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Characterization of Novel Strains of Citrus Canker Bacterium from Citrus in Taiwan[1]

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ABSTRACT

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Two strains of *Xanthomonas axonopodis* pv. *citri* isolated from citrus plants in Taiwan were examined for pathogenicity on the leaves of Mexican lime (*Citrus aurantifolia*), grapefruit (*C. paradisi*), Liucheng (*C. sinensis*), and lemon (*C. limon*). The results showed that two strains induced flated necrotic lesions with watersoaked margin on the leaves of four *Citrus* species. Based on physiological, biochemical, genetic and proteinic characterizations including NaCl tolerance, hydrolysis of gelatin, oxidation of carbon sources, polymerase chain reactions with primers specific to *X. axonopodis* pv. *citri* (*Xac*), rep-PCR, and SDS-PAGE analysis, these two strains were identified as *Xac* and grouped into *Xac*-A^p type. Both strains could be differentiated from *X. axonopodis* pv. *citrumelo* (pathotype E) by oxidation of carbon sources, pectolytic activity, and amplified DNA profiles of PCR, while they could be only differentiated from type *Xac*-A^f strain by a distinct pathogenicity on the leaves of Mexican lime. Our data indicated they appeared to be novel strains of *Xac* in Taiwan. We designated these two atypical symptoms-inducing strains as *Xac*-A^p type which are different from strains of types *Xac*-A , *Xac*-A^f, *Xac*-A^r, *Xac*-A* and *Xac*-A^w described previously.

Key words: Citrus canker, *pthA* gene, Rep-PCR, SDS-PAGE, *Xanthomonas axonopodis* pv. *Citri*.

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INTRODUCTION

There are distinct types of citrus canker disease caused by various pathovars and variants of the bacterium *Xanthomonas axonopdis*. Because symptoms are generally similar, the separation of these forms of the bacterial pathogen is based on host range and other phenotypic and genotypic characteristics of the strains.

The Asiatic type of citrus canker (pathotype A), caused by the *X. axonopodis* pv. *citri* (*Xac*), is by far the most widespread and severe form of the disease. This is the strain that causes the disease most often referred to as 'citrus canker'(Stall & Civerolo 1991). Cancrosis B (pathotype B) caused by the *X. axonopodis* pv. *aurantifolii* is a minor canker disease of diminishing importance on lemons in Argentina, Paraguay, and Uruguay. Mexican lime, sour orange and pummelo are also susceptible (Civerolo 1984). Cancrosis C (pathotype C) also caused by the *X. axonopodis* pv. *Aurantifolii* that can only infect infected Mexican lime in Brazil (Schubert *et al*. 2001). Strains in pathotypes A, B and C induce raised canker lesions on citrus plants. However, Cancrosis E (pathtype E) was originally described in Florida, induce flat necrotic lesions with watersoaked margins on citrus species (Graham & Gottwald 1991; Stall & Civerolo 1991). The disease was renamed citrus bacterial spot (CBS) and the bacterium was reclassified as *X. axonopodis* pv. *citrumelo* (Vauterin *et al*. 1995). The typical canker lesion caused by *X. axonopodis* pv. *citri* is erumpent, with watersoaked, or oily tan or brown colored margin, often surrounded by a chlorotic halo. However, several distinct phenotypes of *Xac* have been reported (Verniere *et al*. 1998; Mohammadi *et al*. 2001; Sun *et al*. 2004; Lin *et al*. 2005). Strains from southwest Asia (*Xac*-A*) and Florida (*Xac*-A^{w}) elicit typical canker lesions on Mexican lime and induce flat watersoaked lesions on grapefruit (Verniere *et al*. 1998; Sun *et al*. 2004). These new strains have a limited host range in Mexican lime that differs from *Xac* strains which have a wide host range. Recently, atypical symptoms-inducing strains of type *Xac*-A^{f} and type *Xac*-A^{r} were identified in Taiwan (Lin *et al*. 2005). A strain of *Xac*-A^{f} induces flat necrotic lesion with watersoaked margin and light chlorotic halo on leaves of grapefruit, lemon and Liucheng, while induces typical canker lesions on Mexican lime. Strains of *Xac*-A^{r} induce restricted and raised corky lesions with no watersoaked margin and light chlorotic halo on leaves of citrus species.

Several approaches have been employed in studying the taxonomy and discriminating between strains of *X. axonopodis* belonging to various pathotypes including physiological and biochemical assays (Verniere *et al*. 1991; Verniere *et al*. 1993), serological approaches (Civerolo & Fan 1982; Alvarez *et al*. 1991), phage typing (Verniere *et al*. 1998), protein profiles (Vauterin *et al*. 1991), fatty acid analysis (Graham *et al*. 1990; Vauterin *et al*. 1991), DNA-DNA hybridization (Vauterin *et al*. 1991), restriction-fragment length polymorphism (Hartung & Civerolo 1989; Graham *et al*. 1990), plasmid DNA fingerprints (Pruvost *et al*. 1992), and specific primer pairs (Hartung *et al*. 1993; Cubero & Graham 2002; Sun *et al*. 2004). In addition, Southern hybridization with a *pth*A probe reveals distinct profiles among strains in pathotype A, pathotype B, pathotype C, and pathotype E (Swarup *et al*. 1992). And rep-PCR with BOX and ERIC primer pairs has been used to separate pathotypes of *Xac* or to differentiate strains in

the same pathotype. The rep-PCR technique also allows to evaluate the diversity of *Xac* in certain geographic areas of the world (Cubero & Graham 2002).

In this study, we examined the pathogenicity of *Xac* strains from Taiwan on the leaves of Mexican lime, grapefruit, Liucheng, and lemon. We found both of the strains could induce atypical symptoms and further characterized these strains based on their physiological, biochemical, genetic, and proteinic characterizations. The results revealed that these atypical symptoms-inducing strains are novel strains of *Xac*.

MATERIALS AND METHODS

BACTERIA STRAINS AND CULTURE CONDITION

A total of six *X. axonopodis* strains were used in this study, including two typical Asiatic type strains XW19 and 2863 (*Xac*-A), one atypical symptoms-inducing strains XW47 (*Xac*-A^f type), one citrus bacterial spot (CBS) strain *X. axonopodis* pv. *citrumelo* F2 (pathotype E) (Lin *et al*. 2005), and newly isolated strains *X. axonopodis* pv. *citri* XL16 and XL38 from Taiwan. All strains were stored in YPD broth (yeast extract 7 g, bactopeptone 7 g, dextrose 7g, distilled water 1000 mL, pH 7.2) (Verniere *et al*. 1991) cotaining 20% glycerol at -80℃. When required, each bacterial strain was streaked out from the glycerol stock at -80℃ and cultured on YPD agar plates at 30℃ for 3 days.

PATHOGENICITY TEST

Citrus plants (*C. paradisi*, grapefruit; *C. aurantifolia*, Mexican lime; *C. sinensis*, Liucheng; *C. limon*, lemon) grown in pots under greenhouse conditions were used for examining the pathogenicity of *X. axonopodis* pv. *citri* strains. To prepare inoculum, bacterial cells grown overnight in YPD broth were harvested by centrifugation and resuspended in sterile distilled water to a concentration of approximately 10^8 colony-forming units (CFU)/mL. Prior to inoculation, six wounds per 1-cm^2 on young fully expanded citrus leaves were made with a standard 26-gauge needle. An aliquot (20 μL) of bacterial suspension was dropped onto each of six wounds on leaves, and the inoculum drops were wiped off with sterile cotton just after inoculation. Citrus plants inoculated with various *X. axonopodis* strains were moved into a growth chamber with 65–90% relative humidity and 12-hr light at 30℃ and 12-hr dark at 25℃. Visual symptom was recorded and examined with dissection microscopy.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZATION

Physiological and biochemical characteristics used to differentiate pathovars of *X. axonopodis* associated with citrus plants were performed to characterize the *X. axonopodis* pv. *citri* strains isolated in Taiwan. Salt tolerance was tested by growing bacteria on YPDA plates containing 3% NaCl (Verniere *et al*. 1998). Hydrolysis of gelatin was performed as described in Lelliott *et al.* (Lelliott *et al*. 1966). Oxidation of carbon sources was carried out on Biolog® GN microplates (Biolog Inc., Hayward, CA) as described by Verniere *et al.* (Verniere *et al*. 1993). Pectolytic activity was tested on Hildebrand's medium with pH values at 5.0, 7.0, and 8.5 (Hildebrand 1971).

TOTAL DNA EXTRACTION

Total DNAs of xanthomonad strains were extracted using the method described by Sambrook *et al.* (1989). PCR was performed with a thermocycler (GeneAmp PCR System 2400, Perkin-Elmer, CT).

IDENTIFICATION OF *Xanthomonas* STRAINS WITH SPECIFIC PRIMER PAIRS

Primer pairs 2/3 and 4/7 designed from sequence of plasmid of the *X. axonopodis* pv. *citri* (Hartung *et al*. 1993), and primer pairs *J-pth1/J-pth2*, *J-RXg/J-RXc2* designed based on sequences of nuclear localization signal in *pthA* (Cubero & Graham 2002) and rDNA (Cubero & Graham 2002) of *X. axonopodis* pv. *citri* were used to identify or differentiate the *Xac* strains tested in this study.

REP- AND ERIC-PCR ANALYSES

The genetic diversity among the *Xac* strains was analyzed by REP- and ERIC-PCR. The REP-PCR was carried out as described by Versalovic *et al.* (Versalovic *et al*. 1991) with some modifications. A 25 µL reaction mixture contained 150 ng template DNA, 1× *Taq* buffer, 3 mM $MgCl_2$, 1.25 µM each of primers REP1-1 and REP2-1, 250 µM each of deoxynucleoside triphosphates and 0.8 U Taq polymerase (DyNAzyme^{TM} II DNA Polymerase, Finnzymes Oy Inc. Finland). The amplification condition consisted of 94℃ for 1 min, 44℃ for 1 min, and 65℃ for 8 mins for 30 cycles plus an initial step of 95℃ for 7 mins and a final step of 65℃ for 15 mins. The ERIC-PCR was carried out with primer pair ERIC1R/ERIC2 in a 25 µL reaction mixtures in which the other ingredients were the same as that for REP-PCR; the amplification condition consisted of 94℃ for 1 min, 52℃ for 1 min, and 65℃ for 8 mins for 30 cycles plus an initial step of 95℃ for 7 mins and a final step of 65℃ for 15 mins. PCR products of the REP- and ERIC-PCR were analyzed by 2% agarose gel electrophoresis in 0.5× TAE buffer at 90 V for 4 hrs and were stained with ethidium bromide. REP- and ERIC-PCR fingerprint profiles were converted to binary form (0 = absence; 1 = presence), and Dice coefficient was calculated to determine the similarity among the bacterial strains tested (Dice 1945).

PATHOGENICITY (*pthA*) GENE DETECTION

The primers pthAP7/AR2 described Lin *et al*. (2005) were used to amplify the region corresponding to nucleotide of *pthA*. Polymerase chain reaction for *pthA* gene was performed in a 50 µL mixtures containing 150 ng template DNA, 1× Taq buffer, 1 µM primer pthAP7, 1 µM primer pthAR2, each deoxynucleoside triphosphate at a concentration of 300 µM, 1U of Taq plus DNA polymerase, and 5 µL DMSO. The amplification condition consisted of 94℃ for 1 min, 63℃ for 1 min, and 72℃ for 5 mins for 35 cycles plus an initial step of 94℃ for 10 mins and a final step of 72℃ for 10 mins.

SDS-PAGE ANALYSIS

Electrophoresis of cellular proteins was carried out in a discontinuous system under the denaturing condition following the method of Laemmli (Laemmli 1970). A bacterial suspension with an optical density of 1.0 at 630 nm was prepared in 1 mL distilled water in an Eppendorf tube from each strain cultured on nutrient agar medium cantaining 1% glucose. Each sample was spun down at 1000 rpm for 5 mins at 4℃. The bacterial pellet was washed in sterile distilled water and then resuspended in sample

buffer at 0.2× volume, boiled for 4 mins and centrifuged at 10000 rpm for 10 mins. Fifty microliters of soluble proteins from each sample was loaded into slots of stacking gel (5% w/v). Proteins were fractioned in 12% resolving gel. Electrophoresis was carried out in a vertical gel slab unit (Sigma Chemical Co., London, UK) at a constant voltage of 140 V untile the tracking dye reached near the bottom. Gel was stained in Coomassie brilliant blue R250 and destained in acetic acid: methanol solution without the dye.

RESULTS

PATHOGENICITY TEST

Based on the symptoms induced on leaves of the four *Citrus* species, the strains XL16 and XL38 tested were grouped into *Xac*-A^p type (Table 1). In the *Xac*-A^p type strains induced flat necrotic lesions with watersoaked margins and light chlorotic haloes on the leaves of Mexican lime (Fig. 1C), grapefruit (Fig. 1F), Liucheng and lemon. The size of lesions induced by strains XL16 and XL38 on leaves of four citrus species was not significantly different from that induced by XW19. The lesion size was in a range from 1.9 to 2.6 mm in diameter 24 days after inoculation.

PHYSIOLOGICAL AND BIOCHEMICAL CHARACTERIZACTION

Type *Xac*-A^p strains XL16 and XL38 were able to grow in the presence of 3% NaCl, possessed hydrolysis ability of gelatin and were positive in utilization of L-fucose, D-galactose and alaninamide in the Biolog® GN plate. They also were positive for pectolytic activity on Hildebrand’s medium at pH 7.0 and pH 8.5 the same as reference strains of types *Xac*-A and *Xac*-A^f (Table 2), whereas *X. axonopodis* pv. *citrumelo* (pathotype E) strain F2 did not utilize L-fucose and with no pectolytic activity on the Hildebrand’s medium at various pH values (Table 2).

IDENTIFICATION OF *Xanthomonas* STRAINS WITH SPECIFIC PRIMER PAIRS

Xac-specific primers 2/3, 4/7, *J-pth1*/*J-pth2* and *J-RXg*/*J-RXc2* were used to identify strains XL16 and XL 38. Specific PCR products were detected from these two strains as well as reference strains of *Xac*-A 19 and 2863. No specific amplified product was detected from DNA of *X. axonopodis* pv. *citrumelo* F2 with these primer pairs (Table 2).

REP- AND ERIC-PCR ANALYSES

The similarity coefficient of both atypical symptoms-inducing strains XL16 and XL38 was 0.9–1.0 to *Xac* reference strains XW19 or 2863 based on REP- and ERIC-PCR analyses (Fig. 2). The size of amplification products ranged from 800 to 2500 base pairs (bp) for ERIC and from 600 to 2000 bp for REP.

PATHOGENICITY (*pthA*) GENE DETECTION

A 3.8 kb DNA product containing a full length of *pthA* gene of *Xac* strain was amplified with primer pair pthAP7/pthAR2 from each of *Xac* strains XL16, XL38 and XW19 but not from *X. axonopodis* pv. *citrumelo* strain F2. (Fig. 3).

SDS-PAGE ANALYSIS OF TOTAL SOLUBLE PROTEINS

The reproducibility of the electrophoresis technique used in this study was checked by preparing protein extracts in duplicate and running all of the extracts in at least two gels. In all cases, a level of reproducibility of more than 0.9 was obtained. A high homogeneity in SDS protein profiles was observed among *Xac* strains XL16, XL38, XW47, and XW19 and there were no detectable differences among these strains tested (Fig. 4).

Table 1. Symptoms induced by *Xanthomonas axonopodis* pv. *citri* strains on four *Citrus* species

Type	Strains	*C. aurantifolii* (Mexican lime)	*C. paradisi* (Grapefruit)	*C. sinensis* (Liucheng)	*C. limon* (Lemon)
A	XW19	Canker [z]	Canker	Canker	Canker
A^f	XW47	Canker	FW	FW	FW
A^p	XL16,XL38	FW	FW	FW	FW

[z] Canker: Typical erumpent canker lesion with watersoaked margin and light chlorotic halo; FW: Flat necrotic lesion with watersoaked margin and light chlorotic halo.

Table 2. Physiological, biochemical and genetic characteristics of *Xanthomonas axonopodis* pv. *citri* type A^p strains XL16 and XL38

Item	XL16	XL38	XW47	XW19 [w]	2863	F2
Growth on NaCl (3%)	+ [v]	+	+	+	+	+
Hydrolysis of gelatin	+	+	+	+	+	+
Oxidation of carbon sources [z]						
D-Galactose	+	+	+	+	+	+
Alaninamide	+	+	+	+	+	+
L-Fucose	+	+	+	+	+	−
Pectolytic activity on [y]						
Hildebrand's medium (pH5.0)	−	−	−	−	−	−
Hildebrand's medium (pH7.0)	+	+	+	+	+	−
Hildebrand's medium (pH8.5)	+	+	+	+	+	−
PCR with primers [x]						
2/3	+	+	+	+	+	−
4/7	+	+	+	+	+	−
J-pth1/J-pth2	+	+	+	+	+	−
J-RXg/J-RXc2	+	+	+	+	+	−

[z] Oxidation of carbon sources was done in the Biolog GN plates as described by Verniere *et al*. (1993).
[y] Pectolytic activity was tested on Hildebrand's medium (Hildebrand 1971).
[x] The primer pairs 2/3 and 4/7 are specific for DNA fragments in the *X. axonopodis* pv. *citri* plasmid (Hartung *et al*. 1993); The primer pair *J-pth1/J-pth2* is specific for the nuclear localization signal in *pthA* of *X. axonopodis* pv. *citri* (Cubero & Graham 2002); The primer pair *J-RXg/J-RXc2* is specific for rDNA of *X. axonopodis* pv. *citri* (Cubero & Graham 2002).
[w] Strains XW19 and 2863 were reference strains of type *Xac*-A for *X. axonopodis* pv. *citri*; XW47 was a reference strain of type *Xac*-A^f for *X. axonopodis* pv. *citri* ; F2 was a reference strain for *X. axonopodis* pv. *citrumelo* (pathotype E).
[v] ＋: positive reaction; －: negative reaction.

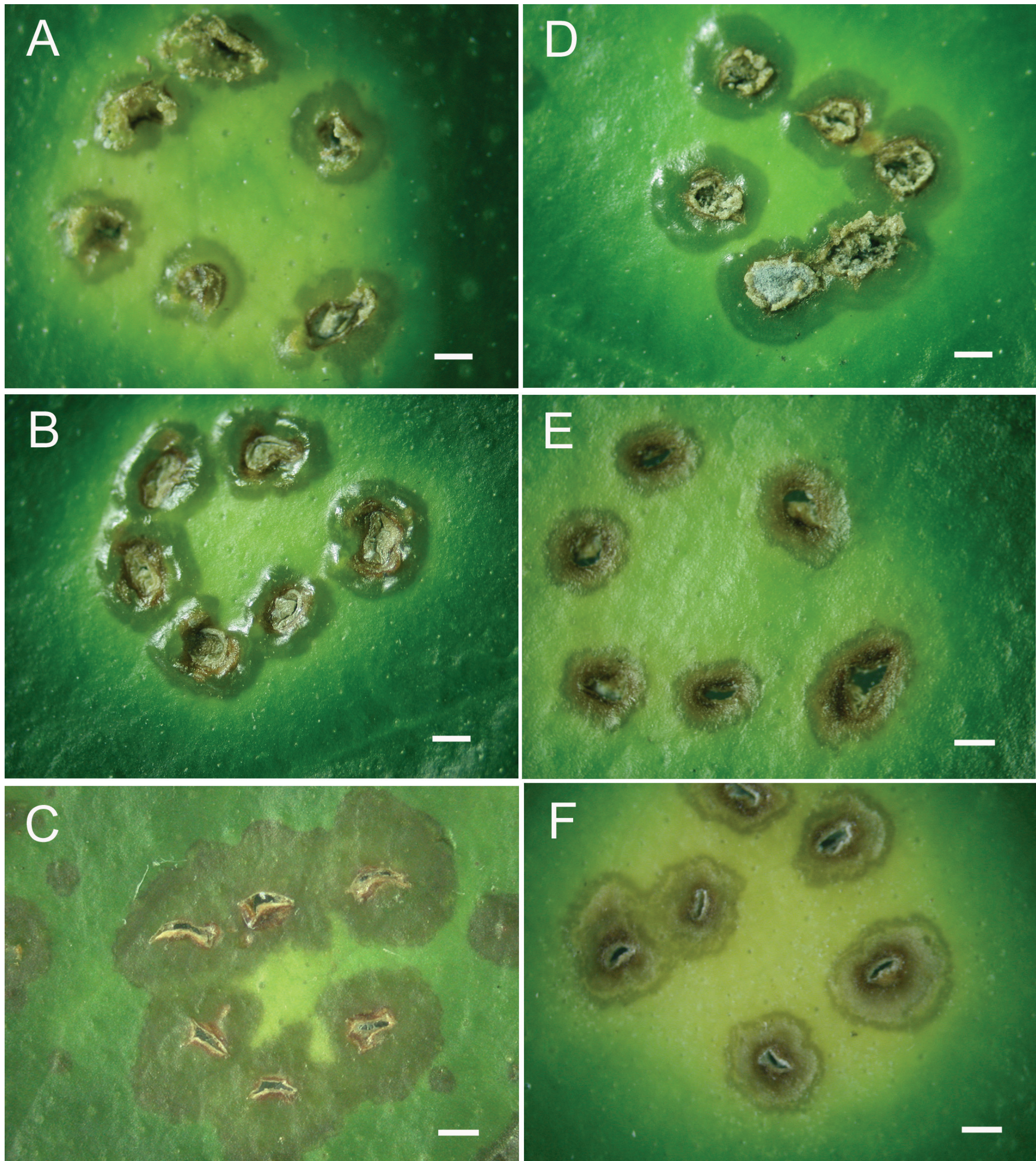

Fig. 1. Symptoms induced on leaves of Mexican lime (A, B, C) and grapefruit (D, E, F) by strains of *X. axonopodis* pv. *citri* 24 days after inoculation. (A and D): Typical erumpent canker lesions with watersoaked margin and light chlorotic halo induced by type *Xac*-A strain XW19; (B): Typical erumpent canker lesions with watersoaked margin and light chlorotic halo induced by type *Xac*-A^f strain XW47; (E): Flat necrosis lesions with watersoaked margin and light chlorotic halo induced by type *Xac*-A^f strain XW47; (C and F): Flat necrosis lesions with watersoaked margin and light chlorotic halo induced by type *Xac*-A^p strain XL38. (Bars = 1 mm).

DISCUSSION

According to symptoms induced by *Xac* on leaves of four *Citrus* species, we found type *Xac*-A^p strains XL16 and XL38 failed to induce any erumpent canker lesions typical of *Xac*-A on the leaves of Mexican lime, grapefruit, Liucheng, and lemon but they induced flat necrotic with watersoaked lesions on the leaves of four *Citrus* species. Symptom induced by type *Xac*-A^p strains on the leaves of Mexican lime were different from those induced by strains of types *Xac*-A, *Xac*-A^f, *Xac*-A^r, *Xac*-A*, and *Xac*-A^w (Verniere *et al*. 1998; Sun *et al*. 2004; Lin *et al*. 2005). To our knowledge, there is no any other *Xac* strain which is pathologically similar to strains of type *Xac*-A^p.

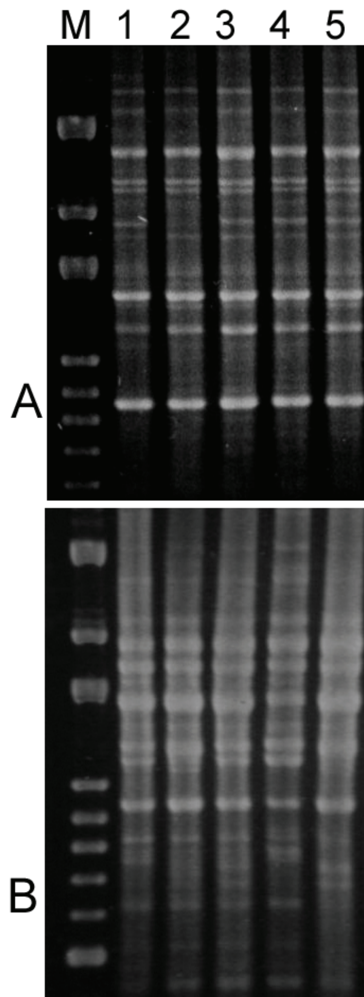

Fig. 2. Rep-PCR patterns of *Xanthomonas axonopodis* pv. *citri* strains using ERIC- (A) and REP- primer pair (B). Lanes 1-5: DNA templates from stains XL16, XL38, XW47, XW19 and 2863, respectively; lane M: Bio 100 DNA ladder.

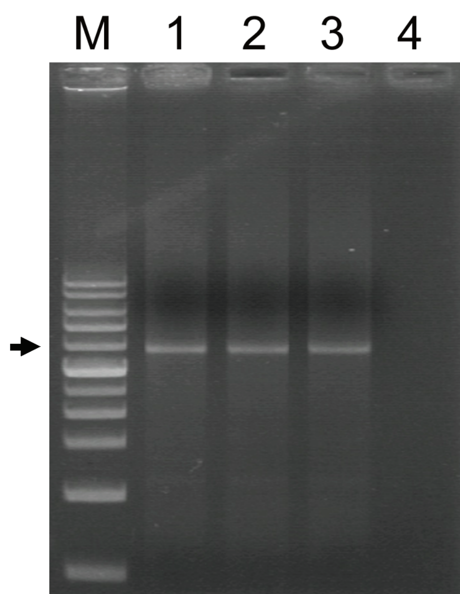

Fig. 3. Amplification profiles of DNAs from *Xanthomonas axonopodis* strains by polymerase chain reaction with primer pair pthAP7/pthAR2 (A). Lanes 1-3: *X. axonopodis* pv. *citri* strains XL16, XL38 and XW19, respectively; lane 4: *X. axonopodis* pv. *citrumelo* F2; lane M: Gen-KB DNA ladder. The predicted 3.8 kb DNA fragment containinng an entire length of *pthA* gene is marked with an arrow in the margin.

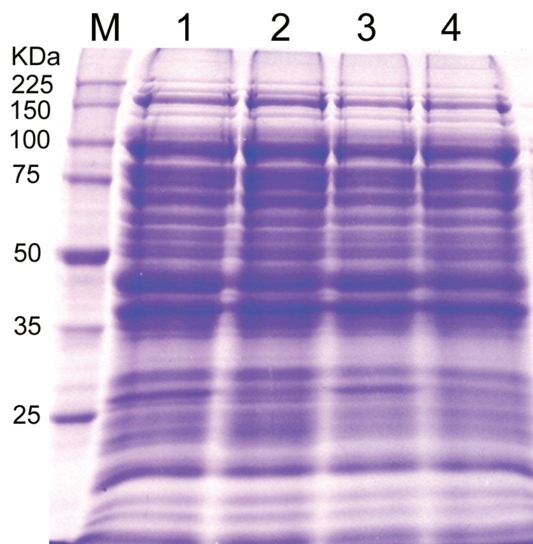

Fig. 4. SDS-PAGE analysis of total soluble proteins from different strains of *Xanthomonas axonopodis* pv. *citri* Lanes 1-4: *X. axonopodis* pv. *citri* strains XL16, XL38, XW47 and XW19, respectively; lane M: the protein molecular weight markers.

In physiological and biochemical tests, the atypical symptoms-inducing strains of type *Xac*-A^p could be grown on 3% NaCl, hydrolyze gelatin and utilize L-fucose, D-galactose and alaninamide. These strains shared typical *Xac* group profiles of assimilation of these three carbon sources as reported by Verniere *et al. (*1993). This study also showed that the reference strain *X. axonopodis* pv. *citrumelo* F2, a CBS strain, could utilize D-galactose and alaninamide but not L-fucose. Therefor, type *Xac*-A^p could be differentiated from *X. axonopodis* pv. *citrumelo* by the carbon source utilization (Table 2).

Similarity coefficients obtained from REP- and ERIC-PCR analyses grouped *Xac*-A^p strains XL16 and XL38 with the reference strains of types *Xac*-A and *Xac*-A^f into the same group. REP- and ERIC-PCR analyses did not display any major difference among the *Xac* strains used in this investigation (Fig. 2). Specific primers have been designed to identify or differentiate of *Xanthomonas* spp. associated with citrus plant (Hartung *et al*. 1993; Cubero & Graham 2002). In this study, strains in *Xac*-A^p could be identified as *Xac* with primer pairs 2/3, 4/7, *J-pth1/J-pth2*, *J-RXg/J-RXc2*, and pthAP7/pthAR2. However, strain F2 can not be detected with these primer pairs. Thus, these primer pairs could be used to differentiate *Xac*-A^p strains from *X. axonopodis* pv. *citrumelo* strain. Strains of *Xac*-A^w can not be detected with primer pairs 2/3 (Cubero & Graham 2002) and 4/7 (Sun *et al*. 2004), and a distinct DNA profiles could be amplified from *Xac*-A* strains with primer pair pthAP7/pthAR2 (Lin *et al*. 2005). Thus, the primer pairs 2/3, 4/7 and pthAP7/pthAR2 could be used to differentiate type *Xac*-A^p strains from types *Xac*-A^w or *Xac*-A* strains.

Sequences of *lrp* gene have been used to characterize the relationship among strains in different pathovars of *X. axonopodis* and other *Xanthomonas* species (Cubero & Graham 2004). Based on the *lrp* gene sequences, atypical symptoms-inducing strains XL16 and XL38 in type *Xac*-A^p were grouped into the same cluster with typical symptoms-inducing strains XW19 and 2863 in type *Xac*-A (unpublished data).

A pathogenicity gene, *pthA*, is required, and present in all *X. axonopodis* strains tested causing citrus canker disease, and absent in non-canker causing xanthomonds isolated from citrus. The result suggested that the presence or absence of a *pthA* allele is therefore diagnostic for strains having capacity to cause erumpent canker lesions on citrus. Althought *Xac*-A^p type strains XL16 and XL38 induced flated necrotic lesions with watersoaked margin on the leaves of four *Citrus* species, a 3.8 kb DNA fragment containing entire *pthA* gene was amplified from total DNAs of type *Xac*-A^p strains XL16 and XL38 with primer pair pthAP7/pthAR2. It has been shown that for *avrBs3* family of avirulence gene, which includes *pthA* from *Xac* (Swarup *et al*. 1991; Swarup *et al*. 1992; AI-Saadi *et al*. 2007) that the central region of such *avr* genes is composed of a number of 102 bp direct repeats are key factors determining the interaction with plant resistance genes (Bonas *et al*. 1993). Novel host specificities have been reported based on *pthA* constructs engineered with altered numbers of 102 bp repeats (Yang & Gabriel 1995). Thus, such a rearrangement, occurring spontaneously in a variant clonal subgroup of *Xac*, could account for the origin of *Xac*-A^p group of strains. The studies on the mechanism of atypical symptoms-inducing between *Xac*-A^p strains with citrus plants have been undertaken.

In the pathogenicity tests, we found new strains in type *Xac*-A^p which induced atypical symptoms on leaves of *Citrus* species tested. Based on physiological, biochemical, genetic and proteinic analyses, strains of type *Xac*-A^p were characterized as *Xac*. Type *Xac*-A^p strains could be differentiated from strains of type *Xac*-A^f, *Xac*-A* and *Xac*-A^w by a distinct pathogenicity on the leaves of Mexican lime. Moreover, *Xac*-A^p can also be differentiated from a pathologically similar strain *X. axonopodis* pv. *citrumelo* by oxidation of carbon sources, pectolytic activity and amplified DNA profiles of PCR with specific primer pair for *Xac*.

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台灣柑橘潰瘍病菌新菌株之特性分析 [1]

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摘　要

林信成、張翔、曾國欽。2008。台灣柑橘潰瘍病菌新菌株之特性分析。台灣農業研究 57:265–278。

將台灣柑橘植株所分離到之柑橘潰瘍病菌 (*Xanthomonas axonopodis* pv. *citri*) 菌株，在墨西哥萊姆 (*Citrus aurantifolia*)、葡萄柚 (*C. paradisi*)、柳橙 (*C. sinensis*) 及檸檬 (*C. limon*) 等四種不同品種之柑橘植株葉片上分別測試其病原性。測試結果顯示這些菌株中有兩株菌株可在測試之四種柑橘植株葉片上，引起具水浸狀邊緣之扁平壞疽病斑。依據耐鹽性、明膠水解作用、碳素源利用、應用柑橘潰瘍病菌專一性引子對之聚合酵素連鎖反應、重複性序列聚合酵素連鎖反應及十二烷基硫酸鈉聚丙烯醯胺膠體電泳 (SDS-PAGE) 分析等生理、生化、基因及蛋白質特性分析，顯示這兩株菌株係屬於 *X. axonopodis* pv. *citri* (*Xac*)，並將歸類為 *Xac*-A^{p} 類型菌株。利用碳素源利用、明膠水解作用及 pthAP7/pthAR2、2/3、4/7、*J-pth1*/*J-pth2*、*J-RXg*/*J-RXc2* 等引子對之聚合酵素連鎖反應的 DNA 圖譜，可將這兩株菌株與 *X. axonopodis* pv. *citrumelo* (E 病原型) 菌株區分開；然而僅能利用在墨西哥萊姆上之病原性差異將這些菌株與 *Xac*-A^{f} 類型菌株區分開。數據顯示出由台灣分離到可引起非典型病徵之菌株為 *X. axonopodis* pv. *citri* 之新特殊菌株，並將其命名為 *Xac*-A^{p} 以有別於 *Xac*-A，*Xac*-A^{f}，*Xac*-A^{r}，*Xac*-A*及 *Xac*-A^{w} 等類型菌株。

關鍵詞：柑橘潰瘍病、*pthA* 基因、重複性序列聚合酵素連鎖反應、聚丙烯醯胺膠體電泳、柑橘潰瘍病菌。

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