. It should be stated that in some cases,
296 original pathovar designation of a given bacterial strain could be incorrect, leading to mislabeling
297 of the same strain when deposited in culture collections. This was the case for the strains CFBP
298 4166 = UPB 928 and CFBP 2539^{PT}. As for the former strain (UPB 928), pathovar designation of the
299 bacterium has been questionable since its original description due to the lack of adequate data
300 (Bragard et al. 1997). In fact, most of the bacterial leaf streak strains isolated in Iran before the
301 genomics era (including UPB 928) were named as *X. translucens* pv. *cerealis* (Alizadeh et al. 1997;
302 Bragard et al. 1997). While *X. translucens* pv. *cerealis* was proposed for UPB 928 at the time of
303 isolation, Bragard et al. (1997) investigated the strain using a combination of pathogenicity tests
304 and DNA fingerprinting assays and concluded that the strain was actually *X. translucens* pv.
305 *undulosa* not *X. translucens* pv. *cerealis*. Mislabeling the *X. translucens* strains in the pathovar
306 level still occurs where Falahi charkhabi et al. (2015) referred to the strain ICMP 11055 as *X.*
307 *translucens* pv. *cerealis*, while complete genome sequencing of the same strain indicated its true
308 pathovar status as *X. translucens* pv. *undulosa* (Falahi Charkhabi et al 2017). In fact, Bragard et al.
309 (1997) have investigated most of the *Xanthomonas* spp. strains used in this study in a
310 comprehensive pathogenicity test and DNA fingerprinting assay where the strains CFBP 3740,
311 CFBP 3749, CFBP 3751, CFBP 3752, CFBP 3753, and CFBP 3761 were assigned to the *X.*
312 *translucens* pv. *undulosa* clade, which is in congruence with the PCR results obtained in this study
313 (Table 1). On the other hand, the strains CFBP 3730, CFBP 3733, CFBP 3734, CFBP 3735, CFBP
314 3737, CFBP 3738, CFBP 3742, CFBP 3743, and CFBP 3744 which were negative in the PCR assay
315 using primer pair XtuF/XtuR were assigned into the *X. translucens* pv. *translucens* clade on the
316 basis of their pathogenicity pattern and host range. Phylogenetic analyses using the
317 concatenated sequences of *gyrB* and *recA* genes confirmed pathovar status of the strains
318 mentioned above (data not shown).

319 As for the pathotype strain of *X. translucens* pv. *secalis* (CFBP 2539^{PT} = ICMP 5749^{PT} = LMG 883^{PT}
320 = NCPPB 2822^{PT}), MLSA/MLST using the nucleotide sequences of four housekeeping genes (*i.e.*
321 *dnaK, fyuA, gyrB,* and *rpoD*) showed that the pathotype strains of *X. translucens* pv. *undulosa*

322 (CFBP 2055^{PT} = ATCC 35935^{PT} = ICMP 5755^{PT} = NCPPB 2821^{PT} = LMG 892^{PT}) and *X. translucens* pv.
323 *secalis* (CFBP 2539^{PT}), both isolated in southern Canada in 1966, belong to the same multilocus
324 haplotype (Khojasteh et al. 2019). Bragard et al. (1997) also found that the two pathovars
325 possess similar pathogenicity pattern and host range. The pathotype strain of *X. translucens* pv.
326 *undulosa* was isolated from *Triticum turgidum*, while the pathotype strain of *X. translucens* pv.
327 *secalis* was isolated from rye (Curland et al. 2018). It is more likely that the two pathotypes
328 originated from the same founding genotype in North America, however, predominance of the
329 "one host–one species" concept in mid-20th century led to the designation of the two strains as
330 two different taxa at that time (Vauterin et al. 1995; Khojasteh et al. 2019). Thus, the PCR
331 primers developed in this study could be reliably used for taxonomic assignment and
332 classification of suspected *Xanthomonas* strains associated with small grain cereals.

333 Although culture based and serological methods have traditionally been used for detection of
334 xanthomonads infecting small grain cereals, none of these methods could specifically identify
335 strains belonging to the pathovar *undulosa* from closely related pathovars (Frommel and Pazos
336 1994; Duveiller and Bragard 2017). For instance, Frommel and Pazos (1994) reported that
337 antiserum prepared against somatic antigens of *X. translucens* pv. *undulosa* (at that time *X.*
338 *campestris* pv. *undulosa*) reacted with cells of *X. translucens* pv. *undulosa*, *X. translucens* pv.
339 *cerealis*, *X. translucens* pv. *translucens,* and X. *translucens* pv. *phleipratensis*, but not with other
340 bacterial species belonging to the genera *Xanthomonas*, *Pseudomonas*, *Agrobacterium*,
341 *Clavibacter*, and *Erwinia*. PCR-based screening method was also available to identify the bacterial
342 strains only at the species level (Maes et al. 1996). Hence, development of the PCR method in
343 this study is a step forward towards precise detection and accurate identification of the bacterial
344 leaf streak pathogen.

345 *Xanthomonas translucens* pv. *undulosa* is an important seed borne pathogen with a considerable
346 negative impact on the yield of small grain cereals *i.e.* wheat and barley (Khojasteh et al. 2019;
347 CABI 2022). The increasing worldwide distribution of the pathogen is strongly driven by the
348 global expansion of international trades of seeds, as well as by the multifaceted impact of climate
349 change on plant-pathogen interaction and on weather conducive conditions (Sapkota et al.

350 2020). The importance of the bacterial leaf streak varies among regions depending on the level of
351 cultivar susceptibility and environmental conditions. Studies have shown that yield losses due to
352 the bacterial leaf streak can range from negligible to as much as 40% (McMullen and Adhikari
353 2011). Yield losses are usually due to the reduction of grain test weight per spike and/or the
354 number of grains per spike. Our results showed that the primer pair XtuF/XtuR could successfully
355 detect the pathogen in the seed extract of naturally infected wheat with high sensitivity and
356 efficiency. No further intervention i.e. pathogen purification, and DNA extraction was needed to
357 detect the pathogen.

358 Until recently, culture-based seed wash test on semi-selective media after tenfold serial dilution
359 plating has been the most commonly used non-destructive procedure for detection of
360 *X. translucens* in small grain cereals (Schaad and Forster 1985). The medium XTS agar containing
361 difco nutrient agar, glucose, cycloheximide, gentamycin and cephalexin was developed for semi-
362 selective detection of the pathogen in seed wash. More than 91% of the seed-associated
363 saprophytic bacteria washed from seeds of wheat and barley lot were inhibited on XTS agar
364 (Schaad and Forster 1985). Duveiller and Bragard (2017) described a detail-oriented seed health
365 test method to be used in pathogen-free certification programs. The number of cfu per gram of
366 seed gives an estimate of the number of living bacterial cells present in the sample. Best estimate
367 is obtained by counting colonies on plates where the number of colonies ranges between 50 and
368 300. The lower limit of detection of *X. translucens* pv. *undulosa* in pure cultures using ELISA was 5
369 $\times 10^3$ cfu/ml. Whereas, a semi-selective enrichment broth (SSEB) improved the recovery of the
370 pathogen in cultures mixed with contaminating bacteria commonly found on wheat seeds
371 (Frommel and Pazos 1994). The availability of accurate and highly specific seed health tests is
372 among the most important and essential means of control for bacterial leaf streak disease
373 (Sapkota et al. 2020). Indeed, dramatic outbreaks can easily be caused by inadequate
374 surveillance and quarantine regulatory procedures, following the entry and establishment of a
375 new and emerging plant pathogen. Detection of *X. translucens* pv. *undulosa* during a port of
376 entry inspection of plant material is extremely difficult, since the infected seeds are almost
377 entirely asymptomatic (Schaad and Forster 1985). Furthermore, the existing assays developed so
378 far for detection of *X. translucens* pv. *undulosa* on plant material require a considerable time and

379 effort (Duveiller and Bragard 2017). Our results revealed that using the primer pair XtuF/XtuR the
380 pathogen was detected in concentrations ranging from 1.1×10^5 cfu/ml to 8.6×10^2 cfu/ml, while
381 the detection threshold for culture-based method was 1.8×10^3 cfu/ml. The combination of high
382 specificity of the PCR primers XtuF/XtuR (in the pathovar level) and high sensitivity of the method
383 would lead quarantine inspectors and plant protection agencies to target the enemy with lower
384 cost and effort.

385 In conclusion, *X. translucens* pv. *undulosa* is one of the very few bacterial pathogens for which no
386 exact distribution map proposed by the EPPO Global Database
387 (https://gd.eppo.int/taxon/XANTTU/distribution). The same situation exists for its accompanying
388 pathovar *X. translucens* pv. *translucens* (https://gd.eppo.int/taxon/XANTTR/distribution). The
389 latter database flagged the following note on the distribution section of this pathogen: "Due to
390 the complex taxonomy of this bacterium and its changes over time, it is difficult to establish an
391 accurate world distribution", which indicates the need for an urgent action for accurate
392 identification of the pathogen in the areas where the bacterial leaf streak occurs. Development
393 of the pathovar specific primer pair XtuF/XtuR could be one of the first efforts for identification
394 of the pathogen in the pathovar level. This will be on one hand paving the way of determining
395 exact geographic distribution of each of the pathovars of *X. translucens*. On the other hand, our
396 primers will help the plant protection officials and inspectors to detect the pathogen with higher
397 accuracy to combat the risk of bacterial leaf streak disease in the 21st century's cereals industry.

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401

402 **Conflict of Interest Statement**

403 The authors declare that the research was conducted in the absence of any commercial or
404 financial relationships that could be construed as a potential conflict of interest.

405

406 **Author Contribution**

407 EO conceived and designed the study. HA and PP carried out the experiments with assistance
408 from SMT, MK, CD, GT, MAJ and TR. EO analyzed and interpreted the data. EO prepared the
409 manuscript with assistance from HA. All the authors revised the final version of the manuscript
410 while EO and PP acted as the corresponding authors.

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415

416 **Biological resources**

417 All CFBP stains are available directly at CIRM-CFBP: https://doi.org/10.15454/E8XX-4Z18

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423

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585

586 **Figures and Tables**

587 **Figure 1:** Sensitivity of the primer pair XtuF/XtuR in detection of *Xanthomonas translucens* pv.
588 *undulosa* in water suspension (A) compared to the culture plate method on YPGA
589 medium (B). The pathogen was detected in concentrations ranging from 1.1×10^5 cfu/ml
590 (A: lane 1) to 8.6×10^2 cfu/ml (A: lane 8) using the PCR primers XtuF/XtuR, while the
591 detection threshold for culture-based method was 1.8×10^3 cfu/ml (B). Lanes 1 – 14 in
592 "A" section show PCR using dilutions (1:1) of bacterial suspension starting from 1.1×10^5
593 cfu/ml (lane 1) to the 13th dilution where no bacterial DNA was detected. The primer pair
594 XtuF/XtuR was also capable of detecting the pathogen in seed wash of naturally infected
595 wheat seeds in concentration as low as 3.5×10^4 cfu/g (C). Culture plate method was able
596 to detect the pathogen in concentration of 50×10^5 cfu/g of the infected seeds (D).

597

598 **Table 1**: Bacterial strains used in this study for screening the *Xanthomonas translucens* pv.
599 *undulosa*-specific primer pair XtuF/XtuR.

600

601 **Figure S1:** Specificity (A) and sensitivity (B) test of the primer pair XtuF/XtuR for detection of
602 *Xanthomonas translucens* pv. *undulosa*. The primer pair XtuF/XtuR directed the
603 amplification of the expected 229 bp DNA fragment in all *X. translucens* pv. *undulosa*
604 strains (lanes 1-3) while no amplification was observed in the closely-related pathovars
605 (lanes 4-5: *X. translucens* pv. *translucens*). No amplification was also observed in the
606 other plant pathogenic *Xanthomonas* spp. strains *i.e.* lane 6: *X. arboricola* pv. *juglandis*;
607 lane 7: *X. citri* pv. *citri*; lane 8: *X. euvesicatoria* pv. *euvesicatoria*; lane 9: *X. euvesicatoria*
608 pv. *perforans*; lane 10: *X. hortorum* pv. *gardneri*; lane 11: *X. hortorum* pv. *pelargonii*; lane
609 12: *X. bonasiae*; and lanes 13-14: *Xanthomonas* sp. Sensitivity test for the primer pair
610 XtuF/XtuR using DNA concentrations of 1.8×10^4 pg/µl to 3×10^{-2} pg/µl showed that the
611 expected DNA fragment was amplified in 4.5 pg/µl of the *X. translucens* pv. *undulosa*
612 pure DNA (B: lane 13). The leftmost lane in both agarose gels is FastGene 100 bp DNA
613 marker.

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615 **Table S1:** Genomic data of the strains of *Xanthomonas translucens* used for targeting and
616 designing the pathovar specific primer pair XtuF/XtuR.

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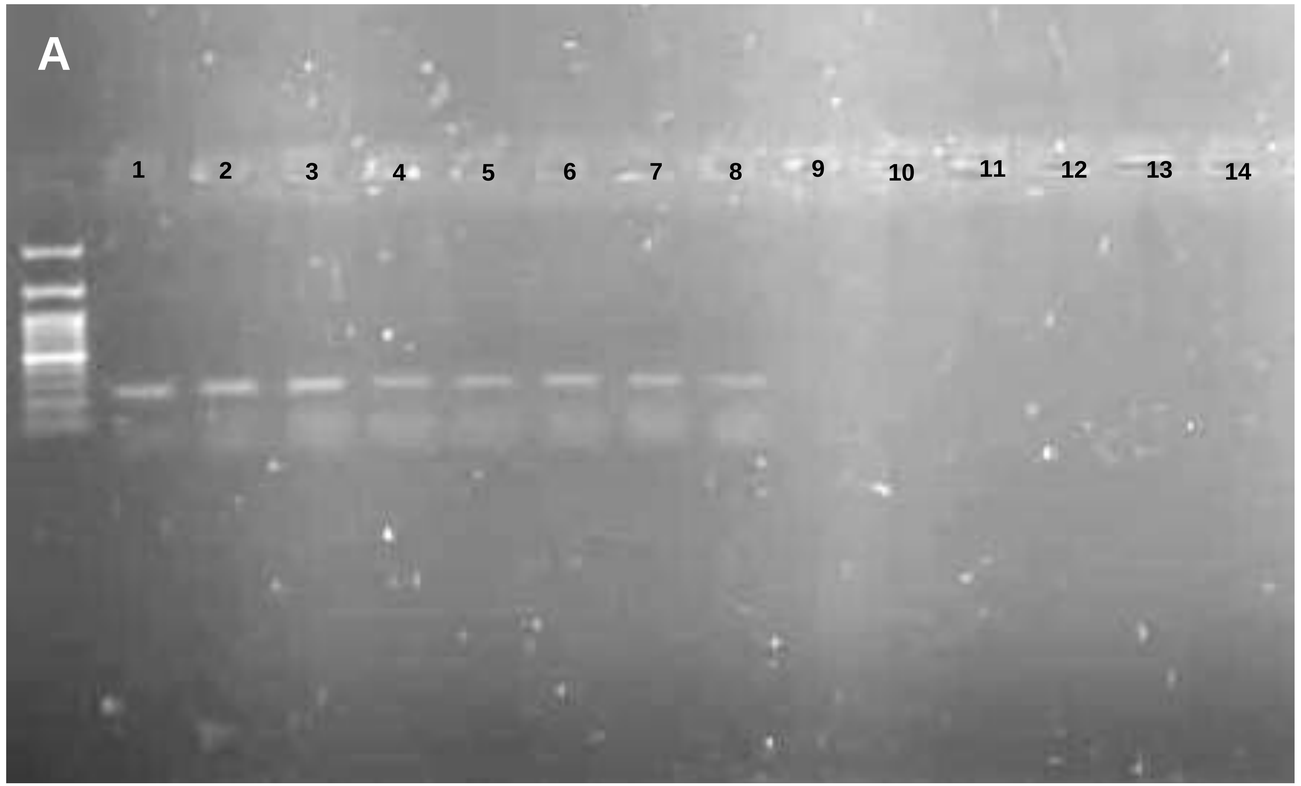
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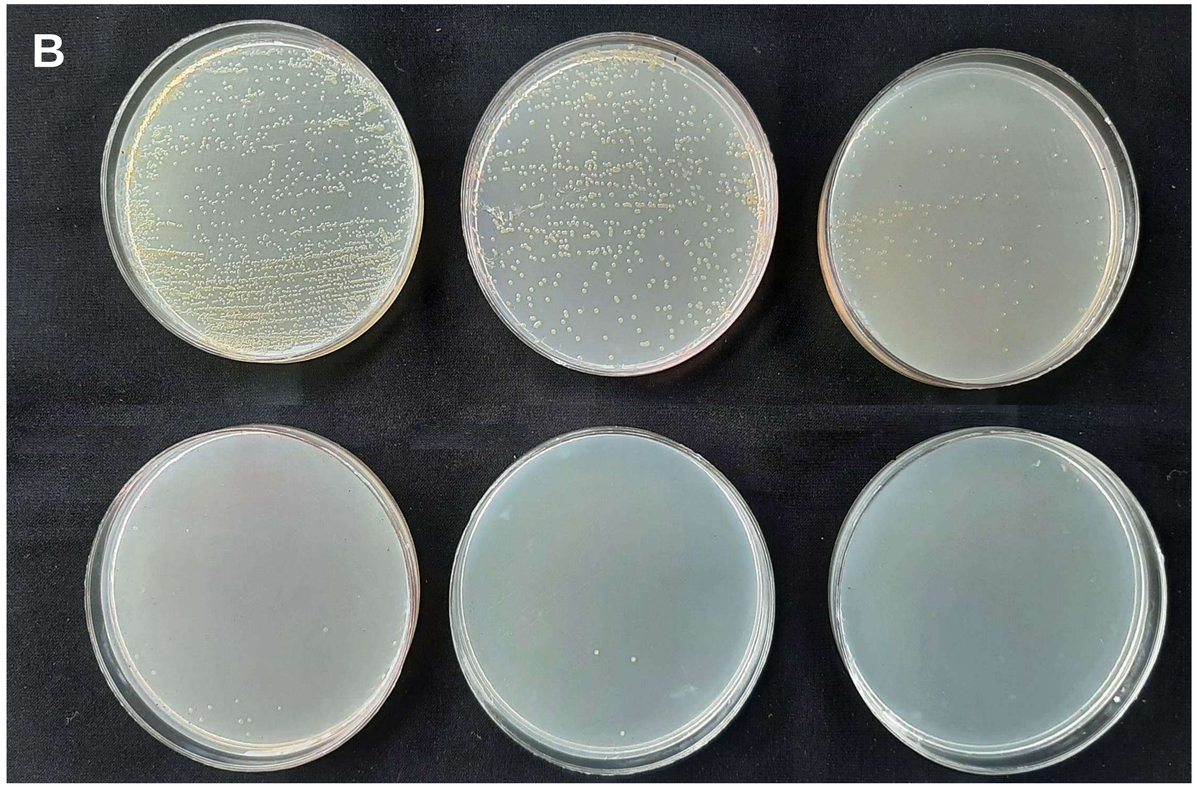
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Table 1: Bacterial strains used in this study for screening the *Xanthomonas translucens* pv. *undulosa*-specific primer pair XtuF/XtuR.

Taxon	Strain	Host of isolation	Country of isolation	Year of isolation	Colony color	Host range	Pathogenic on wheat / barley	PCR result
Xanthomonads associated with small grain cereals								
X. translucens pv. *undulosa*	CFBP 8908	Wheat	Iran	2015	PY	barley , oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 8909	Ryegrass	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 8904	Wheat	Iran	2016	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 8905	Wheat	Iran	2016	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtLr7	Wheat	Iran	2016	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtLr9	Wheat	Iran	2016	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtKm29	Wheat	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtHn3	Wheat	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtHn11	Wheat	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtZa5	Wheat	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	XtBu36	Wheat	Iran	2015	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 2055	Wheat	Canada	1966	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 3085	Wheat	Mexico	1987	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 4157	Wheat	Pakistan	1988	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 4159	Barley	Mexico	1987	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 4160	Wheat	Bolivia	1988	PY	barley, oat, rye and wheat	+/+	_
X. translucens pv. *undulosa*	CFBP 4161	Triticale	Ethiopia	1989	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 4164	Wheat	Madagascar	1991	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *undulosa*	CFBP 6540	Wheat	Mexico	1994	PY	barley, oat, rye and wheat	+/+	+
X. translucens pv. *translucens*	CFBP 8907	Barley	Iran	2014	PY	barley, oat and rye	-/+	-
X. translucens pv. *translucens*	XtKm9	Barley	Iran	2015	PY	barley, oat and rye	-/+	-
X. translucens pv. *translucens*	XtKm18	Barley	Iran	2015	PY	barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 8911	Barley	Iran	2015	PY	barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 2054	Barley	United States	1933	PY	Barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 2544	Barley	India	1970	PY	Barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 4158	Barley	Mexico	1987	PY	Barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 4162	Barley	Paraguay	1990	PY	Barley, oat and rye	-/+	-
X. translucens pv. *translucens*	CFBP 4163	Barley	Libya	1991	PY	Barley, oat and rye	-/+	-
X. translucens pv. *cerealis*	CFBP 2541	*Bromus inermis*	United States	1941	PY	Barley, oat and wheat	+/+	-

X. translucens pv. *cerealis*	CFBP 4165	*Bromus* sp.	Iran	1990	PY	Barley, oat and wheat	+/+	-
X. translucens pv. *cerealis*	CFBP 4166	*Bromus* sp.	Iran	1990	PY	Barley, oat and wheat	+/+	+*
X. translucens pv. *arrhenatheri*	CFBP 2056	*Arrhenatherum* sp.	Switzerland	1982	PY	Forage grasses	-/-	-
X. translucens pv. *arrhenatheri*	CFBP 2605	*Arrhenatherum* sp.	Germany	1986	PY	Forage grasses	-/-	-
X. translucens pv. *arrhenatheri*	CFBP 3631	*Arrhenatherum elatius*	Switzerland	1985	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 1977	*Lolium perenne*	Norway	1978	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 1978	*Dactylis glomerata*	Norway	1978	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 2058	*Lolium* sp.	Unknown	Unknown	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 2259	*Lolium* sp.	France	1983	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 2260	*Bromus cathartics*	France	1983	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 2470	*Lolium* sp.	France	1984	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 3524	*Dactylis glomerata*	Switzerland	1973	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 3630	*Lolium multiflorum*	Switzerland	1993	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6534	*Dactylis glomerata*	France	1987	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6535	*Dactylis glomerata*	France	1986	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6536	*Lolium perenne*	France	1989	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6537	*Lolium multiflorum*	France	1989	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6538	*Lolium perenne*	France	1989	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6547	*Festuca arundinacea*	France	1986	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6678	*Festuca arundinacea*	France	1986	PY	Forage grasses	-/-	-
X. translucens pv. *graminis*	CFBP 6692	*Lolium perenne*	France	1989	PY	Forage grasses	-/-	-
X. translucens pv. *phlei*	CFBP 2062	*Phleum* sp.	Switzerland	1982	PY	Forage grasses	-/-	-
X. translucens pv. *phleipratensis*	CFBP 2540	*Phleum pratense*	United States	1942	PY	Forage grasses	-/-	-
X. translucens pv. *poae*	CFBP 2057	*Poa* sp.	Switzerland	1982	PY	Forage grasses	-/-	-
X. translucens pv. *secalis*	CFBP 2539^{PT}	*Secale cereale*	Canada	1966	PY	Oat, rye, barley and wheat	+/+	+
Xanthomonas sp. **	CFBP 3730	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3731	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3732	*Hordeum vulgare*	Iran	1988	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3733	*Hordeum vulgare*	Iran	1989	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3734	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3735	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3736	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3737	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3738	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3739	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3740	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3741	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3742	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-

Xanthomonas sp. **	CFBP 3743	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3744	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	-
Xanthomonas sp. **	CFBP 3745	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3746	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3747	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3748	*Triticum aestivum*	Iran	1989	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3749	*Triticum aestivum*	Iran	1988	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3751	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3752	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3753	*Triticum aestivum*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3760	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3761	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Xanthomonas sp. **	CFBP 3762	*Hordeum vulgare*	Iran	1990	PY	Barley, oat and wheat	+/+	+
Non-xanthomonad pathogens of small grain cereals								
Clavibacter tessellarius	Ir2126	*Avena sativa*	Iran	2021	O	Barley, oat and wheat	+/+	-
Clavibacter zhangzhiyongii	Ir2130	*Hordeum vulgare*	Iran	2021	O	Barley and oat	-/+	-
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	CFBP 8392	*Triticum aestivum* L.	Brazil	2013	Y	Common bean	-	-
Pseudomonas syringae pv. *atrofaciens*	CFBP 3178	*Triticum aestivum*	Germany	1990	C	ND	+	-
Pseudomonas syringae pv. *coronafaciens*	CFBP 1627	*Avena sativa*	Canada	1954	C	Oat, rye and Italian ryegrass	ND	-
Pseudomonas syringae pv. *striafaciens*	CFBP 1674	*Avena sativa*	Unknown	Unknown	C	Wheat, oat or barley	+/+	-
Rathayibacter sp.	Ir3003	*Triticum aestivum*	Iran	2022	O	Wheat	-/+	-
Other bacterial pathogens								
Agrobacterium shirazense	NCPPB2659	*Rosa sp.*	Iran	2021	C	*Rosa* sp.	-	-
Bacillus subtilis	Koh34ysj2	Tomato *Lycopersicum esculentum*	Iran	2022	C	Non pathogenic	-	-
Cedecea neteri	CFPB 8900	Mushroom	Iran	2018	C	Mushroom	ND	-
Clavibacter michiganensis	M34	*Lycopersicum esculentum*	Iran	2021	Y	Tomato	-	-
Curtobacterium flaccumfaciens pv. *flaccumfaciens*	ICMP 22053	*Capsicum anuum*	Iran	2014	Y	Common bean	-	-
Ewingella americana	1157	Mushroom	Iran	2018	C	Mushroom	ND	-
Mycetocola	CFBP 8709	Mushroom	Iran	2018	C	Mushroom	-	-
Pseudomonas agarici	CFPB 895	Mushroom	Iran	2018	C	Mushroom	-	-
Pseudomonas azotoformans	1163	*Agaricus bisporus*	Iran	2018	C	Non pathogenic	-	-
Pseudomonas syringae pv. *syringae*	55	*Medicago sativa*	Iran	2016	C	ND	-	-

Pseudomonas tolaasii	CFBP 8707	Mushroom	Iran	2018	C	Mushroom	-	-
Ralstonia solanacearum	H31	*Solanum tuberosum*	Iran	2017	C	Potato, tomato	-	-
Serratia marcescens	Sh117	*Cucurbita pepo*	Iran	2018	R	Squash	-	-
X. arboricola pv. *juglandis*	CFBP 8813	*Juglans regia*	Iran	2019	PY	Walnut	-	-
X. bonasiae	CFBP 8700	*Ficus benjamina*	Iran	2019	PY	Fig	-	-
X. citri pv. *citri*	Xc82	*Citrus limon*	Iran	2021	PY	Citrus	-	-
X. euvesicatoria pv. *euvesicatoria*	ICMP 22095	*Capsicum anuum*	Iran	2016	PY	Tomato	-	-
X. hortorum pv. *gardneri*	ICMP 16689	*Lycopersicum esculentum*	Unknown	1953	PY	Tomato	-	-
X. hortorum pv. *pelargonii*	Xhp8	*Pelargonium* sp.	Iran	2020	PY	Pelargonium	-	-
X. euvesicatoria pv. *perforans*	ICMP 16690	*Lycopersicum esculentum*	Unknown	2016	PY	Tomato	-	-

ND: Not determined
PY: pale yellow
O: orange
Y: yellow
C: creamy
R: red
+: positive
-: negative
*: the pathovar status is questionable.
**: the pathovar status is undetermined.

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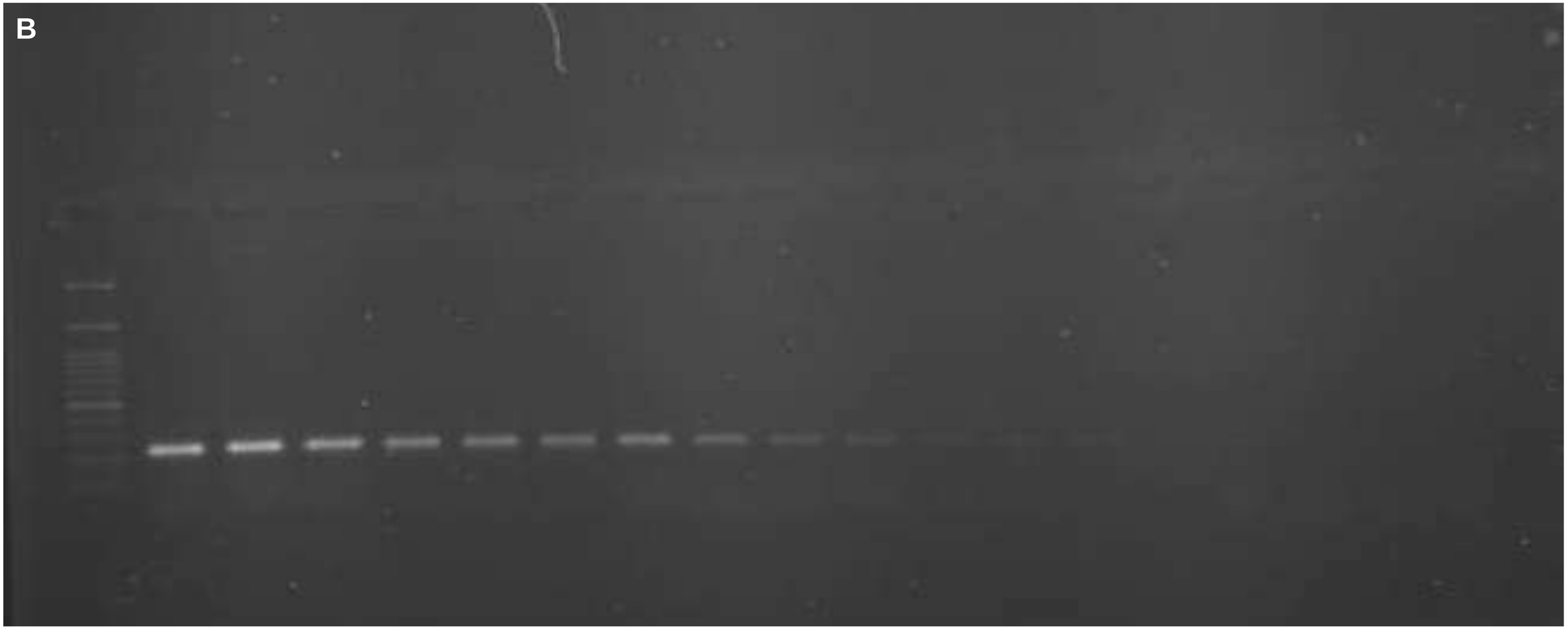
B

Table S1: Genomic data of the strains of *Xanthomonas translucens* used for targeting and designing the pathovar specific primer pair XtuF/XtuR.

Taxon	Strain	Genome size (mbp)	G + C content (%)	Protein-coding genes	RNA genes	Pseudo-genes	GenBank Accession number	Reference
Xanthomonas translucens pv. *undulosa*	Xtu 4699	4,561	68.1	3636	63	205	CP008714	Peng *et al*., 2016
Xanthomonas translucens pv. *undulosa*	ICMP 11055	4,761	67.8	3,835	63	224	CP009750	Falahi charkhabi *et al*., 2017
Xanthomonas translucens pv. *undulosa*	P3	4,618	68.1	3723	63	215	CP043500	Peng *et al*., 2019
Xanthomonas translucens pv. undulosa	LW16	4,746	67.8	3752	64	234	CP043540	Peng *et al*., 2019
Xanthomonas translucens pv. undulosa	CFBP 8908	4,581	68.01	3,654	63	205	CP064000	Shah *et al*., 2021
Xanthomonas translucens pv. undulosa	CFBP 8909	4,560	68.04	3,728	63	211	CP063997	Shah *et al*., 2021
Xanthomonas translucens pv. undulosa	CFBP 8904	4,563	68.04	3,731	63	211	CP063993	Shah *et al*., 2021
Xanthomonas translucens pv. undulosa	CFBP 8905	4,605	68.06	3,699	63	218	CP063996	Shah *et al*., 2021
Xanthomonas translucens pv. translucens	DSM 18974	4,715	67.7	3804	64	190	LT604072	Jaenike *et al*., 2016
Xanthomonas translucens pv. translucens	CFBP 8907	4,792	67.79	3,873	63	208	CP064004	Shah *et al*., 2021
Xanthomonas translucens pv. translucens	XtKm9	4,689	67.87	3,763	64	200	CP064003	Shah *et al*., 2021
Xanthomonas translucens pv. translucens	CFBP 8911	4,680	67.79	3,768	63	185	CP064001	Shah *et al*., 2021
Xanthomonas translucens pv. cerealis	CFBP 2541	4,518	67.34	3609	60	294	NZ_CM003052	Pesce *et al*., 2015
Xanthomonas translucens pv. cerealis	NXtc01	4,622	67.23	3,733	64	256	CP038228	Shah *et al*., 2019