British Microbiology Research Journal
16(5): 1-10, 2016, Article no.BMRJ.27954
ISSN: 2231-0886, NLM ID: 101608140

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Detection, Survival, and Source of Inoculum of *Pseudomonas syringae* pv. *syringae* from Weeds and Plant Debris in Relation to Epidemiology of Bacterial Citrus Blast and Black Pit in Tunisia

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Authors' contributions

This work was carried out in collaboration between both authors. Author IM designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed literature searches. Authors IM and NBM managed the analyses of the study and literature searches. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BMRJ/2016/27954
Editor(s):
(1) Akikazu Sakudo, University of the Ryukyus, Japan.
Reviewers:
(1) Pongsak Rattanachaikunsopon, Ubon Ratchathani University, Thailand.
(2) Ashraf S. Hakim, National Research Centre, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/15895

Original Research Article

Received 27th June 2016
Accepted 4th August 2016
Published 23rd August 2016

ABSTRACT

Background: Citrus blast and black pit caused by *Pseudomonas syringae* pv. *syringae* occur in several citrus growing regions around the world. However, the sources of inoculums of *Pseudomonas syringe* responsible for this disease are not well defined.
Aims: To determine the survival of *P. syringae* pv. *syringae* in weeds and plant debris, evaluate the pathogenicity of *Pseudomonas syringae* on citrus and to assess the potential for plant debris andweeds serving as inoculum sources for this pathogen.
Settings and Design: This study was carried out in the Department of Biological Sciences and Plant Protection at the Institute of Science, Agriculture, Tunisia. For a period of six months.
Methodology: Strains of *Pseudomonas syringae* pv. *syringae* was recovered from plant debris and symptomless weed species growing in citrus orchards. A total of 24 samples of weeds and four samples of plant debris were included in this study. The fluorescent strains cultivated on King's

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medium were identified by LOPAT (Levan production, Oxidase, Pectolytic activity on potato slices, Arginine dehydrolase, HR on tobacco leaves) and GATTa tests (Gelatine hydrolysis, Aesculine hydrolysis, Tyrosine activity, Tarataric Acid usage). Pathogenicity test was carried out on orange (*cv.Navel*) fruits. PCR amplification for the detection of syrB gene was performed with the syrB primers.

Results: Forty six strains isolated from weeds and plants debris were gram negative by KOH test, levan-positive, oxidase-negative, pectolytic activity-negative, arginine dihydrolase-negative and tobacco hypersensitivity-positive. They showed the LOPAT characters of group Ia (+- - -+). Among all, the 46 strains were positive for gelatin liquefaction and aesculin hydrolysis but negative for tyrosinase activity and tartrate utilization (G+A+T-Ta-). Twenty eight of 43 (65.11%) strains were isolated from weeds, 18 of 22 (81.81%) strains isolated from plant debris were, pathogenic on mature orange (cv. Navel) fruits. The PCR amplification with the syrB primers yielded 752-bp fragments confirmed that the syrBgene was present in the 46 isolates. According to biochemical tests (LOPAT and GATTa), pathogenicity assay and PCR amplification with the syrB primers, the forty six isolated bacterial strains were identified as *Pseudomonas syringae* pv. *syringae.*

Conclusion: Forty six isolates are successfully identified as *Pseudomonas syringae* pv. *syringae*.

Keywords: Pseudomonas syringae pv. syringae; weeds; plant debris; syrB gene; source of inoculums.

1. INTRODUCTION

Pseudomonas syringae is a polyphagous phytopathogenic bacterium associated with more than 180 species of both annual and perennial crops, including vegetables, fruits and ornamental plants [1]. This bacterium was found as an epiphyte on the phyllosphere in many geographic areas [2]. Characteristic disease symptoms of blast appear on leaves and twigs. Blast lesions usually develop firstly, on the leaf petiole, or wing of susceptible hosts as small water–soaked or dark spots. These symptoms expand rapidly in both directions, upward toward the leaf mid-vein and downward into the axil and twig. The petiole and twig tissues become severely damaged by girdling, then collapse. The leaves wither, curls, dry become brown, and eventually drop. Necrosis in the twig is generally limited and usually progress slowly. The lesions of infected twigs tissues, beginning at the margins become reddish brown to chestnut colored and may resemble scab or calluses [3]. The disease is associated with cool, damps weather and physical injuries to host caused by wind or hail [4]. Plant disease epidemics are limited by the genetic diversity of host plants, spatial distribution of host plant, energy expenditure to pathogen, and ease of pathogen dispersal. The life cycle of *Pseudomonas syringae* pv. *syringae* strains include in epiphytic phase as well as a pathogenic phase. Blast and black pit strains do not move systematically in citrus hosts beyond the affected tissues [3]. In Tunisia, *Pseudomonas syringae* pv. *Syringae* causing citrus blast of twigs and leaves and black pit of fruit was Reported by Boubaker [5] on sour orange (*citrus aurantium*). Actually, the main problems caused bythis bacterium is blast and black pitof citrus. In fact, the sources of inoculums of *Pseudomonas syringae* responsible of citrus blast and black pit are not well invested. Moreover, there is a little information available on their survival in weeds, and it is unclear whether plant debris also serves as inoculum sources for this pathogen. The role of weeds as alternative source of inoculums for disease epiphytotics has been documented for several plant pathogenic bacteria, including *Xanthomonas campestris* pv. *campestris* [6] and *Pseudomonas syringae* pathogenic to bean [7]. In addition, *Xanthomonas campestris* pv. *campestris* [8], *Xanthomonas campestris* pv. *vesicatoria* [9] and *Pseudomonas syringae* pv. *syringae* the causal agent of bacterial brown spot of bean [10] have been recovered from infected plant debris.

2. MATERIALSANDMETHODS

2.1 Surveys and Sampling

Weeds and plant debris were carried from September 2015 to February 2016 from Tunisian citrus orchard situated in the region of Takelsa. Samples consisting of foliage of weeds species and plants debris collected from within the field, sealed in individual plastic bags and maintained in laboratory at 4℃ until analysis.

2.2 Preparation of Simples and Isolation

Foliar simples were placed in Erlenmeyer flask and covered with 300 ml of sterile phosphate buffer (0.1 M, PH=7). The mixture was shaken

for1h [10] at 200 rpm and serial dilutions were placed on KB Medium [11]. Plates were incubated for 48 h at 25℃.

2.3 Identification of *Pseudomonas syringae*

Plates with individual colonies were examined under binocular microscope. Fluorescents bacterial colonies similar in appearance to *Pseudomonas syringae* were selected and purified on NA amended with 5% of sucrose (SNA). For further analysis, strains were stored in 15% glycerol solution at −20℃.

All strains were identified according to the biochemical and physiological test, according to the Procedures described by [12] and [13]: fluorescence on King's B medium (KB), levan production, oxidase activity, pectolytic activity, arginine dihydrolase activity and tobacco hypersensitivity (LOPAT tests). The Gram reaction of the strains was determined using 3% KOH [14].

2.3.1 LOPAT tests

2.3.1.1 Fluorescent pigmentation test

Strains were developed on King's B medium (KB) at 24-26℃ for 24-48 h then inspected under 366 nm on wavelength ultraviolet light in a dark room for the existence of fluorescent effect. Strains exhibited fluorescent development was recorded as positive.

2.3.1.2 Levan test

Sucrose peptone agar or a nutrient agar medium with 5% sucrose is a suitable substrate for Levan test. For this, a single colony for each isolate was stabbed with a sterilized tooth pick on NA medium containing 5% sucrose. Then the plates were incubated at 25℃ for 2 to 3 days to have distinctive dome shaped colonies [13].

2.3.1.3 Pectolytic activity

Pectolysis of potato slices were washed twice and surface disinfected with alcohol 90%. Standard slices were placed in sterile petri dishes containing a sterile, moistened filter paper [13]. Slices were inoculated with loopful of bacteria previously grown on Nutrient Agar. Then the plates were incubated at 25℃ for 24 - 72 hrs for the detection of soft rot symptoms. A control for each isolate was maintained using Loopful of sterile water. The potato was examined by inserting toothpicks if they are rotten or not.

2.3.1.4 Arginine dihydrolase activity test

The test was performed in Thornley's medium 2A to observe the presence of two enzymes that permits certain Pseudomonads to grow under anaerobic conditions [15]. A fresh culture was stabbed onto a soft agar tube of Thornley's medium, sealed with sterilized mineral oil or melted agar and incubated at 27℃. A color change from faint pink to red within four days is considered as positive reaction.

2.3.1.5 Hypersensitivity response (HR) test

To determine the pathogenic nature of the isolates, hypersensitive reaction was studied on tobacco plants (*Nicotiana tabacum*) by infiltration of bacterial suspension 10^8CFU/ml into the intervening areas of the tobacco leaves [13].

2.3.2 GATTa tests

GATTa tests consisting of gelatine hydrolysis (G), aesculin hydrolysis (A), tyrosinase activity (T) and utilization of tartaric acid (Ta), were carried out as described by [16].

2.3.2.1 Gelatine hydrolysis

A 24-h-old bacterial culture is introduced into a tube with solidified medium containing 0.3%yeast extract, peptone 0.5%, gelatine 12%. After 7-14 days of incubation at 18℃, characteristic liquefaction of gelatine is the indication of a positive reaction [16].

2.3.2.2 Aesculin hydrolysis (A)

The bacteria are inoculated into a semi-solid medium containing peptone 1%, esculine 0.1%, 0.05% ferric citrate, agar 2%. After 24-48 h of incubation at 26-28℃, brown color of the medium proves the presence of the β-glucosidase enzyme [16].

2.3.2.3 Tyrosinase activity (T)

The bacteria are inoculated into a semi-solid medium containing 0.5% sucrose, 1% casamino acid, L-tyrosine 0.1%, 0.05% potassium phosphate, magnesium sulphate heptahydrate 0.0125%, agar 2%, (pH = 7.2). After 7-10 days of incubation at 26-28℃, a color change to red of

the medium shows the presence of tyrosinase [16].

2.3.2.4 Tartrate utilization (Ta)

Bacterial culture are introduced into a liquid medium (pH = 7.0) containing 0.1% ammonium dihydrogen phosphate, potassium chloride 0.02%, magnesium sulfate heptahydrate 0.02%, 1 ml of 4% alcohol bromothymol blue solution. A colour change of the medium from green to blue is a positive test result [16].

2.3.3 Pathogenicity test

Pathogenicity was carried out on mature orange (*cv.Navel*) fruits, purchased in a local market, According to the procedure described by Young [17]. For the inoculum preparation, bacteria were grown on NA for 24h at 27℃, suspended in sterile deionized water and spectrophotometrically adjusted to 10^8 bacterial cells ml^{-1}. Orange fruit surface was sterilized with 0.5% sodium hypochlorite, rinsed twice with sterile distilled water and inoculated with 10 µL of bacterial suspension 10^8 CFU/ml. Fruits treated with sterile distilled water were used as control. All fruits were covered with plastic bags and placed in a humid chamber for five days to one week. The experiment was repeated twice.

2.4 Identification by PCR of *Pseudomonas syringae* pv*. syringae*

2.4.1 Bacterial strains and growth conditions

The *P. syringae* strains used in this study, isolated from different weeds and plant debris in addition, of the strain DAPP-PG115 used as positive control is reported in Table 3. Strains were routinely grown at 25℃, in King's B (KB) medium agar.

2.4.2 DNA preparation

The DNA was obtained by directly sis of single bacterial colonies, picked up from overnight KB medium plate cultures, carefully resuspended in sterile distilled water, incubated at 95℃ and immediately cooled on ice. After a spin in a microcentrifuge to pellet cell debris, 2 µl lysate was directly used in PCR assays as template.

2.4.3 Presence of the syrB

To detect the possible presence of the syrB gene coding for the production of cyclic lipodepsinonapeptides (e.g. syringomycin) in *Pseudomonas syringae* pv. *syringae*, we used theprimerB1(5'-CTTTCCGTGGTCTTGATGAGG-3')and B2(5'-TCGATTTTGCCGTGATGAGTC-3'), which amplify a sequence of 752 bp of the Syr B gene [18]. The syrB1, syrB2 primers and components were mixed in the same amplification reaction containing 10 µM of each primer and 5U/µl of Taq DNA polymerase.

Appropriate thermocycling program was set on Eppendorf Master cycler DNA Engine Thermal Cycler PCR as Pre-PCR 95℃ for 1 min; Thermocycling (35 cycles): Denaturation 94℃ for 40 sec, Annealing 60℃ for 50 sec, Extension 72℃ for 1 min and Final Extension 72℃ for 10 min. The products were then analysed on 1% agarose gels to confirm the presence of a single product of the desired size. The gels were visualised under UV transillumination using a Gel Doc Fujifilm LAS 3000.

3. RESULTS

3.1 Surveys and Sampling

Weeds and plant debris samples were collected periodically from the same site where citrus trees grown. During this study a total of 24 weeds samples (Fig. 1) and 4 plants debris samples were collected.

Weeds collected were assigned to five families as shown in Table 1 in addition of, plant debris sampled from the soil surface of the field. These weeds are commonly encountered in the majority of Tunisian citrus fields.

3.2 Isolation and Biochemical Identification of the Bacterium

A total of 65 oxidase negative and fluorescent on King B medium bacterial strains were isolated from plant debris and symptomless weeds species growing in citrus grove. However, the frequency of bacteria isolation recovered from the weeds samples (*Amaranthus retroflexus, Convolvulus arvensis, Elymus repens, Rumex crispus, Conyza canadensis, Sonchus oleraceus, Lolium perenne, and Glebionis segetum)* was variable.

Bacterial strains were characterized on the basis of LOPAT and GATTa tests. Fifteen of 24 (62.5%) weeds samples, 2 of 4 (50%) plants debris samples yielded oxidase negative and

green fluorescent bacterial strains. Forty six strains were Gram negative by KOH test, oxidase negative, levane positif on NSA medium and induced hypersensitive reaction on tobacco leaves in 24 hr. Forty six strains were arginine dihydrolase and potato rot negative. The 46 strains were all positive for gelatin liquefaction and aesculin hydrolysis while being negative for tyrosinase activity and tartrate utilization (G+A+T-Ta-) and also pathogenic to orange fruits (*cv. Navel*) (Table 2). Five to seven days after the inoculation, pathogenic strains generated necrotic areas in correspondence of the inoculation sites. With regard to patho-genicity, 28 of 43 (65.11%) pseudomonads isolated from weeds, 18 of 22 (81.81%) pseudomonads isolated from plants debris were pathogenic to citrus fruits (*cv.Navel*).The pathogenic strains induced a light brown spots in the inoculated fruit rind. These become later dark brown. Our pathogenic strains to citrus were essentially recovered from *Convolvulus arvensis* (83.33%), *Elytrigia repens* (100%), *Amaranthus retroflexus* (100%), *Conyza canadensis* (80%). Most of strains recovered from plant debris were pathogenic to citrus. Plant debris and weeds could be considered as a reservoir of inocumum for *Pseudomonas syringae.*

3.3 Identification by PCR of *Pseudomonas syringae* pv*. syringae*

3.3.1 Presence of the syrB

Regardless its origin (plant debris or weeds), the 46 selected strains of *Pseudomonas syringae* pv. *syringae* amplified a 752-bp fragment with the syrB primers as the reference strain DAPP-PG115 (Table 3 and Fig. 2).

Thus, PCR amplification with the syrB primers yielded 752-bp fragments from all strains that were confirmed to have the syrB gene. In fact, the PCR employed with specific primers for syringomycinsyrB gene proved that the forty six strains could synthesize the syringomycin. In addition, of the molecular detection of the gene syrB, all strains were identified as *Pseudomonas syringae* pv. *syringae* through biochemical and pathogencity test.

4. DISCUSSION

Weeds and plant debris could be a source of inoculum, but their relative importance in the development of epidemics of citrus blast is not well investigated. It is suitable to know what extent of bacteria that disseminate by splashing rain from weeds and plant debris to citrus. In order to realize a rapid and reliable identification of *P. s*. pv. *syringae* from weeds and plant debris, we studied their pathogenicity, morphological, physiological and biochemical characteristics, and also the presence of syrB gene for toxin production. *Pseudomonas syringae* pv. *syringae* strains isolated from weeds and plant debris pathogenic on orange fruits produced fluorescent pigments on King's B medium. They were levan producers and formed whitish to cream-colored, dome-shaped colonies on nutrient sucrose medium, oxidase negative and induce HR on tobacco leave.

Fig. 1. Weeds and plants debris sampled from within the field. (A) *Amaranthusretroflexus*. (B) *Convolvulus arvensis*. (C) *Elymusrepens* (D) *Rumexcrispus*. (E) *Conyza Canadensis*. (F) *Sonchusoleraceus*. (G) *Loliumperenne*. (H) Plant debris

Table 1. Characterization of weeds species and plant debris

Family	Scientific name	Common name	Characteristics	Sampling data
Convolvulaceae	*Convolvulus arvensis*	Field bindweed	Perennial	2015/2016
Poaceae	*Elymus repens*	Common couch	Perennial	2015/2016
Plant debris	Plant debris	-	-	2015/2016
Asteraceae	*Glebionissegetum*	corn marigold	Annual	2015/2016
Polygonaceae	*Rumex crispus*	Curly Dock	Perennial	2015/2016
Amaranthaceae	*Amaranthus retroflexus*	Red-rootamaranth	Annual	2015/2016
Poaceae	*Lolium perenne*	Perennialrye-grass	Perennial	2015/2016
Asteraceae	*Conyza canadensis*	Horseweed	Annual	2015/2016
Asteraceae	*Sonchus oleraceus*	Common sowthistle	Annual	2015/2016

Table 2. Biochemical characterization and pathogencity of green fluorescent oxidase negative bacteria recovered from weeds and plant debris collected from the field

Source of strains	Oxidase strains		Levane	Pectolytic activity	Arginine-dihydrolase	HR	Pathogenicity on fruits	GATTa	KB test
	Ratio	%							
Convolvulus	5(6)	83.33	+	-	-	+	+	++ - -	+
arvensis	1(6)	16.66	-	+	-	+	-	nd	+
Elymus	5(5)	100	+	-	-	+	+	++ - -	+
repens									
Plant	18(22)	81.81	+	-	-	+	+	++ - -	+
debris	4(22)	18.18	+	-	-	+	-	nd	+
Glebionis	1(6)	16.66	+	-	-	+	+	++ - -	+
segetum	5 (6)	83.33	+	-	-	+	-	nd	+
Rumex	2(4)	50	+	-	-	+	+	++ - -	+
crispus	2(4)	50	+	-	-	+	-	nd	+
Amaranthus	3(3)	100	+	-	-	+	+	++ - -	+
retroflexus					+				
Lolium	5(9)	55.55	+	-	-	+	+	++ - -	+
perenne	4(9)	44.55	+	-	-	+	-	nd	
Conyza	4(5)	80	+	-	-	+	+	++ - -	+
canadensis	1(5)	20	-	+	-	+	-	nd	+
Sonchus	3(5)	60	+	-	-	+	+	++ - -	+
oleraceus	2(5)	40	-	+	-	+	-	nd	+

nd=not determined

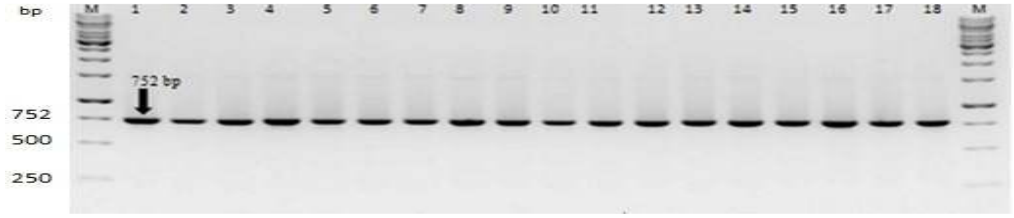

Fig. 2. Agarose gel Electrophoresis of PCR with primers B1and B2 corresponding to gene syrB, 1:strain of *Pseudomonas syringae* pv. *Syringae* DAPP-PG115, 2-18: *P. syringae* pv. *syringae strains* 752-bp, M: 1 Kb DNA molecular marker

Forty six strains were negative for arginine dihydrolase. They showed the LOPAT characters of group Ia (+- - -+). Based on GATTa scheme, we identified 46 *Pseudomonas syringae* pv. *syringae* bacterial strains isolated from weeds and plants debris. The 46 selected strains were positive for gelatin liquefaction and aesculin hydrolysis while being negative for tyrosinase activity and tartrate utilization, Thus, *P. syringae* pv. *syringae* strains are G+A+T-Ta-. Isolates of *P. syringae* pv. *syringae* from weeds and plants debris were pathogenic to orange fruits cultivar (*cv. Navel*), and orange fruits showed necrosis of tissue at the inoculation site five to seven days after inoculation. Moreover, the 46 tested strains amplified a 752-bp fragment with the syrB primers. Those isolates could synthesize this toxin that assists the identification and

characterization of putative *Pseudomoans syringae* pv. *syrinage* strains. In fact, the syrB gene was encoded synthesize syringomycin because these toxins are considered the main virulence factor of *Pseudomonas syringae* pv. *syringae* [17,19,20].

Many strains of *Pseudomonas syringae* pv. *syringae* are known to produce cyclic lipodepsipeptides as secondary metabolites. In addition, the syringomycin has been used as a determinative characteristic in identifying pathogenic strains of *Pseudomonas syringae* pv. *syringae* [17,21].

The PCR amplification of the 752-bp syrB fragment offers rapid and accurate detection of cyclic lipodepsinonapeptide-producing strains. The results obtained by using primers B1 and B2 were in agreement with previous studies. Thus, Cirvilleri et al. [22] reported that the PCR amplification with primer B1 and B2 gave rise to a 752-bp band indicating the syrB gene was present. Najafi Pour et al. [23] also demonstrated the presence of syr B gene in all strains of *Pseudomonas syringae* pv. *syringae* and *Pseudomonas syrinage* pv. *syringae* IVIA 773-1, those amplified a 752-bp fragment with the syrBprimers. Scortichini et al. [24] used the primers B1 and B2 to identify *Pseudomonas syringae* pv. *syringae* and the syrB gene was present in almost all strains.

Those results proved that *Pseudomonas syrinage* pv. *syringae* could synthesize syringomycine, which is very important toxin for pathogenicity induction in plant.

The present study revealed that, *P. syringae* is common phylloplane inhabitant of many weed. The arrival of this pathogen into agricultural fields with rain or snowfall is not really surprising. Rain is the main long-distance vector of other pathogens, such as rusts [25].

In the cropping area, we noticed the excessive presence of weeds and also plants debris. Moreover, the farmers didn't develop any appropriate methods for their control. Camille et al. [26] suggest to suppress weeds around the trees for controlling citrus blast. Anderson and Lindow [27] found that INA bacteria were detected frequently at the border of citrus groves when weeds or other crops bearing *Pseudomonas syringae* were adjacent to the grove.

Table 3. PCR results of 46 isolates by specific primer of syringomycin (syrB) gene

Bacterialstrain	Weeds/ plant debris/ host	Syr B	Bacterial strain	Weeds/ plant debris/ host	Syr B
DAPP-PG 115	*Clementine*	+	Psp 24	Plant debris	+
Psc1	*Convolvulus arvensis*	+	Psp 25	Plant debris	+
Psc 2	*Convolvulus arvensis*	+	Psp 26	Plant debris	+
Psc 3	*Convolvulus arvensis*	+	Psp 27	Plant debris	+
Psc 4	*Convolvulus arvensis*	+	Psp 28	Plant debris	+
Psc 5	*Convolvulus arvensis*	+	Psg 29	*Glebionis segetum*	+
Pse 6	*Elymus repens*	+	Psr 30	*Rumex crispus*	+
Pse 7	*Elymus repens*	+	Psr 31	*Rumex crispus*	+
Pse 8	*Elymus repens*	+	Psa 32	*Amaranthus retroflexus*	+
Pse 9	*Elytmus repens*	+	Psa 33	*Amaranthus retroflexus*	+
Pse 10	*Elymus repens*	+	Psa 34	*Amaranthus retroflexus*	+
Psp 11	Plant debris	+	Psl 35	*Lolium perenne*	+
Psp 12	Plant debris	+	Psl 36	*Lolium perenne*	+
Psp 13	Plant debris	+	Psl 37	*Lolium perenne*	+
Psp 14	Plant debris	+	Psl 38	*Lolium perenne*	+
Psp15	Plant debris	+	Psl 39	*Lolium perenne*	+
Psp16	Plant debris	+	Psc 40	*Conyza canadensis*	+
Psp17	Plant debris	+	Psc 41	*Conyza canadensis*	+
Psp18	Plant debris	+	Psc 42	*Conyza canadensis*	+
Psp19	Plant debris	+	Psc 43	*Conyza canadensis*	+
Psp 20	Plant debris	+	Pss 44	*Sonchus oleraceus*	+
Psp 21	Plant debris	+	Pss 45	*Sonchus oleraceus*	+
Psp 22	Plant debris	+	Pss 46	*Sonchus oleraceus*	+
Psp23	Plant debris	+			

DAPP-PG=Bacterial Collection of the Plant Protection Unit, Department of Agricultural, Nutritional and Environmental Sciences, University of Perugia, Italy

Infact, *P. syringae* was isolated from wild plants and weeds [28,29]. Lindowet al. [30] found that *Pseudomonas syringae* was a widely distributed epiphyte on many plant speciesand speculated that these epiphytes may serve as a source of inoculum for various diseases for either the plant on which they reside or nearby plants. English and Davis [31], isolated green-fluorescent pseudomonads from the surfaces of healthy peach and almond trees, and occasionally from orchard weeds. Some of these were pathogenic to peach and were presumed to be *P. syringae*. In addition, weeds were suggested as a source of *P. syringae* for bacterial canker of stone fruit trees by English and Davis [31], blast of pears [32] and bacterial brown spot of beans [33]. In fact, *P. syringae* pathogenic to stone fruit trees was recovered from weeds in peach and almond orchards and from several apparently healthy woody plants in California [31]. In Michigan, *P. syringae* pv. *syringae* and less frequently, *P. syringae* pv. *morsprunorum*, were isolated from grasses and broad-leaf weeds in sour cherry orchards [34]. Therefore, it seems that the populations of *P. syringae* on weeds are important source of inoculum for brown spot. However, the pathogen can over wintering bean crop residue, and this source of inoculums role in the epidemiology of brown spot of beans. In addition, Lindow [35] found that *P. syringae* was widely distributed as an epiphyte on many plants and, frequently reside epiphytically on non-host plant species that is, on plants that are not known to be susceptible to diseases incited by these pathogens.

Moreover, Ercolani [33] found a correlation between high populations of *P. syringae* pv. *syringae* pathogenic to bean (Psb) on hairy vetch and subsequent brown spot epidemics in adjacent bean fields. Wind-blown rain was suggested as the mechanism by which Psb was disseminated from the non-host to the host plants. Furthermore, Ercolani [36] demonstrated that a cherry strain of *P. syringae* pv. *morsprunorum* and a pear strain of *P. syringae* colonized leaf surfaces only of their respective hosts when they were artificially applied to cherry and pear plants, in spite of, the apparent ability of pv. *morsprunorum* to exist on weeds [34]. *Pseudomonas syringae* pv. *syringae* can survive on crop residue. Plant debris could be a source of inoculums of *Pseudomonas syringae* pv. *syringae* causing blast and black pit of citrus. Thereby, crop debris has been recognized as a reservoir of *P. syringae* for some disease epidemics, but the debris-associated populations decline rapidly [37,38]. Infected plant debris has been considered as a source of inoculum for epipabs [39]. In addition, *P. syringae* pv. *syringae* could be detected in soil containing plant debris collected from a field where beans had been severely attacked with brown spot [40].

Therefore, *X. campestris*pv. *glycines* pathogenic to soybean and *X .campestris*pv. *vesicatoria* the causal agent of bacterial spot of tomato and pepper have also been recovered from infected plant debris [41-43]. Numerous studies have documented that foliar bacterial pathogens can be recovered from infected plant debris or from the rhizosphere. It has been reported that *Pseudomonas syringae* pv. *lacrymans* can survive in plant debris for more than one year if the debris has not disintegrated completely [44].

In addition, the survival of *Pseudomonas syringae* pv. *phaseolicola* and *Pseudomonas syringae* pv. *pisi* was longer when plant debris remained on the soil surface [45,46].

5. CONCLUSION

In this work, we conclude by biochemical, pathogencity test and by PCR detection of syrB gene that the strains isolated from weeds and plant debris are *Pseudomonas syringae* pv. *syringae*. We also conclude that those putative *Pseudomonas syringae* pv. *syringae* strains could survive on weeds and plant debris and could be also a source of inoculums of blast and black pit of citrus.

ACKNOWLEDGEMENTS

The authors are grateful to prof. Roberto Buonaurio for providing us reference strain DAPP-PG 115.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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