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Characterization of *Xanthomonas* Associated with Bacterial Spot of Tomato and Pepper in Taiwan

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ABSTRACT

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Bacterial spot is an important disease of tomato and pepper in Taiwan. Recently, the strains of *Xanthomonas* associated with bacterial spot of tomato and pepper were classified into four species, namely *X. euvesicatoria* (Doidge) Jones *et al*., *X. gardneri* (Sutic) Jones *et al*., *X. perforans* Jones *et al*. and *X. vesicatoria* (Doidge) Vauterin *et al*.. To characterize *Xanthomonas* species associated with bacterial spot of tomato and pepper (BSX) in Taiwan, 53 BSX strains collected from various localities in Taiwan were analyzed. Among them, 13 strains were amylolytic and pectolytic. The 560 bp DNA fragment specific for *X. axonopodis* pv. *vesicatoria* was amplified from 40 strains with primer pair RST13/14, and the 407 bp DNA fragment specific for *X. vesicatoria* was amplified from five strains with primer pair C-2-2L/2R, however no products could be amplified from the other eight strains by either primer pairs. Carbon sources utilization of these BSX strains were tested on Biolog GN2 microplate. Three utilization patterns of 13 differential carbon sources were observed as described for *X. euvesicatoria*, *X. vesicatoria* and *X. perforans*. Based on the characterization results, 40 strains of *X. euvesicatoria*, 8 strains of *X. perforans* and 5 strains of *X. vesicatoria* were identified. No *X. gardneri* strains were found in this study. In addition, the strains identified as *X. perforans* produced bacteriocins that inhibited the growth of *X. euvesicatoria*. This is the first report of *X. perforans* in Taiwan. The distribution and ecological fitness of *X. perforans* strains in Taiwan is worthy of further study.

Keywords: *Xanthomonas euvesicatoria*, *Xanthomonas gardneri*, *Xanthomonas perforans*, *Xanthomonas vesicatoria*, Bacteriocin

INTRODUCTION

Bacterial spot is an important disease of tomato and pepper worldwide. It causes significant economic losses especially in warm and wet weather. The causal agent of this disease was originally named as *Bacterium vesicatorium*. The bacterium was then transferred to *Xanthomonas* as *X. vesicatoria* and later to *X. campestris* as *X. campestris* pv. *vesicatoria* (Doidge) Dye [11]. Based on the phenotypic and genetic analysis, two diverse groups of strains are included in *X. campestris* pv. *vesicatoria* [16]. When species and pathovars of *Xanthomonas* was reclassified based on DNA hybridization, utilization of carbon sources, electrophoresis of whole-cell proteins and cellular fatty acids contents, the strains in these two diverse groups of *X. campestris* pv. *vesicatoria* were separated into two different species, *X. axonopodis* pv. *vesicatoria* (Doidge) Vauterin *et al*. (*X. a*. pv. *vesicatoria*) and *X. vesicatoria* (Doidge) Vauterin *et al.* [19]. The strains of *X. a*. pv. *vesicatoria* are non-amylolytic and non-pectolytic, while strains of *X. vesicatoria* are amylolytic and pectolytic [16, 19]. *Pseudomonas gardneri* Sutic is another bacterial leaf spot pathogen of tomato, it was first isolated from Yugoslavia. This bacterium is phenotypically and phylogenetically related to xanthomonads [2, 7]. However, it has less than 70% DNA related to any of the *Xanthomonas* species. Jones *et al*. reclassified these strains as *X. gardneri* (Sutic) Jones *et al.* [9]. In addition, new amylolytic and pectolytic strains of bacterial spot causing *Xanthomonas* (BSX) were isolated in Florida [12]. However, these strains are different from *X. vesicatoria* serologically and pathogenically [1]. Based on DNA-DNA hybridization and metabolic profiles, Jones *et al*. reclassified the BSX strains into four species *X. euvesicatoria* (Doidge) Jones *et al*., *X. gardneri* (Sutic) Jones *et al*., *X. perforans* Jones *et al*. and *X. vesicatoria* (Doidge) Vauterin *et al*. in 2004 [9]. The metabolic patterns of thirteen carbon substrates on Biolog GN2 Microplate can be used to discriminate the strains of these four species of *Xanthomonas* species associated with bacterial spot of tomato and pepper. The strains of *X. euvesicatoria* can utilize most of the thirteen carbon substrates, and the utilization of *cis*-aconitic acid can be used to differentiate *X. euvesicatoria* from *X. vesicatoria*, the utilization of glycyl-L-aspartic acid can be used to distinguish *X. euvesicatoria* from *X. perforans*, and the utilization of acetic acid is a main characteristic of *X. perforans* to separate it from *X. vesicatoria*, whereas strains of *X. gardneri* can not use any of these thirteen carbon substrates [9]. Based on the pathogenicity tests, these four species of *Xanthomonas* can be corresponded to different tomato races as follows: *X. euvesicatoria* as tomato race 1 (T1 race) strains; *X. gardneri* and *X. vesicatoria* as tomato race 2 (T2 race) strains; *X. perforans* as tomato race 3 (T3 race) or tomato race 4 (T4 race) strains (12, 17). In addition, the strains of T3 (*X. perforans*) can produce the bacteriocin to inhibit the growth of T1 strain (*X. euvesicatoria*), and gain a competitive advantage in the greenhouses and fields [5].

Polymerase chain reaction is a powerful tool which can rapidly identify plant pathogenic bacteria [13]. The primer pair RST 13/14 specific for *X. axonopodis* pv. *vesicatoria* has been developed based on the DNA region involved in expression of lipopolysaccharide [10]. In addition, primer pair C-2-2L/2R specific for *X. vesicatoria* was developed from random amplification DNA polymorphism analysis. With these two primer pairs, strains of *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria* could be rapidly identified by multiplex PCR [14].

The variability of BSX strains in Taiwan were studied in 1998, and two species, *X. axonopodis* pv. *vesicatoria* and *X. vesicatoria* were identified [6]. Since then, there was no further report to characterize the *Xanthomonas* species associated with bacterial spot of pepper and tomato in Taiwan. In this study, 53 *Xanthomonas* strains isolated from bacterial spot of pepper and tomato in Taiwan were characterized by biochemical characteristics and multiplex PCR with specific primers. Based on the classification scheme of Jones *et al*. [9], strains of *X. euvesicatoria*, *X. perforans* and *X. vesicatoria* were found in Taiwan. No *X. gardneri* strains were detected in this study. This is also the first report of *X. perforans* identified in Taiwan.

MATERIALS AND METHODS

Bacterial strains

Fifty-three *Xanthomonas* strains isolated from bacterial spot of tomato and pepper in Taiwan were used in this study (Table 1). All strains were preserved in 15% glycerol at -70℃. They were subcultured on nutrient agar (NA) (beef extract 3 g, peptone 5 g, agar 15 g, distilled water 1 L) (Difco Laboratories, Detroit) plates at 30℃ for

72 hr prior to analysis.

Amylolytic and pectolytic activity

The NA plates containing 1.5% soluble potato starch (Sigma-Aldrich, Inc. MO. USA) were used to test the amylolytic activity [16]. The strains were streaked on the plates and incubated at 30℃ for 72 hr. Plates were then flooded with I-KI solution (iodine 1.0 g, potassium iodine 2.0 g, distilled water 100 ml). A clear zone would be present around the colonies of the strains with amylolytic activity. For pectolytic activity test, strains were spot-inoculated on modified crystal violet pectate (CVP) plate and incubated at 30℃ for 72 hr. Pectolytic strains produce pits on CVP plates [4].

Polymerase chain reaction with primers RST13/14 and C-2-2L/2R

The primers pairs RST13/14 and C-2-2L/2R specific for *X. a.* pv. *vesicatoria* and *X. vesicatoria*, respectively, were used to identify BSX strains by multiplex PCR [10, 14]. To prepare the template for PCR, a single colony of tested strain was picked up by toothpick and suspended in 500 μl double distilled water. A 20 μl reaction mixture was set up as follows: 1 μl bacterial suspension, 0.5 mM dNTP, 0.5 μM each primers, 0.6 unit of GenTaq polymerase (GeneMark Technology, Taiwan), and 1X GenTaq polymerase buffer. Amplification was performed in a thermal cycler (PTC-200 MJ Research cycler, Bio-rad, USA) with the following conditions: one cycle of 10 min at 95℃; 40 cycles of 30 sec at 95℃, 30 sec at 65℃ and 30 sec at 72℃; and a final extension of 10 min at 72℃. Amplification products were separated on a 1.5% agarose gel, stained with ethidium bromide, and visualized under UV light.

Carbon sources utilization

The strains tested were cultured on NA plate and then transferred to Biolog Universal Growth (BUG) (Biolog Inc. CA. USA) medium. Bacterial cells were suspended in sterilized Gram-negative/Gram-positive inoculating fluid (0.4% NaCl, 0.03% Pluronic F68, and 0.02% Gellen Gum) and adjusted the turbidity to 52%±3% T at 590 nm in a spectrophotometer (Biolog Inc. CA. USA). A 150 μl of bacterial suspension was added into each well of the GN2 microplate by multichannel micropipette (Biohit Proline® Helsinki, Finland), and the plates were incubated for 24 hr at 30℃. Positive reaction of a carbon source was indicated by a color appearance. Results were recorded with MicroStation reader (Biolog Inc. CA. USA) to determine the utilization of 95 carbon substrates of each strain. The utilization patterns of 95 carbon substrates were analyzed by NT-SYS pc2.0 (Exeter Software, NY. USA). The similarity coefficient between two strains was calculated using the SimQual program. Cluster analysis by the unweighted pair group method was used in the SAHN program.

Bacteriocin production

The strains identified as *X. perforans* were further assayed on NA plates for bacteriocin production following the method of Vidaver *et al.* [20]. Strains of *X. euvesicatoria* XVT12 and XVT48 were used as the indicators. The surface of each NA plate was spot-inoculated with 20 μl overnight culture of tested strains in LB broth. The inoculated plates were incubated at 30℃ for 24 hr. The plates were then inverted and exposed to vapor from 3 ml chloroform for 30 min to kill the bacterial cells. Residual chloroform vapors were removed by placing the plates with lids off in a laminar flow for 30 min. Each plate was then overlaid with 7 ml molten NA soft agar containing 200 μl of overnight culture of the indicator bacterial strain in LB broth. After incubation at 30℃ for 24 hr, the inhibition zones were examined.

RESULTS

Amylolytic and pectolytic activity

Fifty-three BSX strains were examined for their amylotic and pectolytic activities. A total of 13 strains were both amylolytic and pectolytic; while the other 40 strains did not hydrolyze starch and degrade pectate medium (Table 1).

PCR test with specific primers

Multiplex PCR was performed with primer pairs RST 13/14 and C-2-2L/2R specific for *X. a.* pv. *vesicatoria* and *X. vesicatoria*, respectively, to identify the BSX strains. The 560-bp DNA fragment (Fig. 1) was amplified from 40 strains tested that were non-amylolytic and non-pectolytic. The 407-bp DNA fragment specific for *X. vesicatoria* (Fig.

1) was amplified from five, but not from the other eight strains with amylolytic and pectolytic activities (Table 1 and Fig. 1).

Carbon sources utilization

According to the differentiation scheme of Jones *et al.* [9], the BSX strains in Taiwan were characterized by carbon sources utilization using Biolog GN MicroPlate system. The 53 BSX strains were characterized into three groups which included 40 strains of group I identified as *X. euvesicatoria* (formerly *X. a.* pv. *vesicatoria*), 5 strains of group II identified as *X. vesicatoria* and 8 strains of group III identified as *X. perforans*; and there was no strains characterized as *X. gardneri* in this study (Table 2). These BSX strains were clustered into two groups on the basis of the utilization of the 13 differentiation carbon sources according to Jones *et al.* [9]. Group A consisted of 46 strains which utilized cis- aconitic acid, and group B consisted of 7 strains which utilized gentibiose but not cis-aconitic acid and acetic acid (Fig. 2). In order to reveal a better differentiation, the 13 amylolytic and pectolytic strains were further clustered on the utilization patterns of 13 differential carbon sources. Eight strains (XTN 1, XTN 7, XTN 47, XTN 50, XTN 168, XTN 169, XTN 170 and XTN 171) utilized at least 11 differential carbon sources including dextrin, glycogen, N-acetyl-D-glucosamine, D-galactose, gentibiose, α-D-lactose lactulose, acetic acid, cis-aconitic acid, malonic acid, D-alanine and L-threonine were identified as *X. perforans*. The other five strains (Xv 92, Xv 105, XVT 45, XVT 147 and XVT 148) which utilized dextrin and gentibiose but not N-acetyl-D-glucosamine, acetic acid, cis-aconitic acid, malonic acid and D-alanine were identified as *X. vesicatoria* (Table 2 and Fig. 3).

Bacteriocin production

The strains identified as *X. perforans* in this study were further tested for their bacteriocin activity. The results showed that all *X. perforans* strains produced bacteriocins to inhibit growth of *X. euvesicatoria* XVT48 (Fig. 4) .

DISCUSSION

Fifty-three strains of *Xanthomonas* associated with bacterial spot of tomato and pepper in Taiwan were characterized in this study. Based on amylolytic and pectolytic tests, 40 strains which were non-amylolytic and non-pectolytic belong to *X. a.* pv. *vesicatoria* (Doidge) Vauterin *et al.*, 13 amylolytic and pectolytic strains belong to *X. vesicatoria* (Doidge) Vauterin *et al.* [6]. Jones *et al.* [9] reported that 13 carbon sources can be used to differentiate *Xanthomonas* spp. causing bacterial spot on tomato and pepper. Following this system, strains of *X. euvesicatoria*, *X. vesicatoria*, and *X. perforans* were identified in this study. Strains of *X. euvesicatoria* are predominant and distributed widely in Taiwan. Distribution of *X. vesicatoria* and *X. perforans* appeared to be limited, but requires

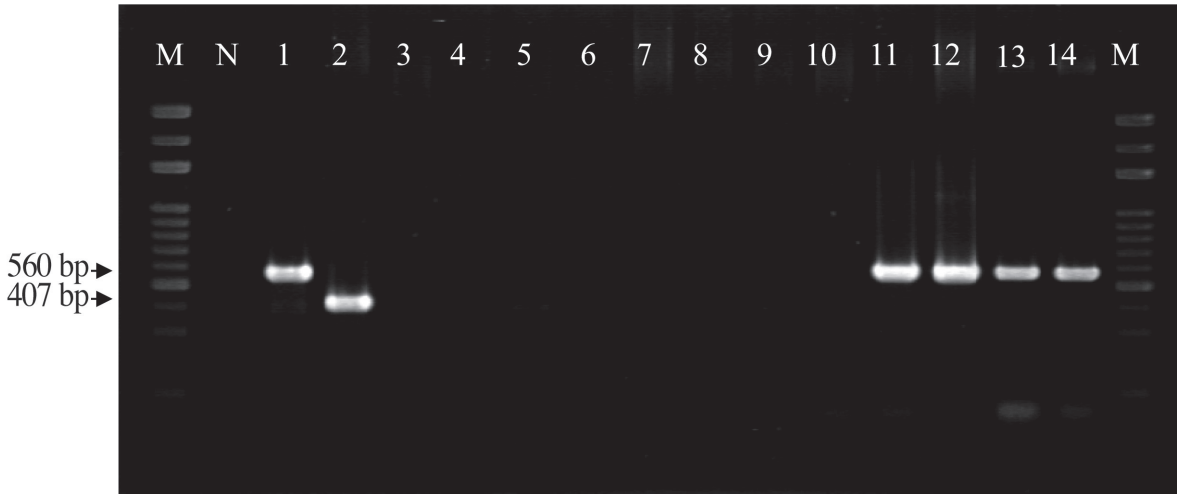

Fig. 1. Amplification of *Xanthomonas* strains isolated from bacterial spot of tomato and pepper in Taiwan by multiplex PCR with RST13/14 and C-2-2L/2R. Lane M, Bio100 DNA Ladder (PROtech Technology, R.O.C.); lane N, sterilized distilled water, lane 1, *Xanthomonas euvesicatoria* Xv 62; lane 2, *X. vesicatoria* Xv 92; lanes 3 ~10, XTN 1, 7, 47, 50, 168, 169, 170, 171; lanes 11~14, XTN 29, 36, 44, 46.

Table 1. Bacterial strains used in this study

Strain	Host	Location	Source [1]	Amylolytic and pectolytic [2]	RST13/14 and C-2-2L/2R(bp) [3]
XPN 2	Pepper	Yunlin	TNDAIS	−	560
XPN 3	Pepper	Yunlin	TNDAIS	−	560
XPN 33	Pepper	Chiayi	TNDAIS	−	560
XPN 34	Pepper	Chiayi	TNDAIS	−	560
XPN 35	Pepper	Chiayi	TNDAIS	−	560
XPN 37	Pepper	Pingtung	TNDAIS	−	560
XPN 38	Pepper	Pingtung	TNDAIS	−	560
XTN 1	Tomato	Yunlin	TNDAIS	+	−
XTN 3	Tomato	Yunlin	TNDAIS	−	560
XTN 7	Tomato	Yunlin	TNDAIS	+	−
XTN 11	Tomato	Chiayi	TNDAIS	−	560
XTN 14	Tomato	Chiayi	TNDAIS	−	560
XTN 17	Tomato	Chiayi	TNDAIS	−	560
XTN 29	Tomato	Chiayi	TNDAIS	−	560
XTN 32	Tomato	Chiayi	TNDAIS	−	560
XTN 36	Tomato	Chiayi	TNDAIS	−	560
XTN 44	Tomato	Chiayi	TNDAIS	−	560
XTN 46	Tomato	Chiayi	TNDAIS	−	560
XTN 47	Tomato	Chiayi	TNDAIS	+	−
XTN 50	Tomato	Chiayi	TNDAIS	+	−
XTN 51	Tomato	Chiayi	TNDAIS	−	560
XTN 64	Tomato	Tainan	TNDAIS	−	560
XTN 76	Tomato	Tainan	TNDAIS	−	560
XTN 78	Tomato	Taitung	TNDAIS	−	560
XTN 79	Tomato	Taitung	TNDAIS	−	560
XTN 80	Tomato	Taitung	TNDAIS	−	560
XTN 168	Tomato	Tainan	TNDAIS	+	−
XTN 169	Tomato	Tainan	TNDAIS	+	−
XTN 170	Tomato	Tainan	TNDAIS	+	−
XTN 171	Tomato	Tainan	TNDAIS	+	−
Xv 12	Tomato	Yunlin	TARI	−	560
Xv 17	Tomato	Changhwa	TARI	−	560
Xv 21	Tomato	Kaohsiung	TARI	−	560
Xv 62	Pepper	Yunlin	TARI	−	560
Xv 64	Pepper	Yunlin	TARI	−	560
Xv 92	Tomato	Nantou	TARI	+	407
Xv 105	Tomato	Nantou	TARI	+	407
XVT 12	Tomato	Taichung	AVRDC	−	560
XVT 28	Tomato	Tainan	AVRDC	−	560
XVT 38	Tomato	Hualien	AVRDC	−	560
XVT 45	Tomato	Nantou	AVRDC	+	407
XVT 48	Tomato	Changhwa	AVRDC	−	560
XVT 57	Tomato	Ilan	AVRDC	−	560
XVT 85	Tomato	Chiayi	AVRDC	−	560
XVT 122	Tomato	Pingtung	AVRDC	−	560
XVT 147	Tomato	Nantou	AVRDC	+	407
XVT 148	Tomato	Nantou	AVRDC	+	407
XVT 185	Tomato	Hsinchu	AVRDC	−	560
XVP 169	Pepper	Nantou	AVRDC	−	560
XVP 182	Pepper	Hualien	AVRDC	−	560
XVP 186	Pepper	Hualien	AVRDC	−	560
XVP 194	Pepper	Tainan	AVRDC	−	560
XVP 197	Pepper	Tainan	AVRDC	−	560

[1] TNDAIS: Tainan District Agricultural Research & Extension Station, TARI: Taiwan Agricultural Research Institue, AVRDC: AVRDC-The World Vegetable Center

[2] Amylolytic activity was tested on Nutrient agar plates containing starch [16]; pectolytic activity was tested on modified CVP medium [4].

[3] A specific 560 bp DNA fragment amplified using primer pair RST 13/14 for *X. euvesicatoria* [10]. A specific 407 bp DNA fragment amplified using primer pair C-2-2L/2R for *X. vesicatoria* [14]. "—" indicates that no amplification products were observed with these two set of primer pairs.

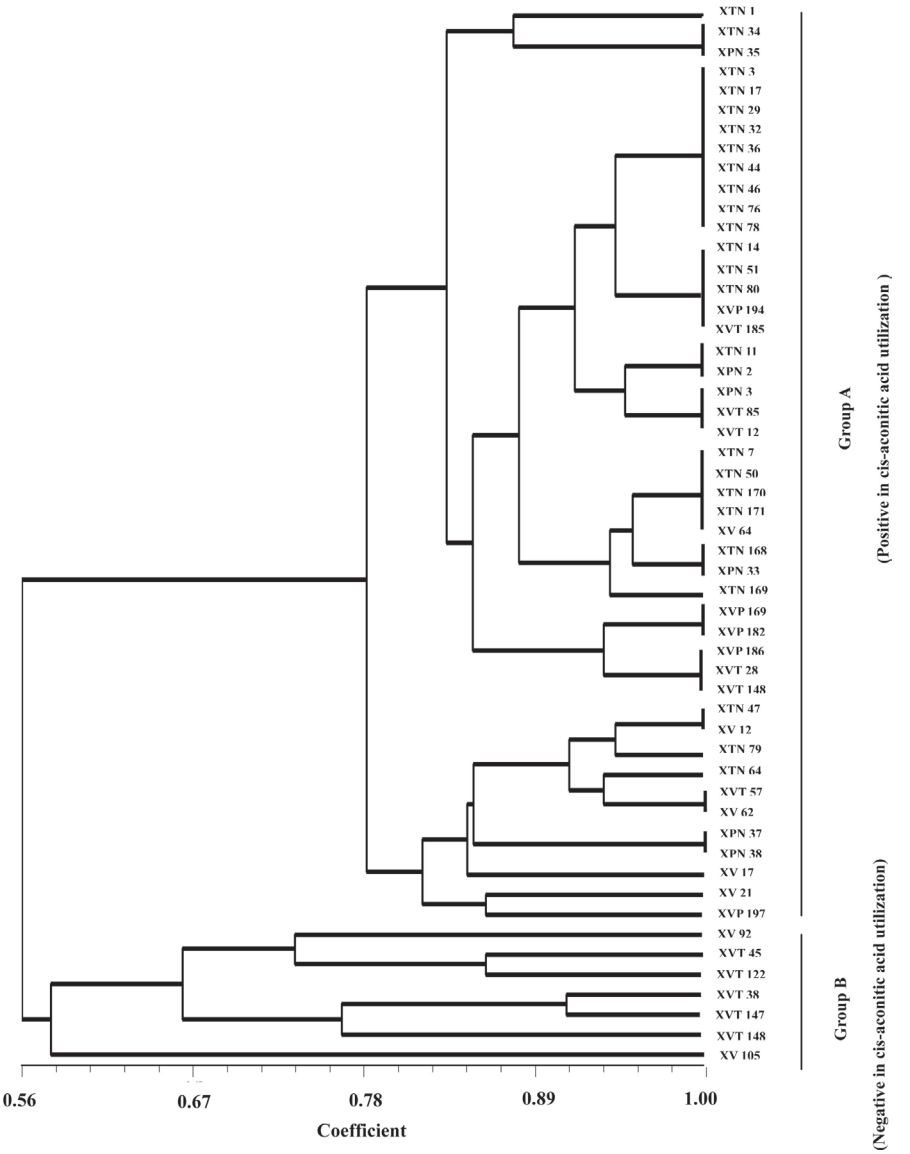

Fig. 2. Grouping of 53 *Xanthomonas* strains isolated from bacterial spot of tomato and pepper in Taiwan on the basis of utilization of 13 differentiation carbon sources on the Biolog GN2 MicroPlate (Jones *et al*., 2004). The tree was derived from cluster analysis of distances among strains using the UPGMA with NT-SYS pc 2.0 (Exeter Software, NY. USA)

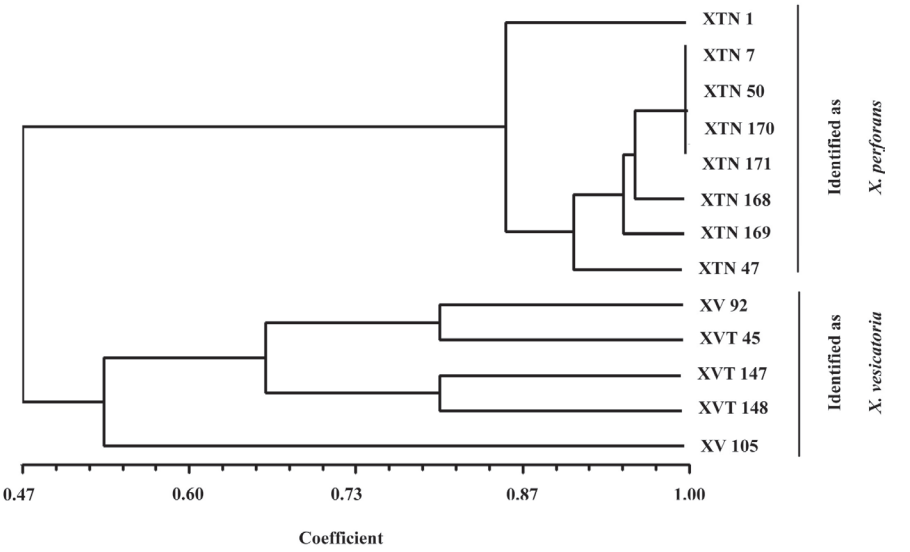

Fig. 3. Grouping of thirteen amylolytic and pectolytic *Xanthomonas* strains isolated from bacterial spot of tomato in Taiwan on the basis of utilization patterns of 13 differentiation carbon sources on the Biolog GN2 MicroPlate (Jones *et al*., 2004). The tree was derived from cluster analysis of distances among strains using the UPGMA with NT-SYS pc 2.0 (Exeter Software, NY. USA)

Table 2. Differentiation of BSX strains in Taiwan on carbon utilization patterns using the Biolog GN2 MicroPlate system

Carbon sources utilization	Group[1]						Carbon sources utilization pattern[2]			
	I (40)		II (5)		III (8)		*X. euvesicatoria*	*X. vesicatoria*	*X. perforans*	*X. gardneri*
Dextrin	+	40[3]	+	5	+	8	+	+	+	−
Glycogen	V-	9	V-	2	+	8	+	V	V	−
N-acetyl-D-glucosamine	+	40	−	0	+	8	+	V	+	−
D-galactose	V	25	V-	1	V	7	+	V-	+	−
Gentibiose	+	40	+	5	+	8	+	V	+	−
α-D-lactose lactulose	V	33	V-	1	V	7	V	V-	+	−
Acetic acid	V	28	−	0	V	7	V	−	+	−
cis-aconitic acid	V	38	−	0	+	8	+	−	V	−
Malonic acid	V	28	−	0	+	8	+	V	+	−
Propionic acid	V-	6	−	0	V	5	V-	V	+	−
D-alanine	+	40	V	3	+	8	V	V	+	−
Glycyl-L-aspartic acid	V-	1	V-	1	−	0	−	V-	+	−
L-threonine	V	35	V-	1	V	7	V	V-	+	−

[1] Forty strains of Group I identified as *X. euvesicatoria*.
Five strains of Group II identified as *X. vesicatoria*.
Eight strains of Group III identified as *X. perforans*.

[2] The data from Jones *et al.* [9]. + = positive reaction by all strains; v = 50% or more of strains utilized compound; v– = <50% of strains utilized compound; and – = none of strains utilized compound.

[3] Number of strains tested in this study showing positive reaction on Biolog GN2 MicroPlate.

further surveys for confirmation. Strains of *X. gardneri* have limited distribution globally, and only been reported in Yugoslavia, Brazil, Ontario in Canada and Florida in United States [3, 7, 15]. In this study, no *X. gardneri* strains was found in Taiwan.

Based on the 13 differential carbon sources utilization patterns, the 53 BSX strains were divided into two groups. The group A contained 46 BSX strains, all of them utilized cis-aconitic acid and formerly considered as *X. a.* pv. *vesicatoria* (Vauterin *et al.*). However, eight strains (XTN 1, XTN 7, XTN 47, XTN 50, XTN 168, XTN 169, XTN 170 and XTN 171) in group A were amylolytic and pectolytic; and no DNA fragment was amplified by multiplex PCR with primer pairs RST13/14 and C-2-2L/2R. These strains can utilize dextrin, glycogen, N-acetyl-D-glucosamine, D-galactose, gentibiose, α-D-lactose lactulose, acetic acid, cis-aconitic acid, malonic acid, D-alanine and L-threonine. Jones *et al.* [9] described 50% or more strains of *X.*

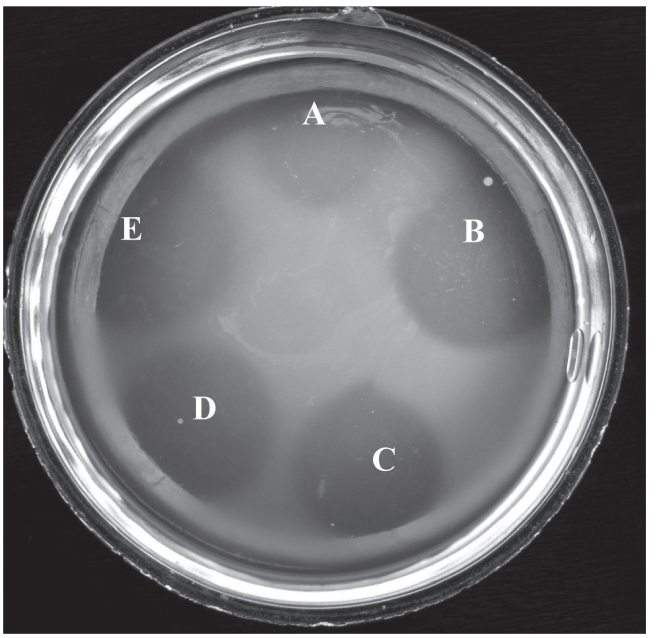

Fig. 4. Inhibition of *Xanthomonas euvesicatoria* XVT 48 by *X. perforans* strains XTN 1 (A), XTN 7 (B), XTN 47 (C), XTN 50 (D) and XTN 168 (E).The bacteriocin producing strains of *X. perforans* were spot-inoculated on the NA plate. The inoculated plate was incubated at 30℃ for 24 hr. The plate was then inverted and exposed to vapor from 3 ml chloroform for 30 min to kill the bacterial cells. The plate was then overlaid with 7 ml molten NA soft agar containing 200 μl of overnight culture of indicator *X. euvesicatoria* XVT 48.

perforans could utilize cis-aconitic acid, and *X. perforans* strains are usually associated with tomato. All of the above eight strains were isolated from tomato. Thus, considering all characteristics, the eight strains should belong to *X. perforans*, while the non-amylolytic and non-pectolytic strains in group A should belong to *X. euvesicatoria*. The remaining 7 strains which did not utilize cis-aconitic acid were clustered in group B and should be considered as *X. vesicatoria*. Two group B strains XVT38 and XVT122 were non-amylolytic and non-pectolytic, and the 560-bp DNA fragment specific for *X. a.* pv. *vesicatoria* (Vauterin *et al.*) was amplified from these two strains. Thus, they could be strains of *X. a.* pv. *vesicatoria* which lost the ability to utilize cis-aconitic acid. Thirteen amylolytic and pectolytic strains of BSX were found in this study, of which five strains (XVT45, XVT147, XVT148, Xv92 and Xv105) were identified as *X. vesicatoria*, because they did not utilize cis-aconitic acid and acetic acid, and the 407-bp DNA fragment specific for *X. vesicatoria* was amplified from their DNAs by multiplex PCR with primer pairs RST13/14 and C-2-2L/2R, while the other 8 strains were identified as *X. perforans* as stated above.

In Florida, *X. euvesicatoria* (formerly *X. a.* pv. *vesicatoria*) was the only pathogen of bacterial spot disease of tomato until the introduction of *X. perforans* in 1991 [8, 12]. Strains of *X. perforans* displaced *X. euvesicatoria* within three years and became the predominant population in commercial tomato fields [8]. The reason for the population shift in Florida could be in part resulted from antagonism of *X. perforans* toward *X. euvesicatoria* [5, 18]. Three kinds of bacteriocins were produced by *X. perforans*, including BCN-A, BCN-B and BCN-C. They provided a competitive advantage over *X. euvesicatoria* in the greenhouses and the fields [5]. In this study, BSX strains identified as *X. perforans* can also produce bacteriocins to inhibit the growth of *X. euvesicatoria*. These *X. perforans* strains may be more competitive than *X. euvesicatoria* strains in the tomato field. Hence, their impacts on the development of bacterial spot disease of pepper and tomato in the field conditions are worthy of further study.

The strains of *X. perforans* had been isolated from seeds produced from Thailand [12]. The infested seeds were the possible inoclum sources for transmitting this bacterium. An accurate and rapid detection technique for this bacterium is important to prevent the introduction of the pathogen to a new region. In this study, primer pairs RST 13/14 and C-2-2L/2R specific for *X. a.* pv. *vesicatoria* (Vauterin *et al.*) and *X. vesicatoria* (Vauterin *et al.*), respectively, were successfully used to identify strains of *X. euvesicatoria* (Jones *et al.*) and *X. vesicatoria* (Vauterin *et al.*). They can be applied simultaneously to detect *X. a.* pv. *vesicatoria* (Vauterin *et al.*) and *X. vesicatoria* in artificially infested tomato and pepper seeds by multiplex PCR [14]. No amplification product was observed from *X. perforans* with these two primer pairs. Thus, primer pair C-2-2L/2R, can be used to rapidly distinguish *X. vesicatoria* (Vauterin *et al.*) from *X. perforans*.

Based on the results of biochemical characteristics and PCR tests, strains of *X. euvesicatoria*, *X. perforans* and *X. vesicatoria* were the causal agents of bacterial spot of tomato and pepper in Taiwan. No *X. gardneri* strain was found in this study. This is the first report of *X. perforans* identified in Taiwan. Strains of *X. perforans* identified in this study can produce the bacteriocins to inhibit the growth of *X. euvesicatoria*. The distribution and ecological fitness of *X. perforans* strains in Taiwan is worthy of further study.

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LITERATURE CITED

1. Bouzar, H., Jones, J. B., Somodi, G. C., Stall, R. E., Daouzli, N., Lambe, R. C., Felix-Gastelum, R., and Trinidad-Correa, R. 1996. Diversity of *Xanthomonas campestris* pv. *vesicatoria* in tomato and pepper fields of Mexico. Can. J. Plant Pathol. 18: 75-77.
2. Bouzar, H., Jones, J. B., Stall, R. E., Hodge, N. C., Minsavage, G. V., Benedict, A. A., and Alvarez, A. M. 1994. Physiological, chemical, serological, and pathogenic analysis of a worldwide collection of *Xanthomonas campestris* pv. *vesicatoria* strains. Phytopathology 84: 663-671.
3. Cuppels, D. A., Louws, F. J., and Ainsworth, T. 2006. Development and evaluation of PCR-based diagnostic assays for the bacterial speck and bacterial spot pathogens of tomato. Plant Dis. 90: 451-458.
4. De Boer, S. H. and Kelman, A. 2001. *Erwinia* soft

rot group. Pages 56-72 *in*: Laboratory Guide for Identification of Plant Pathogenic Bacteria, N. W. Schaad, J. B. Jones and W. Chun, eds. APS, St. Paul, Minnesota.

5. Hert, A. P., Roberts, P. D., Momol, M. T., Minsavage, G. V., Tudor-Nelson, S. M., and Jones, J. B. 2005. Relative importance of bacteriocin-like genes in antagonism of *Xanthomonas perforans* tomato race 3 to *Xanthomonas euvesicatoria* Tomato race 1 strains. Appl. Environ. Microbiol. 71: 3581-3588.
6. Hsu, S. Y., Hsu, S. T., Tzeng, K. C., and Huang, H. C. 1998. Variability and grouping of strains of *Xanthomonas campestris* pv. *vesicatoria* from Taiwan. Plant Pathol. Bull. 7: 208. (In Chinese).
7. Jones, J. B., Bouzar, H., Stall, R. E., Almira, E. C., Roberts, P. D., Bowen, B. W., Sudberry, J., Strickler, P. M., and Chun, J. 2000. Systematic analysis of xanthomonads (*Xanthomonas* spp.) associated with pepper and tomato lesions. Int. J. Syst. Evol. Microbiol. 50: 1211-1219.
8. Jones, J. B., Bouzar, H., Somodi, G. C., Stall, R. E., Pernezny, K., El-Morsy, G., and Scott, J. W. 1998. Evidence for the preemptive nature of tomato race 3 of *Xanthomonas campestris* pv. *vesicatoria* in Florida. Phytopathology 88: 33-38.
9. Jones, J. B., Lacy, G. H., Bouzar, H., Stall, R. E., and Schaad, N. W. 2004. Reclassification of the xanthomonads associated with bacterial spot disease of tomato and pepper. Syst. Appl. Microbiol. 27: 755-762.
10. Jones, J. B., Minsavage, G. V., Stall, R. E., Kelly, R. O., and Bouzar, H. 1993. Genetic analysis of a DNA region involved in expression of two epitopes associated with lipopolysaccharide in *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 83: 551-556.
11. Jones, J. B., Stall, R. E., and Bouzar, H. 1998. Diversity among xanthomonads pathogenic on pepper and tomato. Annu. Rev. Phytopathol. 36: 41-58.
12. Jones, J. B., Stall, R. E., Scott, J. W., Somondi, G. C., Bouzar, H., and Hodge, N. C. 1995. A third tomato race of *Xanthomonas campestris* pv. *vesicatoria*. Plant Dis. 79: 395-398.
13. Louws, F., Rademaker, J., and de Bruijn, F. 1999. The three DS of PCR -based genomic and analysis of phtobacteria: diversity, detection, and disease diagnosis. Annu. Rev. Phytopathol. 37: 81-125.
14. Lue, Y. S., Tzeng, K. C., and Hsu, S. T. 2004. Rapid identification and detection of *Xanthomonas vesicatoria* by polymerase chain reaction. Plant Pathol. Bull. 13: 358. (In Chinese).
15. Quezado-Duval, A. M., Leite, R. P., Truff, i. D., and Camargo, L. E. A. 2004. Outbreaks of bacterial spot caused by *Xanthomonas gardneri* on processing tomato in central-west Brazil. Plant Dis. 88: 157-161.
16. Stall, R. E., Beaulieu, C., Egel, D., Hodge, N. C., Leite, R. P., Minsavage, G. V., Bouzar, H., Jones, J. B., Alvarez, A. M., and Benedict, A. A. 1994. Two genetically diverse groups of strains are included in *Xanthomonas campestris* pv. *vesicatoria*. Int. J. Syst. Bacteriol. 44: 47-53.
17. Stall, R. E., Jones, J. B., and Minsavage, G. V. 2009. Durability of resistance in tomato and pepper to *Xanthomonads* causing bacterial spot. Annu. Rev. Phytopathology 47: 265-284.
18. Tudor-Nelson, S. M., Minsavage, G. V., Stall, R. E., and Jones, J. B. 2003. Bacteriocin-like substances from tomato race 3 strains of *Xanthomonas campestris* pv. *vesicatoria*. Phytopathology 93: 1415-1421.
19. Vauterin, L., Hoste, B., Kersters, K., and Swings, J. 1995. Reclassification of *Xanthomonas*. Int. J. Syst. Bacteriol. 45: 472-489.
20. Vidaver, A. K. and Bauckner, S. 1978. Typing of fluorescent phytopathogenic pseudomonads by bacteriocin production. Can. J. Microbiol. 18: 705-713.

摘　要

呂昀陞[1, 2]、鄧文玲[1]、吳雅芳[3]、鄭安秀[3]、徐世典[1]、曾國欽[1, 4]. 2010. 臺灣 *Xanthomonas* 屬茄科植物細菌性斑點病菌之特性分析. 植病會刊 19: 181-190. ([1]臺中市 國立中興大學植物病理學系；[2]臺中市 行政院農業委員會農業試驗所；[3]臺南市 行政院農業委員會臺南區農業改良場；[4]聯絡作者，電子郵件：kctzeng@nchu.edu.tw；傳眞：+886-4228-54633)

細菌性斑點病爲茄科植物重要細菌性病害之一，最近引起番茄與甜椒細菌性斑點病之 *Xanthomonas* 細菌已被重新分類爲 *Xanthomonas euvesicatoria* (Doidge) Jones *et al*.、*X. gardneri* (Sutic) Jones *et al*.、*X. perforans* Jones *et al*. 與 *X. vesicatoria* (Doidge) Vauterin *et al*. 等四種。爲了解臺灣 *Xanthomonas* 屬茄科植物細菌性斑點病菌之特性與種類，本研究利用生化特性、PCR 反應與碳素源利用等，分析臺灣各地分離之 53 個茄科植物細菌性斑點病菌菌株，結果顯示其中 40 個菌株不具澱粉與果膠分解能力，其餘 13 個菌株則同時具有澱粉與果膠分解能力；以 *X. euvesicatoria* (Doidge) Jones *et al*. 之專一性引子對 RST 13/14 與 *X. vesicatoria* (Doidge) Vauterin *et al*. 之專一性引子對 C-2-2L/2R 進行多引子聚合酵素連鎖反應 (multiplex PCR) 測試，發現 40 菌株可增幅出 560 bp 之 *X. euvesicatoria* 專一性 DNA 片段，5 個菌株可增幅出 407 bp 之 *X. vesicatoria* 專一性 DNA 片段，但有8個菌株無法以此 2 組引子對增幅出預期之產物；利用 Biolog 鑑定系統進行菌株碳素源利用測試，並以 NT-SYS pc 2.0 分析，結果顯示，供試之 53 個 *Xanthomonas* 菌株，有 40 株爲 *X. euvesicatoria*，8 株爲 *X. perforans* 及 5 株爲 *X. vesicatoria*。本研究測試之菌株中並未有 *X. gardneri* 特性之菌株。此外本研究鑑定爲 *X. perforans* 之菌株，皆可產生對 *X. euvesicatoria* 菌株生長具抑制作用之細菌素。本篇爲臺灣 *X. perforans* 之首次報導。*Xanthomonas perforans* 在臺灣之分佈及其生態特性值得進一步探討。

關鍵詞：*Xanthomonas euvesicatoria*、*Xanthomonas gardneri*、*Xanthomonas perforans*、*Xanthomonas vesicatoria*、細菌素