Short Communication

Different Sequevars of *Ralstonia pseudosolanacearum* Causing Bacterial Wilt of *Bidens pilosa* in ChinaYonglin He, Yixue Mo, Dehong Zheng, Qiqin Li, Wei Lin, and Gaoqing Yuan[†]

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Abstract

Bidens pilosa is an invasive weed that threatens the growth of crops and biodiversity in China. In 2017, suspected bacterial wilt of *B. pilosa* was discovered in Qinzhou and Beihai, Guangxi, China. A variety of weeds are considered as reservoirs harboring bacterial wilt pathogens, but most do not show obvious symptoms in the field. Identifying the classification status of the *B. pilosa* bacterial wilt pathogen and exploring its geographical origin might be helpful for clarifying the role of weeds in the circulation of the disease. Phylotyping, sequevar analysis, and cross inoculation of pathogens isolated from *B. pilosa* and nearby peanut (*Arachis hypogaea*), balsam gourd (*Momordica charantia*), and eucalyptus (*Eucalyptus robusta*) plants were carried out. Three isolates of *B. pilosa* (Bp01, Bp02, and Bp03) were identified as *Ralstonia pseudosolanacearum*, race 1, biovar 3, and phylotype I, and belonged to sequevars 17 and 44, and an unknown sequevar. The sequevars isolated

from *B. pilosa* were not completely consistent with those of the nearby hosts, and the virulence of these isolates differed when cross inoculated. The Bp03 sequevar was different from peanut isolate sequevars in the same field and was not identical to any previously designated sequevars. The isolates from *B. pilosa* and other nearby hosts displayed low or no virulence toward their cross hosts (with wilt incidences less than 33.33%). An exception to this was the isolates from *B. pilosa*, which displayed high virulence toward eucalyptus (with a wilt incidence of 70.00 to 100.00%). This is the first report of different sequevars of *R. pseudosolanacearum* causing typical bacterial wilt symptoms in *B. pilosa* in the field.

Keywords: *Bidens pilosa*, *Ralstonia pseudosolanacearum*, bacterial wilt, sequevar

Bacterial wilt, caused by the *Ralstonia solanacearum* species complex (RSSC), is one of the most destructive bacterial plant diseases (Mansfield et al. 2012). The pathogen is widespread in tropical and subtropical regions and can infect over 450 plant species representing 54 botanical families (Hayward 1991; Wicker et al. 2007). The RSSC is an extremely diverse group and has multiple taxonomic classifications based on divergent physiological properties, host ranges, geographic regions, or genetic profiles. The RSSC is traditionally subdivided into five races based on host range and six biovars based on carbohydrate catabolism (Buddenhagen et al. 1962; Hayward 1991; He et al. 1983). In 2005, according to the sequences of the 16S-23S rRNA internal transcribed spacer (ITS) region, and the endoglucanase (*egl*), *hrpB*, and *mutS* genes, Fegan and Prior proposed a more phylogenetically meaningful classification scheme for the RSSC (Fegan and Prior 2005). The RSSC comprises species, phylotypes, sequevars, and clones in this new hierarchical classification. The four known phylotypes were phylotypes I (Asia), II (IIA, IIB) (Americas), III (Africa), and IV (Indonesia and Japan), each corresponding to a different geographic region. Each phylotype was subsequently divided into an increasing number of sequevars. Recently, the RSSC was separated into three genospecies based on DNA-DNA hybridization analyses combined with phenotypic analyses, and modern genomic and

proteomic techniques. The three genospecies were *R. solanacearum* (phylotype II strains), *R. pseudosolanacearum* (phylotype I and III strains), and *R. syzygii* (phylotype IV strains) (Prior et al. 2016; Safni et al. 2014).

The host range of the RSSC is expanding rapidly (Wicker et al. 2007), and new hosts are constantly being found, which fully reflects the strong host and ecological adaptability of the RSSC. In China, bacterial wilt has been found in 30 provinces, especially in southern China (Guangxi, Guangdong, Hainan, and Fujian) (Jiang et al. 2017). There are at least 90 plant species belonging to 39 botanical families infected by RSSC, including many crops, woody and shrub plants, ornamental plants, and medicinal plants. Guangxi is located in a subtropical region with a warm climate and abundant rainfall, which is very suitable for the occurrence of bacterial wilt (Jiang et al. 2017). In 2017, suspected bacterial wilt of *Bidens pilosa* was discovered in Qinzhou and Beihai, Guangxi. *B. pilosa* belongs to the family Asteraceae and is an annual or perennial herbaceous plant native to Central America. *B. pilosa* is distributed in the tropics and subtropics of Asia and the Americas, and is now also widely distributed in east, central, south, and southwest China. The weed competes with crops for light, water, nutrition, and space. Its invasiveness and environmental adaptability are strong, resulting in enormous losses to agriculture, forestry, and livestock industries, and causing significant ecological changes in China (Shang and Zhu 2019). Crop rotation is an effective method of controlling bacterial wilt, but the widespread presence of latent infection in weeds prevents rotations from controlling the disease (Tusiime et al. 1998). To date, there have been no reports of typical symptoms of bacterial wilt in *B. pilosa*. The recent occurrence of this disease indicates that the *B. pilosa* weed has become more susceptible to the pathogen, which will increase the difficulty of controlling bacterial wilt in the field.

Whether the pathogen causing the disease in the *B. pilosa* plants is consistent with the reported pathogen of bacterial wilt is unknown. Studying the classification status and geographical origin

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of the pathogen causing *B. pilosa* bacterial wilt would be useful for understanding the complex epidemiology of RSSC and for developing disease management. In this study, the causal agent of the *B. pilosa* bacterial wilt was identified. The phylotype and sequevar of isolates from *B. pilosa* and other nearby wilted crops were determined. The virulence of these isolates was then analyzed through cross inoculation to explore the source of the *B. pilosa* pathogens and the association between them and other nearby crop pathogens.

Materials and Methods

Pathogen isolation. Wilted *B. pilosa* plants and adjacent crops were collected from Qinzhou and Hepu, Guangxi, China. For the isolation of the bacterial wilt pathogen, brown vascular bundles of stems 1 to 2 cm above the ground were cut into fragments. The fragments were surface sterilized with 75% ethanol for 30 s, and 0.1% mercury dichloride for 30 s, rinsed with sterile water three times, and then mashed and macerated in sterile distilled water for 2 to 3 min. The suspensions were diluted with sterile water and streaked on nutrient agar (NA) and triphenyltetrazolium chloride (TTC) medium plates (Kelman 1954), and incubated at $28 \pm 2^\circ\text{C}$ for 48 h. Colonies with a typical RSSC phenotype were purified and stored in 20% glycerol at -80°C . When further research is required, these isolates can be revived by plating on NA.

Pathogenicity and race determination. In order to determine the pathogenicity and race of the isolates from *B. pilosa*, a total of eight plants were inoculated using the soil-drenching or stem-injecting method. Inoculation was performed on the wounded roots of *B. pilosa*, tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*), and tobacco (*Nicotiana tabacum*) plants at 4 weeks old (the 4 to 6 leaf stage). Each seedling was soil-drenched with 30 ml of the bacterial suspension at 1×10^8 cfu ml $^{-1}$. For the injection method, 100 μl of bacterial suspension (1×10^8 cfu ml $^{-1}$) was injected into the young stems of potato (*Solanum tuberosum*), ginger (*Zingiber officinale*), mulberry (*Morus alba*), and banana (*Musa nana*) plants. Seedlings drenched or injected with sterile water were used as blank controls. Sixteen replicate plants were inoculated with each isolate, and the experiment was repeated three times. The inoculated plants were incubated at $28 \pm 2^\circ\text{C}$ with 75 to 80% relative humidity and were recorded as wilted plants when they wilted completely. The pathogen was reisolated from the diseased *B. pilosa* stems by using the method described above. The wilt incidence (WI) of inoculated plants was calculated as $\text{WI} (\%) = 100 \times \text{number of completely wilted plants} / \text{total number of inoculated plants}$.

Biovar determination. Biovars of the isolates from *B. pilosa* were determined based on their capacity to utilize three hexose alcohols (mannitol, sorbitol, and dulcitol) and three disaccharides (lactose, maltose, and cellobiose) (Hayward 1964). Ten microliters of bacterial suspension (1×10^8 cfu ml $^{-1}$) were inoculated on Hayward's medium. Each test was repeated three times, and liquid culture media inoculation was used as a negative control. All inoculated tubes were incubated at $28 \pm 2^\circ\text{C}$ for 7 days. Color changes of the medium from green to yellow were recorded as positive.

DNA extraction and sequencing of the 16S rRNA gene. Total DNA of each isolate was extracted using a Bacterial Genomic DNA Extraction Kit (Sangon Biotech [Shanghai] Co., Ltd.). The genomic DNA of the isolates was used as a template for PCR amplification of the 16S rRNA genes using the universal primers 27F/1492R (Lane 1991). Approximately 50 ng of DNA template was added to 12.5 μl Taq PCR Master Mix, and 10 pmol of the primers 27F and 1491R, and ddH $_2$ O was added to make a final volume of 25 μl . The PCR program consisted of an initial denaturation at 96°C for 5 min, followed by 30 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 10 min. Then, 5 μl of PCR product was subjected to electrophoresis on 1.0% agarose gel, stained with ethidium bromide, and visualized using a UV-transilluminator. The PCR products were purified and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China).

Phylotype-specific multiplex PCR (Pmx-PCR) amplification. The phylotypes of the isolates were identified using multiplex PCR as described by Fegan and Prior (2005). Approximately 50 ng of DNA template was added to 12.5 μl of Taq PCR Master Mix, 4 pmol of the species-specific primers 759 and 760 (Opina et al. 1997), 6 pmol of the phylotype-specific primers Nm21:1F, Nm21:2F, Nm23:AF, Nm22:InF, and Nm22:RR (Fegan and Prior 2005), and ddH $_2$ O was added to make a final volume of 25 μl . The PCR program included an initial denaturation at 95°C for 5 min, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 90 s, and a final extension at 72°C for 10 min. Then, 5 μl of PCR product was subjected to electrophoresis on 1.0% agarose gel, stained with ethidium bromide, and visualized using a UV-transilluminator. The size of the amplified fragments was estimated by comparison with a 1 kb marker ladder. Multiplex PCR amplifications were performed in a Biometra TProfessional thermocycler. Identification of phylotypes I, II, III, and IV was based on the amplification of the expected 144, 372, 91, and 213 bp fragments, respectively. Meanwhile, the 280 bp amplicon confirmed the RSSC identity.

Sequevar determination. PCR amplification of the *egl* gene was performed using the primer pair Endo-F (5-ATGCATGCCGCTGGTCGCCG-3) and Endo-R (5-GCGTTGCCCGGCACGAACACC-3) (Fegan et al. 1998). The PCR products were purified and sequenced by Sangon Biotech Co. Ltd. (Shanghai, China). All *egl* sequences from the RSSC isolates in this study were deposited in GenBank (<https://www.ncbi.nlm.nih.gov/>; Table 1). Thirteen reference strain sequences were used in the phylogenetic analysis (Table 2). Sequences were edited with BioEdit 7.0.5.1 and aligned using the Clustal W program of MEGA version 6.0. Phylogenetic trees were constructed using neighbor-joining (NJ) methods embedded in MEGA version 6.0, and bootstrapping was performed with 1,000 replicates for the NJ tree.

Virulence assays for cross inoculation. The isolates from *B. pilosa* and nearby peanut (*Arachis hypogaea*), balsam gourd (*Momordica charantia*), and eucalyptus (*Eucalyptus robusta*) plants were inoculated on their own hosts and cross hosts. *B. pilosa*, peanut, and balsam gourd plants were inoculated using the same soil-drenching method described above. Injection was performed on the

Table 1. *Ralstonia pseudosolanacearum* isolates used in this study

Origin	Isolate code	Host scientific name (common name)	Phylotype/sequevar	GenBank accession no. (<i>egl</i>)
Jishui village, Xiaodong town, Qinzhou city	Bp01	<i>Bidens pilosa</i>	I/17	MH021274
	Pn36	<i>Arachis hypogaea</i> (Peanut)	I/44	MG999623
	Ec06	<i>Eucalyptus robusta</i> (Eucalyptus)	I/17	MH005161
	Ec07			MH005163
Shiwan town, Hepu county, Beihai city	Bp02	<i>B. pilosa</i>	I/44	MH021276
	Pn37	<i>A. hypogaea</i> (Peanut)	I/44	MG999624
	Pn38			MG999625
Zhushan village, Nameng town, Qinzhou city	Bp03	<i>B. pilosa</i>	I/unknown sequevar	MH021278
	Pn32	<i>A. hypogaea</i> (Peanut)	I/44	MG999619
	Pn33			MG999620
	Bg07	<i>Momordica charantia</i> (Bitter melon)	I/44	MH050780

axillary bud of eucalyptus at the 4 to 6 leaf stage. Seedlings drenched or injected with sterile water were used as blank controls. Each strain was inoculated on 16 plants and the experiment was repeated three times. The methods used for incubating plants and calculating WI were the same as described above.

Data analysis. Statistical analysis was performed using the software SPSS version 20, and mean comparisons among treatments were performed using Tukey's test at level 0.05.

Results

Symptoms. The first symptoms in *B. pilosa* included the drooping of the top green leaves and petiole browning. This was followed by the wilting of the whole plants within a few days, leading to total plant collapse and death (Fig. 1a and b). Pith tissues turned brown, especially at the stem base. Bacterial ooze was observed on the diseased stem by squeezing.

Identification of pathogens and phylotypes. A total of 11 isolates were obtained. Three bacterial strains were isolated from the stems of *B. pilosa* plants, named Bp01, Bp02, and Bp03. The strains Pn36, Ec06, and Ec07 were isolated from the peanut and the eucalyptus, which were collected from the field next to the host of Bp01. Pn37 and Pn38 were isolated from peanuts next to the host of Bp02. Pn32 and Pn33 were isolated from peanuts collected from the same field as the host of Bp03. Bg07 was isolated from bitter gourd next to the host of Bp03 (Table 1). Colonies grown on NA plates at 28°C for 48 h were round or irregular, slightly raised, white, and translucent, with strong fluidity. All isolates showed typical colony characteristics of *Ralstonia pseudosolanacearum* on TTC medium, which were fluidal white with a red center (Fig. 1c). Furthermore, the biochemical features of the isolates Bp01, Bp02, and Bp03 matched those of *R. pseudosolanacearum*.

The 16S rRNA gene sequences of Bp01, Bp02, and Bp03 (GenBank accession nos. MN124452, MN124453, and MN124454) were 100% similar to those of *R. pseudosolanacearum* (accession no. KY346976), and standard strain GMI1000 (accession no. NC_003295) from GenBank. Using the Pmx-PCR protocol, each of the 11 isolates generated the expected 280 bp species-complex-specific fragment and a 144 bp fragment specific to phylotype I of *R. pseudosolanacearum*. This indicated that all the isolates from wilting *B. pilosa*, peanut, bitter gourd, and eucalyptus were *R. pseudosolanacearum*. The typical wilt symptoms of *B. pilosa* were observed 5 days after inoculation, similar to those in the field, with wilt incidences of 50.00 to 83.33% 21 days after inoculation (Fig. 1d, Table 3). No symptoms developed in the control plants (Fig. 1e). The colony morphologies and 16S rRNA gene sequences of the isolates from the inoculated plants were identical to those of isolates from the field samples, thus fulfilling Koch's postulates. Based on morphological and molecular analyses, it was proved that the bacterial wilt of *B. pilosa* was caused by *R. pseudosolanacearum*.

Sequevar analysis. Partial nucleotide sequences of the *egl* gene from 11 isolates were aligned. The phylogenetic relationships according to *egl* gene sequences were analyzed together with 13 reference

strains (Table 2). Sequences from reference strains were added to the trees in order to position the new strains within the known phylogenetic structure. Based on the phylogenetic tree of *egl* partial sequences (Fig. 2), all isolates were classified as phylotype I, which was consistent with the Pmx-PCR. The *egl*-tree branching pattern within phylotype I showed a split with a 96% bootstrap value, which partitioned isolates from *B. pilosa* in this study into groups comprising sequevars 17 and 44, and an unknown sequevar. Although collected from the adjacent fields, *B. pilosa* isolate Bp01 and eucalyptus isolates Ec06 and Ec07 were clustered within sequevar 17; however, the nearby peanut isolate Pn36 belonged to sequevar 44. *B. pilosa* isolate Bp02 and nearby peanut isolates were all clustered within sequevar 44. The sequevar of Bp03 from *B. pilosa* was different from that of peanut isolates in the same field, and was not identical to any previously designated sequevar.

Race and biovar determination. After 7 days for plants injected with bacterial suspension and 21 days for plants subjected to wounded root with bacterial suspension, wilting plants were investigated and counted. All isolates from *B. pilosa* caused infection in tomato, eggplant, tobacco, and potato plants, with wilt incidences of 50.00 to 100.00%, but could not infect banana plants. The three isolates caused mulberry wilt via the injection inoculation method, with a wilt incidence of 20.00 to 40.00%. However, only the Bp01 isolate infected ginger, with a wilt incidence of 40.00%. Moreover, Bp01, Bp02, and Bp03 isolates utilized three hexose alcohols and three disaccharides. Therefore, the three isolates from *B. pilosa* were determined to be race 1, biovar 3 (Buddenhagen et al. 1962; Buddenhagen and Kelman 1964; He et al. 1983).

Cross-inoculation analysis. The isolates of peanut, bitter gourd, and eucalyptus adjacent to wilted *B. pilosa* weeds were reinoculated into their own hosts and displayed high virulence, with wilt incidences of 50.00 to 90.00%. However, the isolates displayed low virulence toward *B. pilosa*, with a wilt incidence of 13.89 to 33.33%, which was significantly lower than the virulence of *B. pilosa* isolates toward *B. pilosa*. The isolates from *B. pilosa* displayed high virulence toward eucalyptus, but low or no virulence toward peanut (with wilt incidences of 0.00 to 16.67%), and no virulence toward bitter gourd. Bp03 and Pn33 were collected from the same field. However, not only were they of different sequevars, but the virulence of their cross inoculations also differed (Table 3).

Discussion

The RSSC is highly variable and adaptable as attested by its worldwide distribution and expanding host range. Phylotype I strains have been shown to be phylogenetically diverse, including at least 21 sequevars (Kyaw et al. 2017; Santiago et al. 2016; She et al. 2017, 2018). In this study, *egl* sequences of three *B. pilosa* isolates were not identical, representing different sequevars. Bp01 was clustered within sequevar 17 and was not consistent with the adjacent peanut isolate Pn36. Bp02 belonged to sequevar 44, but Bp03 was not identical to any previously designated sequevar. In recent years, research on RSSC sequevars has been concentrated, so some RSSC isolates

Table 2. Reference *Ralstonia solanacearum* species complex (RSSC) strains

Strain	Host	Origin	Phylotype/sequevar	GenBank accession no. (<i>egl</i>)	References
P11	Peanut	China	I/17	FJ561068	Xu et al. 2009
O3	Olive tree	China	I/44	FJ561069	Xu et al. 2009
Pe3	Pepper	China	I/44	FJ561155	Xu et al. 2009
SXXX-2	Tobacco	China	I/44	KT961490	Liu et al. 2017
GMI8254	Tomato	Indonesia	I/47	GU295014	Cellier and Prior 2010
M2	Mulberry	China	I/48	FJ561067	Xu et al. 2009
Ca-Gy-05-2	Pepper	China	I/57	KC441923	She et al. 2018
IPO1609	Potato	Netherlands	IIB/1	EF371814	Wicker et al. 2007
CFBP2047	Tomato	United States	IIA/7	AF295262	Poussier et al. 2000
CMR33	Tomato	Cameroon	III/20	GU295007	Cellier and Prior 2010
DGBBC1125	Potato	Guinea	III/43	GU295008	Cellier and Prior 2010
R28	<i>Syzygium aromaticum</i>	Indonesia	IV/9	DQ011552	Fegan and Prior 2006
Psi7	Tomato	Indonesia	IV/10	EF371804	Wicker et al. 2007

with completely different genetic backgrounds are named as the same sequevars. The sequevars 54 to 57 were reported by Li, Liu, and She to belong to phylotype I (Li et al. 2016; Liu et al. 2017; She et al. 2017, 2018), but were reported by Santiago to belong to phylotype II (Santiago et al. 2016). In addition, the sequevars of some isolates have not been determined (Kyaw et al. 2017; Liliam et al. 2017). Therefore, the new *R. pseudosolanacearum* sequevar found in this study was not named.

There have been some reports on the role of weeds in the occurrence and spread of crop bacterial wilt. The RSSC infects the roots of many plants that were previously not considered to be hosts, and the infection ability might be closely related to its long-term survival ability (Granada and Sequeira 1983), and may even play a decisive role in the maintenance of soil infestation (Amat et al. 1978). Under natural conditions, these weeds usually serve as symptomless carriers of the pathogen (Sunaina et al. 1989; Swanepoel 1992).

However, it has been shown that if the biovar 3 strains of the RSSC were inoculated artificially, a few kinds of weeds developed wilt symptoms (Swanepoel 1992). *B. pilosa* is one of the weeds widely distributed in farmland and woodland in China and can grow and reproduce all year round, causing significant ecological changes in China (Shang and Zhu 2019). Lin et al. and Tusiime et al. reported that a variety of weeds, including *B. pilosa*, were symptomless hosts of RSSC strains and were highly infested with the pathogen. It is likely that the host-pathogen-environment interaction plays a role in the suppression of wilt symptoms in these weeds (Lin et al. 1999; Tusiime et al. 1998). In a study of the population dynamics of RSSC strains in the roots of weeds and the ability of weeds to spread tobacco bacterial wilt, Fang et al. found that the pathogen could proliferate and overwinter in weed roots. The weeds that the pathogen could overwinter in included *Alopecurus aequalis*, *Polygonum orientale*, and *Alternanthera philoxeroides*, but the pathogen

Fig. 1. Symptoms of *Ralstonia pseudosolanacearum* infection on *Bidens pilosa* and colony characteristics of the isolate Bp01 on TTC medium. **a** and **b**, Symptoms associated with bacterial wilt of *B. pilosa* in the field; **c**, colony characteristics of the isolate Bp01 on TTC medium; **d**, symptoms 12 days after inoculation with the suspension of isolate Bp01; **e**, control plants, inoculated with sterilized water.

Table 3. Virulence of isolates from *Bidens pilosa* and adjacent plants assessed by cross inoculations

Host	Isolate	Wilt incidence of inoculated plants (%) ^z			
		<i>B. pilosa</i>	Peanut	Bitter gourd	Eucalyptus
<i>B. pilosa</i>	Bp01	83.33 ± 3.61 a	16.67 ± 0 b	0 b	90.00 ± 10.00 ab
	Bp02	50.00 ± 6.25 b	10.00 ± 0 b	0 b	70.00 ± 10.00 b
	Bp03	62.50 ± 6.25 b	0 c	0 b	100.00 ± 0 a
Peanut	Pn33	19.44 ± 4.81 d	80.00 ± 10.00 a	–	–
Bitter gourd	Bg07	33.33 ± 0 c	–	50.00 ± 8.33 a	–
Eucalyptus	Ec06	13.89 ± 4.82 d	–	–	90.00 ± 10.00 ab

^z Wilt incidence ± standard deviations ($n = 3$ replicates, 16 plants per replicate). Wilt incidence was calculated as the percentage of inoculated plants that were completely wilted. Values followed by the same letter in the same column are not significantly different (Tukey's test, $\alpha = 0.05$).

Fig. 2. The phylogenetic tree of *egl* gene partial sequences constructed with MEGA 6.0 using the neighbor-joining method. The numbers at the tree branch points indicate the percent bootstrap support for 1,000 iterations. ● Present reference sequevars; ▲ the *Bidens pilosa* isolate sequevars.

was only noted on the root surface of *Bidens bipinnata* and *Phytolacca acinosa*. According to the amount of bacteria carried and the ability to transmit the diseases, these weeds were divided into different grades (Fang et al. 2013).

There is no doubt that weeds, as the bridge hosts of bacterial wilt, play an important role in increasing the amount of RSSC in soil and continuing its survival after the harvest of cultivated crops. The pathogen that caused the *B. pilosa* bacterial wilt found in China was *R. pseudosolanacearum*, biovar 3. Although *B. pilosa* isolates infected mulberry or ginger weakly when the inoculate was injected, the isolates infected tomato, eggplant, tobacco, and potato plants strongly, so the *B. pilosa* isolates belonged to race 1 (Buddenhagen et al. 1962; Buddenhagen and Kelman 1964; He et al. 1983). In contrast to previous reports that RSSC strains only caused a latent infection in *B. pilosa*, the plants in Guangxi could produce typical symptoms of bacterial wilt. The transition from latent infection to obvious symptoms of the host indicated that the adaptability of pathogens to the host may be enhanced and the quantities of pathogens in the soil will increase accordingly. This suggests that the role of the pathogen in the disease cycle may be further enhanced and bacterial wilt management may become increasingly difficult.

Although the latent infection of RSSC strains in weeds and its role in the circulation of bacterial wilt have been widely recognized, the classification status and geographical origin of the weed pathogens are not clear. In this study, the isolates from *B. pilosa* (Bp01, Bp02, and Bp03) were identified as phylotype I, including sequevars 17 and 44, and an unknown sequevar. The *B. pilosa* isolate sequevars were not identical to those of nearby host isolates, and the virulence of these isolates was low when cross inoculated, even if the isolates were collected from the same field. This indicated that the bacterial wilt pathogen of *B. pilosa* weeds did not necessarily come from a host in the same or adjacent fields. These isolates provide more resources to explore host specificity within RSSC. The sequevar is related to the geographic region and distribution of the RSSC. The finding of different sequevars among the *B. pilosa* isolates from three different regions contributes to information on RSSC epidemiology and management. This is the first report of *R. pseudosolanacearum* on *B.*

pilosa in Guangxi, China, but the source of the pathogen remains to be identified. Although it has been reported that weeds are intermediate hosts that need to be considered in the circulation and agricultural control of bacterial wilt, our research suggests that the study of the role of weeds in the complex epidemiology, geographical origin, and host specificity of the RSSC needs to be strengthened. Further work is required to determine the function of weeds in the evolution of the RSSC.

Literature Cited

- Amat, Z., Alborno, A., Hevesi, M., and Stefanova, M. 1978. *Pseudomonas solanacearum* detected in a naturally infested soil containing a new wild host. Pages 869-873 in: Proceedings of the Fourth International Conference on Plant Pathogenic Bacteria. Angers, France.
- Buddenhagen, I., and Kelman, A. 1964. Biological and physiological aspects of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 2:203-230.
- Buddenhagen, I., Sequeira, L., and Kelman, A. 1962. Designation of races in *Pseudomonas solanacearum*. Phytopathology 52:726.
- Cellier, G., and Prior, P. 2010. Deciphering phenotypic diversity of *Ralstonia solanacearum* strains pathogenic to potato. Phytopathology 100:1250-1261.
- Fang, S. M., Gu, G., Chen, Y. S., Huang, C. M., and Chen, S. H. 2013. Colonization and infection of *Ralstonia solanacearum* in weed roots. Acta Tabacaria Sin. 19:72-81 (In Chinese).
- Fegan, M., and Prior, P. 2005. How complex is the *Ralstonia solanacearum* species complex? Pages 449-461 in: Bacterial Wilt: The Disease and the *Ralstonia solanacearum* Species Complex. C. Allen and A. C. Hayward, eds. APS Press, St. Paul, MN.
- Fegan, M., and Prior, P. 2006. Diverse members of the *Ralstonia solanacearum* species complex cause bacterial wilts of banana. Australas. Plant Pathol. 35:93-101.
- Fegan, M., Taghavi, M., Sly, L. I., and Hayward, A. C. 1998. Phylogeny, diversity and molecular diagnostics of *Ralstonia solanacearum*. Pages 19-33 in: Bacterial Wilt Disease: Molecular and Ecological Aspects. P. Prior, C. Allen, and J. Elphinstone, eds. Springer, New York.
- Granada, G. A., and Sequeira, L. 1983. Survival of *Pseudomonas solanacearum* in soil, rhizosphere and plant roots. Can. J. Microbiol. 29:433-440.
- Hayward, A. C. 1964. Characteristics of *Pseudomonas solanacearum*. J. Appl. Bacteriol. 27:265-277.
- Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. Annu. Rev. Phytopathol. 29:65-87.
- He, L. Y., Sequeira, L., and Kelman, A. 1983. Characteristics of strains of *Pseudomonas solanacearum* from China. Plant Dis. 67:1357-1361.
- Jiang, G. F., Wei, Z., Xu, J., Chen, H. L., Zhang, Y., She, X. M., Macho, A. P., Ding, W., and Liao, B. S. 2017. Bacterial wilt in China: history, current status, and future perspectives. Front. Plant Sci. 8:1549.
- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium chloride medium. Phytopathology 44: 693-695.
- Kyaw, H. W. W., Tsuchiya, K., Matsumoto, M., Iiyama, K., Aye, S. S., Zaw, M., Kurose, D., Horita, M., and Furuya, N. 2017. Genetic diversity of *Ralstonia solanacearum* strains causing bacterial wilt of Solanaceous crops in Myanmar. J. Gen. Plant Pathol. 83:216-225.
- Lane, D. J. 1991. 16S/23S rRNA sequencing. Pages 115-175 in: Nucleic acid techniques in bacterial systematics. E. Stackebrandt and M. Goodfellow, eds. Wiley, Chichester, UK.
- Li, Y., Feng, J., Liu, H., Wang, L., Hsiang, T., Li, X., and Huang, J. 2016. Genetic diversity and pathogenicity of *Ralstonia solanacearum* causing tobacco bacterial wilt in China. Plant Dis. 100:1288-1296.
- Lilam, G., Juan, H., Elizabeth, F., Jan, K., and Hannele, L. K. 2017. Diversity, pathogenicity, and current occurrence of bacterial wilt bacterium *Ralstonia solanacearum* in Peru. Front. Plant Sci. 8:1-12.
- Lin, J. Q., Xu, S. D., and Zeng, G. Q. 1999. Weed host of *Ralstonia solanacearum* in Taiwan. J. Plant Prot. Sci. 41:277-292 (In Chinese).
- Liu, Y., Wu, D., Liu, Q., Zhang, S., Tang, Y., Li, S., and Jiang, G. 2017. The sequevar distribution of *Ralstonia solanacearum* tobacco-growing zones of China is structured by elevation. Eur. J. Plant Pathol. 147: 541-551.
- Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., Machado, M. A., Toth, I., Salmond, G., and Foster, G. D. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol. Plant Pathol. 13:614-629.
- Opina, N., Tavner, F., Hollway, G., Wang, J. F., Li, T. H., Maghirang, R., Fegan, M., Hayward, A., Krishnapillai, V., Hong, W., Holloway, B., and Timmis, J. 1997. A novel method for development of species and strain-specific DNA probes and PCR primers for identifying *Burkholderia solanacearum* (formerly *Pseudomonas solanacearum*). Asia Pac. J. Mol. Biol. Biotechnol. 5:19-30.
- Poussier, S., Prior, P., Luisetti, J., Hayward, C., and Fegan, M. 2000. Partial sequencing of the *hrpB* and endoglucanase genes confirms and expands the known diversity within the *Ralstonia solanacearum* species complex. Syst. Appl. Microbiol. 23:479-486.

- Prior, P., Ailloud, F., Dalsing, B. L., Remenant, B., Sanchez, B., and Allen, C. 2016. Genomic and proteomic evidence supporting the division of the plant pathogen *Ralstonia solanacearum* into three species. *BMC Genomics* 17:90.
- Safni, I., Cleenwerck, I., De, V. P., Fegan, M., Sly, L., and Kappler, U. 2014. Polyphasic taxonomic revision of the *Ralstonia solanacearum* species complex: proposal to emend the descriptions of *Ralstonia solanacearum* and *Ralstonia syzygii* and reclassify current *R. syzygii* strains as *Ralstonia syzygii* subsp. *syzygii* subsp. nov., *R. solanacearum* phylotype IV strains as *Ralstonia syzygii* subsp. *indonesiensis* subsp. nov., banana blood disease bacterium strains as *Ralstonia syzygii* subsp. *celebesensis* subsp. nov., and *R. solanacearum* phylotype I and III strains as *Ralstonia pseudosolanacearum* sp. nov. *Int. J. Syst. Evol. Microbiol.* 64:3087-3103.
- Santiago, T. R., Lopes, C. A., Caetano-Anollés, G., and Mizubuti, E. S. G. 2016. Phylotype and sequevar variability of *Ralstonia solanacearum* in Brazil, an ancient centre of diversity of the pathogen. *Plant Pathol.* 66:383-392.
- Shang, C. Q., and Zhu, X. Z. 2019. Invasion mechanisms, control and utilization of alien plant species *Bidens pilosa*. *Pratacultural Sci.* 36:47-60 (In Chinese).
- She, X. M., He, Z. F., and Li, H. P. 2018. Genetic structure and phylogenetic relationships of *Ralstonia solanacearum* strains from diverse origins in Guangdong Province, China. *J. Phytopathol.* 166:177-186.
- She, X. M., Yu, L., Lan, G. B., Tang, Y. F., and He, Z. F. 2017. Identification and genetic characterization of *Ralstonia solanacearum* species complex isolates from *Cucurbita maxima* in China. *Front. Plant Sci.* 8:1794.
- Sunaina, V., Kishore, V., and Shekhawat, G. S. 1989. Latent survival of *Pseudomonas solanacearum* in potato tubers and weeds. *Z. Pflanzenschutz* 96:361-364.
- Swanepoel, A. E. 1992. Survival of South African strains of biovar 2 and biovar 3 of *Pseudomonas solanacearum*, in the roots and stems of weeds. *Potato Res.* 35: 329-332.
- Tusiime, G., Adipala, E., Opio, F., and Bhagsari, A. S. 1998. Weeds as latent hosts of *Ralstonia solanacearum* in Highland Uganda: implications to development of an integrated control package for bacterial wilt. Pages 413-419 in: *Bacterial Wilt Disease*. P. Prior, C. Allen, and J. Elphinstone, eds. Springer, Berlin, Heidelberg, Germany.
- Wicker, E., Grassart, L., Coranson-Beaudu, R., Mian, D., Guilbaud, C., Fegan, M., and Prior, P. 2007. *Ralstonia solanacearum* strains from Martinique (French West Indies) exhibiting a new pathogenic potential. *Appl. Environ. Microbiol.* 73:6790-6801.
- Xu, J., Pan, Z. C., Prior, P., Xu, J. S., Zhang, Z., Zhang, H., Zhang, L. Q., He, L. Y., and Feng, J. 2009. Genetic diversity of *Ralstonia solanacearum* strains from China. *Eur. J. Plant Pathol.* 125:641-653.