DOI: 10.1111/epp.12927

EPPO STANDARD ON DIAGNOSTICS

PM 7/43 (2) *Pseudomonas syringae* pv. *persicae*

Specific scope: This Standard describes a diagnostic protocol for *Pseudomonas syringae* pv. *persicae*.[1]
This Standard should be used in conjunction with PM 7/76 Use of EPPO diagnostic protocols.
Specific approval and amendment: Approved in 2004–09. Revised in 2023–03.
Authors and contributors are given in the Acknowledgements section.

1 | INTRODUCTION

Bacterial dieback of peach caused by strains of the *Pseudomonas syringae* complex identified as *Pseudomonas syringae* pv. *persicae* (EPPO/CABI, 1997) was described for the first time in 1967 on nectarine (*P. persica* subsp. *nucipersica*) and peach (*Prunus persica*) in France and almost simultaneously on nectarine, peach and Japanese plum (*Prunus salicina*) in New Zealand (Young, 1987, 1988). The pathogen has since then also been detected in Croatia, but its distribution remains limited in Europe.[2] For an updated geographical distribution consult EPPO Global Database (EPPO, 2022). The disease occurs mainly on the aboveground parts of trees and affects shoots, branches, leaves and fruits. Not all affected stone fruit species display the recognized symptoms. In addition, other pathovars of *Pseudomonas syringae* complex may cause similar symptoms on stone fruits hosts. The pathovars *syringae* and *morsprunorum* are widely spread in Europe. *P. syringae* pv. *syringae* may infect all stone fruits whereas *P. syringae* pv *morsprunorum* mainly infects sweet and sour cherry (*Prunus avium* and *Prunus cerasus*), plum (*Prunus domestica*) and apricot (*Prunus armeniaca*).

According to *rpoD* sequence similarity, *Pseudomonas syringae* pv. *persicae* clusters in phylogroup 1 (equivalent to genomospecies 3) (Parkinson & Elphinstone, 2010). This phylogroup comprises a few other pathovars of *P. syringae*, including *P. syringae* pv. *morsprunorum* and *P. syringae* pv. *avii*. Whole genome sequencing of *P. syringae* pv. *persicae* and *P. syringae* pv. *avii* also supports the fact that the two pathovars are extremely close phylogenetically (Ruinelli et al., 2019, S. McGreig, Fera, GB, personal communication). In vitro pathogenicity tests show that *P. syringae* pv. *avii* can be pathogenic when inoculated on peach and almond leaves (Ruinelli et al., 2019), but young trees of peach, apricot and plum inoculated with *P. syringae* pv. *avii* only exhibited restricted cankers, without subsequent development (Ménard et al., 2003).

Because of their similar host range, symptomatology and physiological, biochemical, and genetic characteristics, distinction of these pathovars is challenging but may be necessary depending on the regulatory status of these pathovars.

2 | IDENTITY

Name: *Pseudomonas syringae* pv. *persicae* (Prunier, Luisetti & Gardan, 1970; Young, Dye & Wilkie, 1978)
Synonyms: *Pseudomonas morsprunorum* f.sp. *persicae* (Prunier et al., 1970)
Taxonomic position: *Bacteria*, *Gammaproteobacteria*, *Pseudomonadales*, *Pseudomonadaceae*, *Pseudomonas* (Garrity, 2005)
EPPO Code: PSDMPE
Phytosanitary categorization: EPPO A2 list no.145, EU Regulated non quarantine pest (Annex IV)

3 | DETECTION

3.1 | Disease symptoms

In nectarine and peach, symptoms include shoot dieback, branch and root damage, tree death, leaf spots and fruit lesions. On Japanese plum, symptoms are mainly confined to dieback, occasional branch death, and leaf spots (Young, 1995). Dieback of terminal shoots can occur in autumn and in spring following the development of girdling lesions from nodal infections. Small elliptical lesions may develop at internodes. The rootstock can also be infected

[1]Use of brand names of chemicals or equipment in these EPPO Standards implies no approval of them to the exclusion of others that may also be suitable.

[2]Note that a similar strain, isolated in the UK in 1966 on *Prunus cerasifera*, was initially identified as *P. syringae* pv. *persicae* but sequencing of the *rpoD* gene has since then shown these isolates to be slightly different (pers. comm., A. Aspin [Fera, GB]).

showing symptoms similar to those on woody shoots. Leaf infection results in small, angular, water-soaked spots, the tissue of which becomes brown (Figure 1a). The necrotic tissue subsequently falls out, causing a 'shot hole' effect. On fruits, small, round, dark, oily spots occur. These can be spread within the fruit tissue, causing sunken, deforming lesions that ooze gum (Figure 1b).

Some symptoms of bacterial dieback due to *P. syringae* pv. *persicae* can be confused with those of bacterial canker of stone fruits (*Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *morsprunorum*) and symptoms of leucostoma canker (*Leucostoma* spp.) or frost injury. Distinctive characteristics of dieback are discoloration of wood in branches above the necrosis and the absence of an obvious boundary between the morbid and healthy bark in the lower parts of the tree (Figure 1c). Bacterial dieback can be disseminated with infected plants for planting or contaminated pruning tools.

3.2 | Isolation

Bacteria can be isolated directly from diseased tissue by cutting out the tissue from the border between apparently healthy tissue and the necrotic area. Prior to removal, the tissue should be disinfected. Small pieces of such tissue are crushed in a small amount (e.g. 1–2 mL) of sterile water. After around 10 min, the suspension is streaked onto King's B (Lelliott & Stead, 1987), SNA (Lelliott & Stead, 1987) and any of the following media: Modified King's B (Kałużna et al., 2012); CSGM (Lelliott & Stead, 1987); and CSGA (Luisetti et al., 1972). After 3–4 days' incubation at 25°C colonies on King's B, Modified King's B, CSGM, and CSGA are irregular, small (2–3 mm in diameter), grey, flat and translucent. It grows significantly more slowly on King's B medium than *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum*. When observed under UV light the *P. syringae* pv. *persicae* colonies on King's B will not fluoresce, but most colonies of *P. syringae* pv. *persicae* on Modified King's B, CSGM and CSGA will fluoresce (80% of the strains tested, Luisetti, 1988). *P. syringae* pv. *persicae* produces small 'Levan-type' colonies on SNA after 4–6 days' incubation at 25°C which is slower than *P. syringae* pv. *syringae* and *P. syringae* pv. *morsprunorum* (Gašić et al., 2012) (Figure 2). Comparison with the reference strain on the same medium is recommended.

FIGURE 1 Symptoms of *P. syringae* pv. *persicae* on a peach leaf (a), fruit (b) and tree trunk (c). Photo courtesy: INRAE (FR), E. Osdaghi (University of Tehran, IR) and Landcare Research (NZ, under CC BY 4.0).

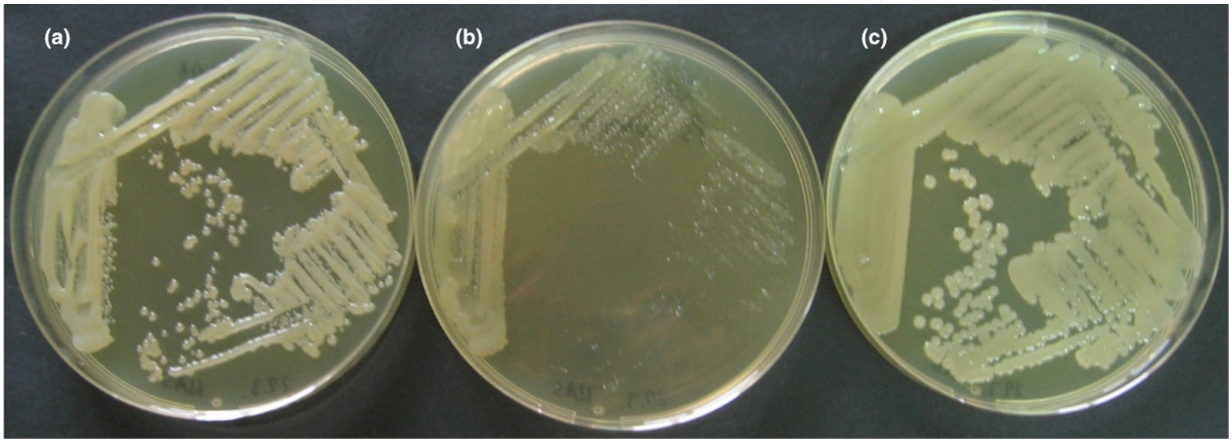

FIGURE 2 'Levan-type' colonies on SNA medium (a) *P. syringae* pv. *morsprunorum* (KFB 0101), (b) *P. syringae* pv. *persicae* (KFB 0102), (c) *P. syringae* pv. *syringae* (KFB 0103). Photo courtesy: K. Gašić (IPPE, Serbia).

3.3 | Description of the pathogen

Gram-negative short rod, motile due to 3–6 polar flagella, obligatorily aerobic with an optimum growth temperature of about 24°C.

4 | IDENTIFICATION

Pure cultures of presumptive *P. syringae* pv. *persicae* isolates should be identified using at least two tests, based on different biological principles, or targeting different genetic loci. For critical cases (EPPO, 2018), a confirmative pathogenicity test is recommended.

4.1 | Molecular tests

4.1.1 | Rep-PCR

P. syringae pv. *persicae* can be distinguished from the other pathovars attacking stone fruits on the basis of rep-PCR profiles or other fingerprinting methods i.e. PCR Melting Profile (Kałużna et al., 2010; Masny & Płucienniczak, 2003). *P. syringae* pv. *persicae* can be differentiated from *P. syringae* pv. *avii* based on BOX and ERIC-PCR profiles but not REP-PCR profiles (Ménard et al., 2003). Descriptions of Rep-PCR tests are available in PM 7/100 *Rep-PCR tests for identification of bacteria* (EPPO, 2010).

4.1.2 | DNA sequencing method

Analysis of the 16S rRNA gene sequence may be used to differentiate *Pseudomonas* from other genera but cannot distinguish all the different species/pathovars within that genus (Lu et al., 2017). A protocol for routine barcoding of bacteria using 16S rDNA is described in appendix 2 of the EPPO Standard PM 7/129(2) *DNA barcoding as an identification tool for a number of regulated pests* (EPPO, 2021).

Based on partial *rpoD* (described by Parkinson & Elphinstone, 2010) gene sequences, it is possible to differentiate strains of *P. syringae* pv. *persicae* from *P. syringae* pv. *morsprunorum* but not from *P. syringae* pv. *avii* (Inman et al., 2011; Lu et al., 2017; Parkinson et al., 2011). However, in silico analysis suggests the *rpoD* primers described by Hwang et al. (2005) can differentiate *P. syringae* pv. *persicae* from *P. syringae* pv. *avii* (S. McGreig, Fera, GB, personal communication). Sequencing of the *gyrB* gene can be used to check that no homologous recombination between the housekeeping genes of strains belonging to different species has occurred (M. Fischer-Le Saux, INRAE, FR, personal communication).

Tests based on the analysis of *rpoD* sequences and *rpoD* and *gyrB* sequences are described in Appendices 2 and 3. Sequence analysis should follow the guidelines described in appendices 7 and 8 of the EPPO Standard PM 7/129 (EPPO, 2021).

4.2 | Phenotypic tests

P. syringae pv. *persicae*, along with *Pseudomonas syringae* pv. *syringae*, *Pseudomonas syringae* pv. *morsprunorum* and *Pseudomonas syringae* pv. *avii*, belongs to LOPAT Group Ia of the determinative scheme of Lelliott et al. (1966). Among *P. syringae* pathovars only *P. syringae* pv. *persicae* produced the exotoxin persicomycin (Ballio et al., 1994; Barzic & Guittet, 1996). However, the production of this toxin is thermoregulated and its role in pathogenicity is unclear (Barzic, 1999).

Detailed differences between *P. syringae* pv. *persicae*, *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syringae* pv. *morsprunorum* are shown in Table 1. Biochemical characters of *Pseudomonas syringae* pv. *avii*

TABLE 1 Biochemical characters of *Pseudomonas syringae* pv. *persicae* in comparison with pathovars *syringae* and *morsprunorum*.

Test[a]	*P. syringae* pv. *persicae*	*P. syringae* pv. *syringae*	*P. syringae* pv. *morsprunorum*
Fluorescence on King's B medium	−	+	+ or −
Fluorescence on CSGM[b]	+	+	+
Levan production	+	+	+
Gelatine hydrolysis	−	+	+
Aesculin hydrolysis	−	+	+
Acid production from			
Inositol	−	+	+
Sorbitol	+	+	+
Erythritol	−	+ or −	+ or −
Utilization of			
DL lactate	−	+ or −	−
D(−) tartrate	−	+ or −	−
L(+) tartrate	−	−	+

[a]Fluorescence – appearance of green or blue pigment which diffuses into medium visible under UV-light; levan production – occurrence of mucoid colonies on sucrose-rich medium; gelatin hydrolysis – liquefaction of solid medium; aesculin hydrolysis – dark brown discoloration of the medium; remaining tests – yellow discoloration of medium. For preparation of media and performance of tests, see Lelliott and Stead (1987), Fahy and Persley (1983), and Schaad (1988).

[b]Casamino–sucrose–gelatin medium (Lelliott & Stead, 1987).

can be found in Ménard et al. (2003). Biochemical differentiation can be done using Biolog GEN III.

4.3 | Pathogenicity tests

4.3.1 | Preparation of inoculum

For preparation of inoculum, 24–48 h cultures of the tested isolate grown on King's B medium (or any other non-selective (rich) medium) are used. Bacteria are rinsed from the medium surface with sterile water. The suspension should be adjusted to approximately 10^7 cfu mL^{-1} with a turbidimeter/spectrophotometer or by eye to a slightly turbid suspension. Cell numbers in the bacterial suspension can be determined afterwards by dilution plating.

4.3.2 | Hypersensitivity test on tobacco

The bacterial suspension is injected into the interveinal, intercellular spaces of a tobacco leaf (cv. 'White Burley' or 'Hicks') with a needleless syringe. Fully developed leaves can be more easily injected than younger ones. After injection, the intercellular spaces become water-soaked for a short time but within about 1 h the leaf regains its original state. The test result is considered positive if the injected tissue becomes flaccid or necrotic in 24 h or less. The reference strain should be used as a positive control and sterile distilled water should be used as a negative control and the negative control should remain symptomless.

4.3.3 | Pathogenicity on shoots

The pathogenicity of the bacterial isolate can be determined by inoculation of young dormant (one-year-old) shoots of young trees of susceptible cultivars of peach, nectarine or plum, growing under standard conditions in a non-heated glasshouse (during the period from mid September to the end of January). Shoots can be inoculated by introduction of a drop of bacterial suspension (approximately 10^7 cfu mL^{-1}) onto a wound made to the xylem by a single transverse incision with a scalpel or on a fresh leaf scar. The inoculated wound or leaf scar should be wrapped in plastic tape for 5 days. Necrosis should be observed and measured in the following spring, by comparison with the controls (treatment of wound or leaf scar with sterile water, and with the reference strain). For each isolate, at least 5 inoculations should be done.

5 | REFERENCE MATERIAL

Type strain LMG 5184 (CFBP 1573; = ICMP 5846; = NCPPB 2761).

6 | REPORTING AND DOCUMENTATION

Guidelines on reporting and documentation are given in EPPO Standard PM 7/77 Documentation and reporting on a diagnosis.

7 | PERFORMANCE CHARACTERISTICS

When performance characteristics are available, these are provided with the description of the test. Validation data is also available in the EPPO Database on Diagnostic Expertise (http://dc.eppo.int), and it is recommended that this database is consulted as additional information may be available there (e.g. more detailed information on analytical specificity, full validation reports, etc.).

8 | FURTHER INFORMATION

Further information on this organism can be obtained from:

Aspin A, Fera Science Ltd, Sand Hutton, YO41 1LZ (United Kingdom). E-mail: andrew.aspin@fera.co.uk

9 | FEEDBACK ON THIS DIAGNOSTIC PROTOCOL

If you have any feedback concerning this Diagnostic Protocol, or any of the tests included, or if you can provide additional validation data for tests included in this Protocol that you wish to share please contact diagnostics@eppo.int.

10 | PROTOCOL REVISION

An annual review process is in place to identify the need for revision of Diagnostic Protocols. Protocols identified as needing revision are marked as such on the EPPO website. When errata and corrigenda are in press, this will also be marked on the website.

ACKNOWLEDGEMENTS

This protocol was originally drafted by Dr P. Sobiczewski (Research Institute of Pomology and Floriculture, Skierniewice, PL) and Dr L. Gardan (INRAE Beaucouzé, FR). Revision of the protocol was prepared by Monika Kaluzna (Research Institute of Pomology and Floriculture, Skierniewice, PL) and Andy Aspin (Fera Science, Ltd, GB). Ms Fischer-Le-Saux (INRAE, FR) contributed to the preparation of Appendix 3. It was reviewed by the Panel on Diagnostics in Bacteriology.

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How to cite this article: EPPO (2023) PM 7/43 (2) *Pseudomonas syringae* pv. *persicae*. *EPPO Bulletin*, 53, 277–284. Available from: https://doi.org/10.1111/epp.12927

APPENDIX 1 – PREPARATION OF MEDIA AND BUFFERS

All media are sterilized by autoclaving at 121°C for 15 min, except when stated otherwise.

1. Media

King's B medium (Lelliott & Stead, 1987)

Microbiological grade agar	15.0 g
Bacto proteose peptone no. 3	20.0 g
K_2HPO_4	1.5 g
$MgSO_4$	1.5 g
Glycerol	10 mL
Distilled water	1 L

Adjust pH to 7.2

Modified King's B Medium (Kałużna et al., 2012)

Microbiological grade agar	15.0 g
Bacto proteose peptone no. 3	20.0 g
K_3PO_4	1.8 g
$MgSO_4 \cdot 7H_2O$	1.5 g
Glycerol	10 mL
Distilled water	1 L

Adjust pH to 7.2

Casamino-sucrose-gelatin medium (CSGM) (Lelliott & Stead, 1987)

Microbiological grade agar	20.0 g
Gelatin	30.0 g
Casaminio acid (vitamin free)	10.0 g
Sucrose	10.0 g
$MgSO_4 \cdot 7H_2O$	1.0 g
Distilled water	1 L

Adjust pH to 7.2

Casamino-sucrose-gelatin agar (CSGA) (Luisetti et al., 1972)

Microbiological grade agar	20.0 g
Gelatin	30.0 g
Casaminic acid (vitamin free)	10.0 g
Sucrose	10.0 g
K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	1.0 g
Distilled water	1 L

Adjust pH to 7.2

Sucrose Nutrient Agar (SNA) (Lelliott & Stead, 1987)

Nutrient agar (Oxoid CM0003)	28.0 g
Sucrose	50.0 g
Distilled water	1 L

The pH will be 7.4 (±0.2) and should not need adjustment.

2. Buffers

Phosphate Buffer Saline

10 mM phosphate buffered saline (PBS) solution	
NaCl	8 g
KCl	0.2 g
$Na_2HPO_4 \cdot 12\ H_2O$	2.9 g
KH_2PO_4	0.2 g
Distilled water	To make up to 1 L

Adjust pH to 7.2 and autoclave.

APPENDIX 2 – SEQUENCING OF *rpoD* GENE (PARKINSON & ELPHINSTONE, 2010)

The test below differs from the one described in the original publication.
Other equipment, kits or reagents may be used provided that a verification (see PM 7/98) is carried out.

1. General Information

1.1. This test is suitable for the identification of *P. syringae* pv. *persicae* cultures by sequencing.
1.2. This test is using primers developed by Parkinson & Elphinstone, 2010.
1.3. This test targets a sequence located in the *rpoD* gene of *Pseudomonas* spp.
1.4. Oligonucleotides and amplicon size:

	Primer	Sequence (5′→3′)	Amplicon size (bp)
Forward primer	PsrpoD FNP1	5′-TGA AGG CGA RAT CGA AAT CGC CAA-3′	700
Reverse primer	PsrpoDnprpcr1	5′-YGC MGW CAG CTT YTG CTG GCA-3′	

2. Methods

2.1. Nucleic Acid Extraction and Purification
Add bacteria to 100 µL sterile distilled water to give a light turbidity (~0.1–0.2 absorption reading on the spectrophotometer at 650 nm). Heat at 95–100°C for 5–10 min (Parkinson, personal communication, 2012). Alternatively, suspend the cells in 300 µL of 6% Chelex 100 suspension by vortexing. Boil at 100°C for 8 min. Immediately following boiling, vortex at high speed for 10 s and chill on ice or in a frozen tube rack. Centrifuge the chilled microtube at 18 000 *g* for 5 min before transferring 200 µL of the supernatant to a clean microtube. Use this supernatant as template DNA.

2.2 The lysate can be stored at approximately −20°C.
2.3 Polymerase chain reaction
2.3.1 *Master Mix*

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	19.0	N.A.
PCR Master Mix (Thermo Fisher)	2×	25.0	1×
Forward primer PsrpoDFnp1	10 µM	2.0	0.4 µM
Reverse primer PsrpoDnprpcr1	10 µM	2.0	0.4 µM
Subtotal		48.0	
Nucleic acid extract		2	
Total		50	

2.3.2 *PCR cycling conditions*: 2 min at 94°C, 34 cycles of 45 secs at 94°C, 1 min at 47°C and 1 min at 72°C, with a final extension step for 7 min at 72°C.
2.3.3 Sanger sequencing was performed on forward and reverse strands.

3. Essential Procedural Information

3.1. Controls
For a reliable test result to be obtained, the following (external) controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification preferably of clean extraction buffer.
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the target organism.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This can include nucleic acid extracted from the target organism, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product).

3.2 Interpretation of results

Verification of the controls

- NIC and NAC: no band is visualized
- PIC, PAC a band of the expected size is visualized.

When these conditions are met

- A test for which a band of the expected size is visualized is used for sequencing.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance characteristics available

See Parkinson & Elphinstone, 2010, Parkinson et al., 2011, Inman et al., 2011.

APPENDIX 3 – SEQUENCING OF *rpoD* AND *gyrB* GENE (HWANG ET AL., 2005)

The test below differs from the one described in the original publication.
The test below is described as it was carried out to generate the validation data provided in Section 4. Other equipment, kits or reagents may be used provided that a verification (see PM 7/98) is carried out.

1. General Information

1.1. These tests are suitable for the identification of *P. syringae* pv. *persicae* cultures by sequencing
1.2. These tests are based on the primers developed by Hwang et al., 2005
1.3. These tests target sequences located in the *rpoD* gene and *gyrB* genes of *Pseudomonas* spp.
1.4. The results can be individually analysed or combined for multilocus sequence analysis
1.5. Oligonucleotides and amplicon size:

	Primer	Sequence (5′→3′)	Amplicon size (bp)[a]
Forward primer	rpoD+364s	5′-GYG AAG GCG ARA TYG RAA TCG-3′	898
Reverse primer	rpoD−1222ps	5′-CCG ATG TTG CCT TCC TGG ATC AG-3′	

[a] Including primers.

Primer		Sequence (5′→3′)	Amplicon size (bp)[a]
Forward primer	gyrB+271ps	5′-TCB GCR GCV GAR GTS ATC ATG AC-3′	749
Reverse primer	gyrB-1022ps	5′-TTG TCY TTG GTC TGS GAG CTG AA-3′	

[a] Including primers.

2. Methods

2.1. Nucleic Acid Extraction and Purification

2.1.1. For crude DNA extraction from presumptive *P. syringae* pv. *persicae* cultures and from cultures of reference strains, suspend approximately 1 µL of cell material (e.g., using a 1 µL disposable inoculating loop) or one colony in 100 µL of sterile distilled water. Heat in closed microvials at approximately 95–100°C for 5–10 min. A freezing step before the heating may be performed.

2.1.2. The lysate can be stored at approximately −20°C.

2.2. Polymerase chain reaction

2.2.1. *Master Mix*

Reagent	Working concentration	Volume per reaction (µL)	Final concentration
Molecular grade water	N.A.	25.6	N.A.
$MgCl_2$	25 mM	3.0	1.5 mM
dNTPs	2.5 mM each	4.0	0.2 mM each
Forward primer	20 µM	1.0	0.4 µM
Reverse primer	20 µM	1.0	0.4 µM
Green Flexi GoTaq reaction buffer (Promega)	5×	10	1×
GoTaq Flexi DNA polymerase (Promega)	5 U/µL	0.4	0.04 U/µL
Subtotal		45	
Nucleic acid extract		5	
Total		50	

2.2.2 *PCR cycling conditions*: 2 min at 95°C, 35 cycles of 45 s at 95°C, 45 s at 64°C and 1 min at 72°C, with a final extension step of 5 min at 72°C.

2.2.3 Sanger sequencing was performed on forward and reverse strands.

3. Essential Procedural Information

3.1. Controls

For a reliable test result to be obtained, the following (external) controls should be included for each series of nucleic acid extraction and amplification of the target organism and target nucleic acid, respectively.

- Negative isolation control (NIC) to monitor contamination during nucleic acid extraction: nucleic acid extraction and subsequent amplification preferably of clean extraction buffer.
- Positive isolation control (PIC) to ensure that nucleic acid of sufficient quantity and quality is isolated: nucleic acid extraction and subsequent amplification of the target organism.
- Negative amplification control (NAC) to rule out false positives due to contamination during the preparation of the reaction mix: application of the amplification procedure to molecular grade water that was used to prepare the reaction mix.
- Positive amplification control (PAC) to monitor the efficiency of the amplification: amplification of nucleic acid of the target organism. This can include nucleic acid extracted from the target organism, whole genome amplified DNA or a synthetic control (e.g. cloned PCR product).

3.2 Interpretation of results

Verification of the controls

- NIC and NAC: no band is visualized.
- PIC, PAC a band of the expected size is visualized.

When these conditions are met

- A test for which a band of the expected size is visualized is used for sequencing.
- Tests should be repeated if any contradictory or unclear results are obtained.

4. Performance characteristics available

Validation data were obtained from CIRM-CFBP (FR). Using the strains available in this collection (including 135 strains of *P. syringae* pv. *persicae*, 4 strains of *P. syringae* pv. *avii* and the type strain of *P. syringae pv. morsprunorum*), it is possible to differentiate strains of *P. syringae* pv. *persicae* from *P. syringae* pv. *morsprunorum* type strain but not from *P. syringae* pv. *avii* strains using (i) *rpoD* alone, (ii) *gyrB* alone or (iii) with both (MLSA) (Fischer-Le-Saux M, personal communication, 2021). The test may have been adapted further and validated or verified using other critical reagents, instruments and/or further modifications. If so, the corresponding test descriptions and validation data can be found in the EPPO database on diagnostic expertise (section validation data http://dc.eppo.int/validationlist.php).