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Isolation and identification of *Pseudomonas syringae* facilitated by a PCR targeting the whole *P. syringae* group

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Running title: PCR for the entire *P. syringae* group

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Abstract

We present a reliable PCR-based method to avoid the biases related to identification based on the conventional phenotypes currently used in the identification of *Pseudomonas syringae sensu lato,* a ubiquitous environmental bacterium including plant pathogens. We identified a DNA target suitable for this purpose by applying a comparative genomic pipeline to *Pseudomonas* genomes. We designed primers and developed PCR conditions that led to a clean and strong PCR product from 97 % of the 185 strains of *P. syringae* strains tested and gave a clear negative result for the 31 non-*P. syringae* strains tested. The sensitivity of standard PCR was determined with pure strains to be 10^6 bacteria mL^{-1} or 0.4 ng of DNA μL^{-1}. Sensitivity could be improved with the touchdown method. The new PCR-assisted isolation of *P. syringae* was efficient when deployed on an environmental sample of river water as compared to the isolation based on phenotypes. This innovation eliminates the need for extensive expertise in isolating *P. syringae* colonies, was simpler, faster and very reliable. It will facilitate discovery of more diversity of *P. syringae* and research on emergence, dispersion, and evolution to understand the varied functions of this environmental bacterium.

Introduction

There is growing concern worldwide about environmentally persistent pathogens. This new dimension of research on pathogens is making considerable progress for human pathogens (Aujoulat *et al.*, 2012) but it has received little attention for plant pathogens (Morris*, et al.*, 2009, Vayssier-Taussat, 2014, Bartoli *et al.*, 2015). For such studies it is essential to have reliable techniques for the isolation and/or identification of natural populations that can be present at low concentrations in substrates other than infected tissues such as rivers (Selezska*, et al.*, 2012) or insects (Carolan *et al.*, 2014). To promote research on the environmental persistence of plant pathogens and the role of environmental reservoirs in emergence of plant diseases, we have developed a technique to facilitate the tracking and identification of the ubiquitous plant pathogen, *Pseudomonas syringae sensu lato* (Morris*, et al.*, 2013). *P. syringae sensu lato* (named *P. syringae* or *P. syringae* group in the text) currently consists of a phylogenetic lineage containing *P. syringae*-related species (Mulet et al., 2010) together with *P. syringae* populations classified into phylogroups by MLSA (multi locus sequence analysis) (Berge *et al.*, 2014). The metapopulation of *P. syringae* comprises strains that are involved in various crop diseases (Lamichhane *et al.*, 2014, Lamichhane *et al.*, 2015), but many strains have been frequently isolated all along the water cycle both in and outside of agricultural zones and from a range of substrates. The environmental populations of *P. syringae* constitute a reservoir of plant pathogens (Monteil *et al.*, 2013, Bartoli *et al.*, 2015) and at the same time they belong to microbial communities and are likely to participate in the functioning and evolution of these communities. Among the various

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environmental impacts of *P. syringae*, strains of this bacterium are purported to contribute to meteorological phenomena via their ice nucleation activity that can trigger rain and snow fall from cloud droplets under specific conditions (Amato *et al.*, 2007, Morris *et al.*, 2014). It is important to be able to isolate the widest diversity of these populations but screening isolates is difficult because of the large genotypic and phenotypic diversity represented by this group of bacteria (Berge *et al.*, 2014). Actually the culture approach extensively used for isolating putative *P. syringae* strains is still the method with the highest sensitivity but it contains biases linked to the use of phenotypic properties. Moreover, strains from the *P. syringae* group have extensive diversity in virulence gene repertoires like those for effectors, toxins or plant hormone production (Baltrus *et al.*, 2011) that cannot be used to characterize the whole *P. syringae* group. Some molecular tools have been proposed for the specific detection of single pathogenic varieties so-called pathovars (Kong *et al.*, 2004, Gervasi & Scortichini 2009, Cho *et al.*, 2010, Gallelli *et al.*, 2011), a group of pathovars (Tegli *et al.*, 2010, Popovic *et al.*, 2014, Vaseghi *et al.*, 2014) or one phylogroup (Clarke *et al.*, 2010, Cottyn *et al.*, 2011). All these PCR-tools have in common that only few phylogroups and fewer than 50 strains were used to test their specificity. There is currently no molecular probe that targets the whole *P. syringae* group and this is reflected in the routinely conventional isolation of *P. syringae* mainly based on phenotypic tests (Kaluzna *et al.*, 2012). Our objective is to develop a reliable molecular probe for the entire *P. syringae* group and to illustrate the effectiveness of this probe with an environmental sample of river water from one of the sites where the greatest diversity of *P. syringae* in a single sample has been reported (Morris *et al.*, 2010).

Materials and methods

Bacterial strains

Bacterial strains used in this study are listed in supporting Table S1. For *P. syringae*, 185 strains were selected that represent the diversity of the 13 phylogenetic groups of *P. syringae* as recently described (Berge *et al.*, 2014). An additional 33 reference strains from outside the *P. syringae* group were used to determine the specificity of the PCR-based method (information about strains provided in Table S1). All strains were stored in nutrient broth containing 20 % glycerol at – 85°C and were cultivated on King's medium B (KB) (King *et al.*, 1954) at 25°C for 48 h before use.

Sequence analysis and primer design

Primer design for the specific detection of strains of the *P. syringae* group required targeting discriminating regions of the genome. In an initial step, this was accomplished with an in-house pipeline designed to take as inputs the genome sequences of strains DC3000, B728A and Pph1448A (http://www.ncbi.nlm.nih.gov/) and to scan DNA sequences to constitute what

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we call the ORFeome-like. The ORFeome-like corresponds to the entire set of extended ORFs defined as the DNA segments occurring between two stop codons in the six reading frames and exceeding 100 nucleotides. The ORFeome-like allows the exploration of the whole genome including intergenic regions, without any further assumption about the presence or not of coding sequences. For instance, the ORFeome-like from *P. syringae* DC3000 includes 111175 sequences. A pairwise sequence alignment was performed based on all-against-all BLAST comparisons. The results were stored in a MySQL database, and parameters such as identity percentage, alignment length, mismatches, gap openings, e-value and bit score were saved. We filtered the data to select the sequences with an identity percentage and an alignment length greater than 98% and 250 nucleotides respectively. Analysis of the genomic regions in 24 *P. syringae* genomes (including DC3000, B728A and Pph1448A) representing a wide diversity in the *P. syringae* group, together with 11 genomes of *Pseudomonas* sp. that are clearly outside the *P. syringae* group (additional information about strains is provided in Table S1) led to the identification of a common short sequence (166 bp) in *P. syringae* genomes beginning at position 4342391 in the *P. syringae* DC3000 genome (Fig. S1). This sequence was positioned astride two contiguous housekeeping genes *plsX* and *rpmF* coding respectively for the fatty acid/phospholipid synthesis protein PlsX and the ribosomal protein L32 (Fig. S2). Primers were designed manually across discriminant positions (Fig. S1): Psy_F 5'-ATG ATC GGA GCG GAC AAG 3' and Psy_R 5' GCT CTT GAG GCA AGC ACT 3' and allowed the amplification of a 144-bp DNA fragment. This PCR was named Psy-PCR.

P. syringae specific polymerase chain reaction (Psy-PCR)

Psy-PCR standard reactions were conducted in a final volume of 25 µL using the ready-to-use GoTaq reagents (Promega, France). Each reaction mix contained 14.17 µl of milliQ water, 5.0 µl of colourless GoTaq PCR buffer (5 x containing $MgCl_2$), 1.5 µL of $MgCl_2$ (25 mM), 0.2 µl of dNTP mix (25 mM each), 1 µl of each primer (10 µM), 0.13 µl of GoTaq DNA polymerase (5 units μl^{-1}) corresponding to final concentrations of dNTPs 0.2 mM, $MgCl_2$ at 1.5 mM, each primer at 0.4 µM, 1 x GoTaq flexi buffer and 0.65 units of GoTaq G2 flexi DNA polymerase; 2.0 µl of DNA template was added to this mixture. In routine tests, DNA template was replaced by cell suspensions adjusted to 10^8 colony-forming units (cfu mL^{-1}) with a spectrophotometer (A_{580} = 0.06). To evaluate the sensitivity of Psy-PCR, subsequent decimal dilutions of suspensions corresponding to 10^2 to 10^8 cfu mL^{-1} were tested as template. Strains used to test the specificity and the sensitivity of Psy-PCR are listed in supporting Table S1. Cell concentrations were checked on KB medium. In some cases DNA was extracted from 2 mL broth culture with a kit (QIAamp DNA minikit – Cat 51304) and used as template. DNA concentration was determined with a Nanodrop ND-1000

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spectrophotometer then diluted in sterile milliQ water to obtain 0.1 to 10 ng μl^{-1}. PCR was conducted in an Mastercycler® epGradient (Eppendorf) with initial polymerase activation for 5 min at 96°C followed by 30 cycles at 94°C for 30 s, 61°C for 30 s, 72°C for 30 s and a final extension at 72°C for 10 min. The Psy-PCR product detection was performed by electrophoresis through 1.5 % agarose gel with ethidium bromide at 0.5 µg mL^{-1} (Euromedex, EU0070) and then visualized under ultraviolet light. A preliminary touchdown-PCR (TD-PCR) was also performed to test its sensitivity using a sub-collection of 29 strains (additional information about strains is provided in Table S1). TD-PCR is a simple and rapid method to optimize PCR, increasing specificity, sensitivity and yield by using a high annealing temperature during the first cycles to enhance stringency and therefore enhancing the specificity of amplification. Hybridisation temperature is then gradually lowered during the following cycles to have a better efficiency of the PCR. TD-PCR is particularly useful for templates that are difficult to amplify but is also currently used to enhance specificity and product formation. TD-PCR was performed with the same mix but dNTPs were at 0.25 mM and primers at 0.6 µM with an initial step of 95°C for 5 min followed by 20 cycles of 94°C for 30 s, annealing temperatures starting at 62°C for 30 s then decreasing 0.5°C per cycle, and 72°C for 30 s for extension. This step was followed by 20 cycles at 94°C for 30 s, 55°C for 30 s, 72°C for 30 s and finally 72°C for 7 min.

Deployment of Psy-PCR to isolate *P. syringae* from environmental samples

Water was collected in September 2013 from the Tarn River (Castelbouc, Lozère, France, GPS coordinates: 44.339905, 3.465330). The microbiology of this river is of considerable importance because of the occasional presence of toxic cyanobacteria that can cause death of wild and domesticated animals when these animals drink river water during periods of blooms (Quiblier *et al.*, 2013). Hence, this river is regularly monitored for indicators of these blooms. Upstream of the site sampled here, a wide diversity of *P. syringae* (phylogroups 1, 2, 7, 9 and 10 (Berge *et al.*, 2014)) has been observed (Morris *et al.*, 2010). Microbial isolation from the water sample was conducted following previously-described procedures (Morris *et al.*, 2008) based on cultivation on a modified KB medium (KBC) containing selective agents (Mohan & Schaad, 1987). One bulk water sample was selected to test the method. Two replicates of the water sample (500 mL) were each filtered across a membrane (0.22 µm pore diameter – Millipore France GSWP 047 00) to concentrate bacteria before isolation. Each filter was agitated in 2.5 mL of filtrate to concentrate bacteria by a factor of 200. These suspensions were then plated on KBC medium and incubated for 4 days at 25°C.

The two replicates were then subjected to two different procedures to isolate colonies of *P. syringae*. For one of the replicates, colonies were collected and purified with the conventional screen based on expertise of the operator that involves recognition of phenotypes (typical

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colony morphology, production of a pale-blue pigment fluorescent under UV light, testing for cytochrome C oxidase activity and arginine dihydrolase activity). For the second replicate, all colonies growing on the KBC medium were screened with the Psy-PCR before purification. All these colonies were also purified by streaking on KB medium, tested again with the Psy-PCR and the phenotypes used in the conventional screen were assessed. Strains isolated through the conventional and the Psy-PCR screens were named "TAW" (for Tarn water) and "S2W" (for Strategy-2 water), respectively. To further validate the identity of Psy-PCR positive strains, their phylogenetic context was determined based on partial sequences of the citrate synthase (*cts*) housekeeping gene as previously described (Berge *et al.*, 2014). Primers Cts-FP (forward): 5' AGT TGA TCA TCG AGG GCG C(AT)G CC 3' and Cts-RP (reverse): 5' TGA TCG GTT TGA TCT CGC ACG G 3' (Sarkar & Guttman 2004, Morris *et al.*, 2010) were used for DNA amplification and primer Cts-FS (fwd): 5'-CCC GTC GAG CTG CCA ATW TTG CTG A-3' for sequencing. Strains that were negative for Psy-PCR were submitted to box-PCR fingerprinting (Versalovic *et al.*, 1994) so that we could select only one representative of each box-fingerprint to identify by sequencing of their *cts* or 16S rRNA gene. 16S rDNA was amplified as previously described (Berge *et al.*, 2002) using the primers Fd1 (forward): 5' AGA GTT TGA TCC TGG CTC AG 3' (Weisburg *et al.*, 1991) and S17 (reverse): 5' GTT ACC TTG TTA CGA CTT 3' (Achouak *et al.*, 1999). *Cts* and 16S rDNA sequences were deposited in the European Nucleotide Archive respectively under the accession numbers LN875503 to LN875544 and LN870360 to LN870385. Phylogenetic analysis of partial *cts* gene sequences was performed as described previously using *P. syringae* reference strains (Berge *et al.*, 2014). Alignment of sequences was made by using DAMBE (version 5) and a Neighbour joining tree was built with Mega (version 4).

Results

Detection of *P. syringae* with Psy-PCR is specific

For the 33 strains outside of the *P. syringae* group that were tested here, no visible PCR products were obtained (Table1, Fig. 1). For the 185 *P. syringae* strains, a PCR product of the expected size was obtained for 97% (180) of the strains. A PCR product could not be obtained for fewer than 3 % (5) of the strains (Table 1). Strains for which a PCR product could not be obtained comprised 1 strain from each of phylogroups 7 and 11 and all of the three strains tested for phylogroup 12. For the other ten phylogroups represented in this analysis (phylogroups 1 to 6, 8 to 10 and 13), 100 % of strains were positive for the Psy-PCR test (Table 1). The small number of strains currently available for phylogroup 12 limits the scope of the findings for this group. In phylogroup 11, one strain (83.1) among the 7 tested was negative, but we obtained a positive amplification (single weak product band) by increasing cell concentrations (>10^8 cfu mL^{-1}) (Fig 1).

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Sensitivity of Psy-PCR

The Psy-PCR tested on a range of decimal dilutions of *P. syringae* cultures with the standard protocol showed that for cell suspensions of concentrations less than 10^6 cfu mL^{-1}, corresponding to 2 10^3 cfu in a reaction mixture of 25 µL, no visible product was obtained. Using purified DNA, the minimum concentration to obtain a positive PCR was 0.4 ng $µL^{-1}$ corresponding to 0.8 ng per PCR reaction.

A touchdown Psy-PCR was tested and it improved the specificity of the test, by allowing the detection of two strains from phylogroup 12 (PG12) and one strain from phylogroup 11 (PG11) that were negative with the standard protocol (Table 2). For these three strains and for one more strain from PG11,a product band was visible with a cell suspension at 10^6 or 10^7cfu mL^{-1} while for the other *P. syringae* strains the minimal concentration to obtain a visible product was between 10^3 to 10^5 cfu mL^{-1} (corresponding to 2 to 200 cfu per 25 µL reaction). Strain PV612 was the only strain that gave negative results for all PCR using either standard or TD-PCR. Using purified DNA, the minimum concentration needed to obtain a positive result with TD-PCR was less than 0.1 ng $µL^{-1}$.

Characterization of strains giving negative results with standard Psy-PCR

We investigated why some strains (PV612, 83.1, GAW0112 and 113, see Table S1) did not amplify with the standard Psy-PCR. Primers were designed to the conserved sequences flanking the region amplified by the diagnostic primers and used with these strains. PCR products were sequenced and aligned with sequences of control strains (*P. syringae* and non-*P. syringae Pseudomonas* strains). This revealed between 1 to 3 mismatches in the sequence corresponding to the Psy-PCR reverse primer (Figure S3). A PCR with the forward primer Psy_F combined with a degenerated reverse primer (CGC YCT TKM GGC WAG CAC HC) that should amplify the sequences of these strains, was tested with the standard protocol. This PCR gave false positive responses and worked only with an expensive Taq DNA polymerase used for multiplex PCR. A more intensive exploration is needed to elaborate a protocol that yields no false responses.

The use of Psy-PCR improves the isolation efficiency of *P. syringae* from environmental samples

P. syringae was isolated from Tarn river water after concentration of samples via filtration. Using the conventional screening procedure, 24 colonies were identified as putative *P. syringae* strains among the 234 colonies that grew on the three plates of KBC medium (Table 3). Expertise of the operator was used to detect the putative *P. syringae* based mainly on colony morphology, pigments and the absence of cytochrome C oxidase. Some non-

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fluorescent but morphologically-typical colonies were also selected among these 24 colonies (Table 3). Phylogenetic analysis of partial *cts* gene sequences confirmed that 22 of these 24 strains were *P. syringae* and 2 strains (TAW80 and TAW100) belonged to other *Pseudomonas* species (Table 3, Fig 2). Psy-PCR was positive for the 22 *P. syringae* strains and negative for TAW080 and TAW100 strains. Overall for the conventional isolation method, 9.4 % of the colonies obtained on KBC medium were identified as *P. syringae*.
For the Psy-PCR-assisted screening procedure, all 95 colonies growing on one plate of KBC medium were analyzed with Psy-PCR conducted directly with cells from the colonies. Among these 95 colonies, 13 gave positive results in a preliminary Psy-PCR (Table S2). All 95 colonies were purified, yielding 108 strains. Among them 23 were no longer culturable after one sub-culturing (S2W-13, 18, 19, 22, 30, 34, 36 to 39, 42 to 47, 58 to 60, 70, 72, 73B, 81B). The non-culturability of most environmental bacteria has been brought to light previously in fresh water in particular (Amann *et al.*, 1995). Morphological differences after purification revealed that some of the colonies initially obtained on KBC medium were mixtures of strains. The Psy-PCR-assisted method indicated that among the 85 culturable strains 17 gave a positive response. Phylogenetic classification of positive strains confirmed these 17 strains to be *P. syringae.* One of these strains (S2W-16B) originated from a mixed isolate that tested negative in the first preliminary Psy-PCR (Table S2, Fig 2). One isolate (S2W-7) that tested positive in the first screen did not yield a *P. syringae* strain after purification (Table S2). Overall for the PCR-assisted method of isolation, 15 % of the colonies on KBC medium were identified as *P. syringae* in that they represented single strains of *P. syringae* or mixtures of *P. syringae* with other bacteria. Phenotypic tests of these *P. syringae* strains were variable particularly for strains belonging to phylogroup 7 (*P. viridiflava*) that could be non-fluorescent and variable for the oxidase test (Table S2). When comparing efficiency, Psy-PCR was better than the conventional method in particular for its higher relative accuracy and sensitivity (Table S3). The intraspecific diversity of the isolated *P. syringae* strains was comparable between the two protocols (Fig. 1). Altogether strains belonged to 5 *P. syringae* phylogroups among the 13 groups currently known and were dominated by phylogroups PG02 and PG07.

Discussion

We have developed a molecular probe for rapid, specific and sensitive identification of strains in the entire *P. syringae* group. Psy-PCR can be used directly with cells from colonies thereby permitting unbiased sorting of colonies for further characterization. This eliminates the need for expert recognition of *P. syringae* colonies or the bias that can be caused due to unexpected phenotypic variability in traits that have been used in the past to select putative *P. syringae* strains (Bartoli *et al.*, 2014, Berge *et al.*, 2014). We have also shown that typical

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P. syringae colony morphology can be masked by the mixture of *P. syringae* with other bacteria. This probe has been developed and validated on the basis of strains from the collection of *P. syringae* chosen for their geographical origin and isolation substrate (Table S1) and representing the full genetic diversity of the 13 phylogroups (Parkinson *et al.*, 2011, Berge *et al.*, 2014). This PCR tool will greatly facilitate the isolation of *P. syringae* especially from substrates where this bacterium is not very frequent. A direct PCR on DNA from substrates could be used to detect the presence of *P. syringae* especially when populations are high such as in plants or after an enrichment when populations are low (data not shown). A QRT analysis of DNA using the same DNA region could be employed to estimate the total *P. syringae* population size within the samples. However, in this paper we chose to develop a method combining the cultivation of bacteria and PCR detection. Cultivating bacteria is justified by the fact that a very efficient isolation method based on cultivation is available and able to detect low densities of this bacterium from environmental samples. By concentrating water samples by a factor of 100 x, for example, densities of cells as low as 10^2 cells of *P. syringae* L^{-1} can be detected with cultivation methods (Morris *et al.*, 2008). Direct PCR applied to the same concentrated samples would require about 10 times more bacteria per sample because of the smaller volumes of sample that are typically processed in PCR reactions than in dilution plating. In addition, obtaining isolated strains is necessary to determine the ensemble of traits associated with a given strain (pathogenicity, ice nucleating activity, etc). Psy-PCR is a powerful, effective tool that opens up many possibilities for exploring the diversity of *P. syringae* and its link to disease epidemiology.

Diagnosis of plant disease and the development of molecular tools to assist this diagnosis generally focus on one or a limited number of lineages that have been isolated from the populations that are dominant in infected tissue. As a consequence, highly specific probes limited to a narrow genetic range of strains have been proposed for diagnosis. Examples for the case of phytopathogenic *P. syringae sensu lato*, including *P. cichorii* (Hseu *et al.*, 2006), *P. savastanoi* (Penyalver *et al.*, 2000), *P. s.* pv. *tomato* (Zaccardelli *et al.*, 2005), *P. s.* pv. *actinidiae* (Mazzaglia *et al.*, 2011) and *P. s.* pv. *phaseolicolae* (Schaad *et al.*, 1995). These markers are useful to show whether a common strain is found during an outbreak or to trace pathogens in a regulatory legal framework. In contrast to this highly specific approach at the strain or the small group level, a more global method could be used in surveillance of reservoirs that are suspected to harbor a diversity of strains that are potential pathogens. This would be especially important for strains with broad host range as in the case of strains of phylogroup 1 from water (Bartoli *et al.*, 2015) or for which host range is not readily predictable from other phenotypes such as strains in phylogroups 7 and 8 (Bartoli *et al.*, 2014). A more global approach has been developed for some human pathogens that are ubiquitous in the environment such as *P. aeruginosa* (Selezska *et al.*, 2012) and for which a

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PCR that targets the species has been used (De Vos *et al.*, 1997). Likewise a detection method based on PCR was developed for *Ralstonia solanacearum* validated on diverse strains of the bacterium in several countries and laboratories (Opina et al., 1997) and for *Erwinia amylovora* causing fire blight in plants in the *Rosaceae* family, dispersed by insects and aerosols (Buhlmann *et al.*, 2013). For *P. syringae* a key to understanding the role of environmental populations in diseases of crop plants is to clearly account for their diversity, the fluctuations of their populations and their evolution. The corollary to the notion that environmental populations have roles in disease is that these environmental populations themselves play other roles in environmental processes. The most-frequently cited example for *P. syringae* is its potential role in rainfall due to its ice nucleation activity (Morris *et al.*, 2014). Detection tools that do not place special importance on markers associated with epidemic potential allow this latter concept to be explored more fully.

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Conflict of interest. None declared

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Table 1. Specificity of standard Psy-PCR based on 185 strains from the 13 *P. syringae* phylogroups and on 31 non-*P. syringae* strains. Additional information about strains is provided in Table S1

P. syringae phylogroups or other species*	N° of strains	Positive Psy-PCR reaction	% positive strains
P. syringae PG01	24	24	100
P. syringae PG02	47	47	100
P. syringae PG03	19	19	100
P. syringae PG04	4	4	100
P. syringae PG05	2	2	100
P. syringae PG06	1	1	100
P. syringae PG07	32	31	97
P. syringae PG08	5	5	100
P. syringae PG09	10	10	100
P. syringae PG10	25	25	100
P. syringae PG11	7	6	86
P. syringae PG12	3	0	0
P. syringae PG13	6	6	100
Total *P. syringae* strains	185	180	**97**
Pseudomonas aeruginosa group	1	0	0
Pseudomonas fluorescens group	16	0	0
Pseudomonas lutea group	2	0	0
Pseudomonas stutzeri group	2	0	0
Pseudomonas putida group	1	0	0
Pectobacterium sp.	3	0	0
Agrobacterium sp.	1	0	0
Erwinia sp.	3	0	0
Burkholderia	1	0	0
Dickeya	1	0	0
Pantoea sp.	1	0	0
Escherichia coli	1	0	0
Total Non-*P. syringae* strains	33	0	**0**

* the *P. syringae* phylogroups and *Pseudomonas* groups refer to the current classifications (Mulet*, et al.*, 2010, Berge*, et al.*, 2014)

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Table 2. Specificity and sensitivity of touch-down Psy-PCR. Fourteen strains from the 13 *P. syringae* phylogroups and 14 non-*P. syringae* strains were used. For all tested strains, decimal intervals of concentrations from 10^2 to 10^9 cells mL^{-1} were tested. Additional information about strains is provided in Table S1.

Strain name	Identification*	Psy-PCR product	Minimal concentration to obtain a visible product	
			Cfu mL^{-1}	ng DNA μL^{-1}
CFBP 1657	*P. syringae*-PG01	+	10^3	< 0.1
CFBP 1392	*P. syringae*-PG02	+	10^5	< 0.1
Pph1448A	*P. syringae*-PG03	+	10^5	< 0.1
CFBP 1634	*P. syringae*-PG04	+	10^5	< 0.1
CFBP 2341	*P. syringae*-PG05	+	10^5	< 0.1
CFBP 2067	*P. syringae*-PG06	+	10^3	< 0.1
CFBP 2107	*P. syringae*-PG07	+	10^3	< 0.1
CMO0085	*P. syringae*-PG08	+	10^5	< 0.1
CC1418	*P. syringae*-PG09	+	10^5	< 0.1
USA0032	*P. syringae*-PG10	+	10^4	< 0.1
CFBP 4407	*P. syringae*-PG11	+	10^4	< 0.1
83.1	*P. syringae*-PG11	+	10^7	< 0.1
IBSBF 1274	*P. syringae*-PG11	+	10^6	< 0.1
GAW0112	*P. syringae*-PG12	+ (weak)	10^7	< 0.1
GAW0113	*P. syringae*-PG12	+ (weak)	10^7	< 0.1
CCE0668	*P. syringae*-PG13	+	10^5	< 0.1
PV612	*P. syringae*-PG07	-	None	None
NFMJ 134	*P. stutzeri*	-	None	None
6B4	*P. rhizosphaerae*	-	None	None
37B2	*P. poae*	-	None	None
38B9	*P. graminis*	-	None	None
CFBP 1387	*P. marginalis* pv. *marginalis*	-	None	None
CFBP 2022	*P. salmonii*	-	None	None
CFBP 2066	*P. putida* bv A	-	None	None
CFBP 2068	*P. tolaasi*	-	None	None
CFBP 2102	*P. fluorescens*	-	None	None
CFBP 2124	*P. fluorescens*	-	None	None
CFBP 2133	*P. aureofaciens*	-	None	None
NCPPB 2445	*P. corrugata*	-	None	None
CFBP 2466	*P. aeruginosa*	-	None	None
CFBP 5593	*P. brassicacearum*	-	None	None

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* the *P. syringae* phylogroups refer to the current *P. syringae* classification (Berge*, et al.*, 2014)

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Table 3. Characteristics and identification of putative *P. syringae* strains isolated from Tarn river water with the conventional procedure based on phenotype screening

Strain	Fluorescence (KB medium)	Oxidase	PsyPCR product	Identification*
TAW078, 79, 86, 87, 90, 92, 96, 97, 99, 126	+	-	+	*P. syringae*-PG02
TAW081, 82, 83, 84, 85, 88, 89, 98, 130	+	-	+	*P. syringae*-PG07
TAW127	-	-	+	*P. syringae*-PG07
TAW129	-	-	+	*P. syringae*-PG13
TAW091	+	-	+	*P. syringae*-PG09
TAW080	W*	-	-	*Pseudomonas sp.*
TAW100	+	-	-	*Pseudomonas sp.*

* Identification was performed using the phylogenetic analysis of partial *cts* sequences (see Fig. 2). *P. syringae* phylogroups refer to the current *P. syringae* classification (Berge*, et al.*, 2014).W : weak

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FIGURES

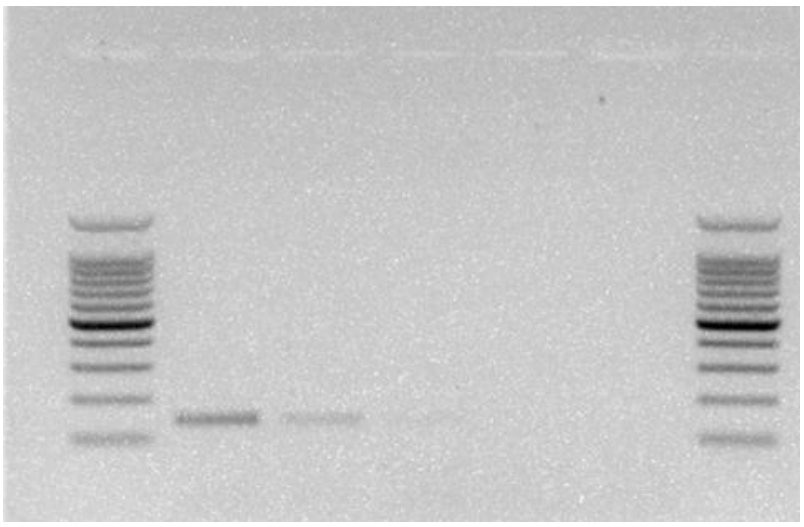

Fig. 1: Detection of *P. syringae* strain with PSY-PCR.

Lane 1 shows positive response, lane 2 and 3 show a single weak product band (obtained for example with strain 83.1 or with strains of PG12 with Touch-Down-PCR), lane 4 shows a negative response.and lane 5 is the control (water). A 100 bp molecular weight ladder was used.

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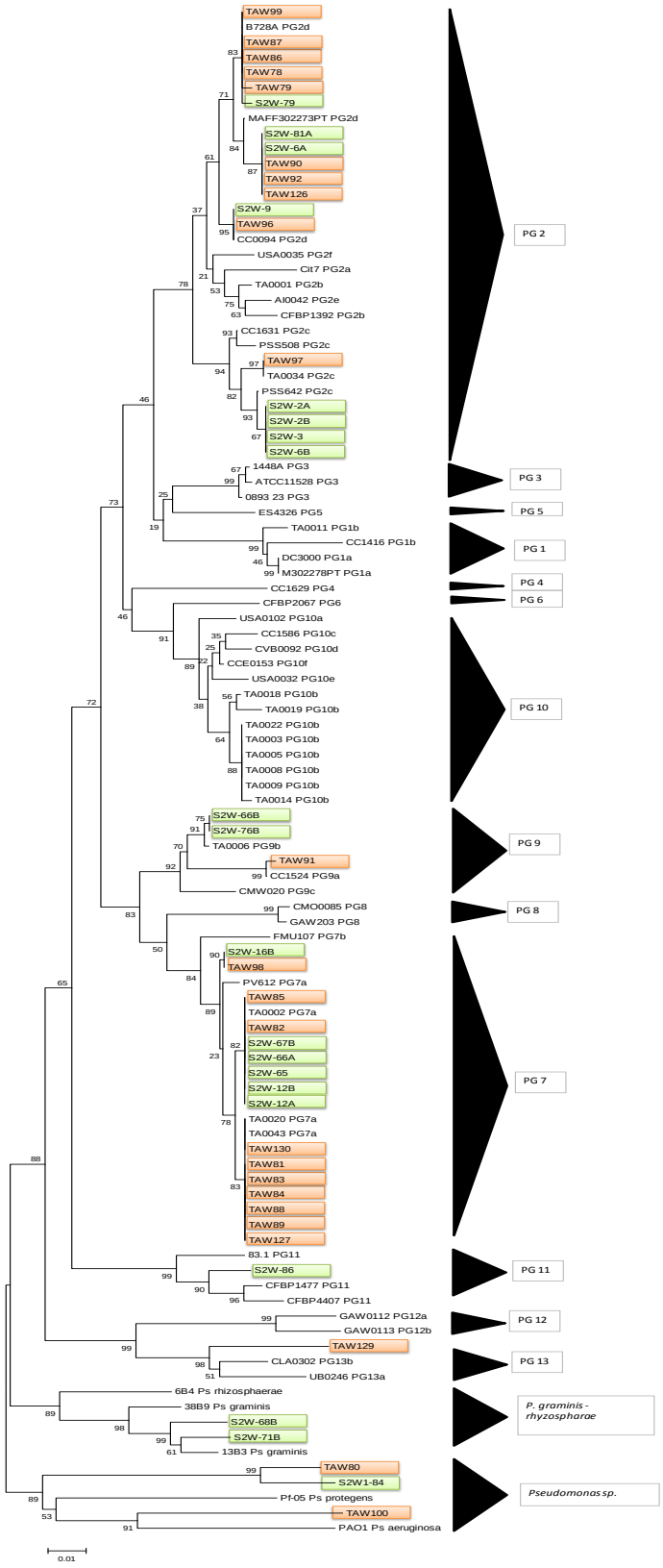

Fig. 2 : Phylogenetic tree of *P. syringae* strains isolated from Tarn river water.

Strains were screened based on expertise of the operator ("TAW" strains for TArn Water) or based on Psy-PCR-assisted screening ("S2W" strains for Strategy-2 Water). Tree was constructed with Neighbor-joining method based on *cts* partial sequences.

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