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Physiological and molecular characterizations of banana finger-tip rot and onion decay pathogens in Taiwan

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ABSTRACT

Banana finger-tip rot pathogen had biochemical and physiological characteristics very similar to those of onion decay pathogen, but the isolates of onion decay pathogen were yellow-pigmented and could not grow at 42℃ in LA agar medium. The *recA* PCR-RFLP analyses indicated that all tested isolates of banana finger-tip rot pathogen belonged to genomovar III, and that isolates of onion decay pathogen belonged to genomovar I of *Burkholderia* cepacia complex, which were named as *B. cenocepacia* and *B. cepacia*, respectively. Accordingly, banana finger-tip rot and onion decay in Taiwan are caused by two separate pathogens. The *recA* PCR-RFLP tests further showed that genetic variability of the *B. cenocepacia* isolates, which contained *recA* IIIA and IIIB lineages. In addition, PCR and PCR-RFLP assays were developed in this study for the detection and identification of these two pathogens. In *bcscV* PCR assay, all tested isolates of banana finger-tip rot pathogen were PCR positive but the isolates of onion decay pathogen were negative for *bcscV*. The *bcscV* probe hybridized only to *B. cenocepacia* but not to *B. cepacia*. The other *uvrB* PCR test amplified a 995-bp DNA fragment for all isolates of *B. cenocepacia* and *B. cepacia* tested. In addition, discovery of a unique *MluI* restriction site of *uvrB* of onion decay pathogen led to the development of a PCR-RFLP assay able to discriminate onion decay pathogen isolates from banana finger-tip rot pathogens. No amplification was observed with other plant pathogenic bacteria in the *bcscV* and *uvrB* PCR assays. The *bcscV* and *uvrB* PCR and PCR-RFLP assays developed in this study will be highly effective for the detection and identification of *B. cenocepacia* and *B. cepacia* in the epidemiological studies.

Key words : Banana finger-tip rot; *Burkholderia cenocepacia*; *B. cepacia*; *bcscV*; onion decay; *uvrB*.

INTRODUCTION

Banana finger-tip rot disease was observed in commercial fields in Pingtung County, Taiwan. The pathogen was identified as *Burkholderia cepacia* and belongs to genomovar III of *B. cepacia* complex [6,7]. *B. cepacia* was originally reported as the causal agent of onion decay [2], an endemic disease of Taiwan. In Taiwan, major cultivation areas of banana and onion are located in the southern counties, such as Kaohsiung and Pingtung. *B. cepacia* found in banana may be related to isolates from onions. *B. cepacia* could be spread between these areas on infested bananas and onions, transportation vehicles, or animals, such as insects and birds. As a result, inoculum sources of banana finger-tip rot may come from onion decay, and vice versa.

The *B. cepacia* complex is a very diverse group of bacteria [17], consisting of nine genomovars that were recently elevated to species status [3,15,16,18,19], pathogens of banana finger-tip rot and onion decay in Taiwan may not belong to the same genomovars. Since the identification of inoculum sources for the diseases is important for disease management, the relationships between *B. cepacia* isolates from bananas and onions should be determined. In this

paper, it was found that the strains of *B. cepacia* isolated from bananas or onions belonged to different genomovars and could be differentiated by physiological and molecular characteristics.

The ability of *B. cepacia* to cause disease is not limited to plant hosts, as these bacteria are also important human opportunistic respiratory pathogens [4,8]. The bacterium can survive on the rhizosphere and in inner tissues of wheat, lupine, and maize [1]. Therefore, epidemiological distributions of banana finger-tip rot and onion decay pathogens and impacts on public health should be further considered. In this study, we also successfully used the *bcscV* and *uvrB* to develop the sensitive and specific PCR and PCR-RFLP assays for the detection and differentiation of the *B. cepacia* isolates from bananas and onions, which could be used for future epidemiological studies.

MATERIALS AND METHODS

Bacterial strains, culture conditions, and characterizations

The finger-tip rot and onion decay pathogenic bacteria, *B. cepacia*, were isolated from diseased banana fruit fingers and onion bulbs. The collection of *B. cepacia* isolates used in this study is shown in Table 1. *B. cepacia* and other plant pathogenic bacteria were routinely cultured on Luria-Bertani agar (LA) or broth medium at 28 °C. Biochemical and physiological characterizations were conducted according to Schaad et al. [13] The API 20E system test (BioMerieux Vitek Inc., Hazelwood, MO) was used for some biochemical tests.

General DNA manipulations

Mini-scale preparations of *E. coli* plasmid DNA, total genomic DNA isolation of *B. cepacia*, restriction endonuclease treatments, DNA ligation, transformation, and agarose gel electrophoresis were done as described by Sambrook et al. [12] For cloning and sequencing of PCR-amplified DNA fragments, the DNA fragments were ligated into pGEM®-T Easy vectors (Promega Corp. Madison, WI). *Escherichia* coli DH5 α strain was used for gene cloning and was cultured in Luria-Bertani agar (LA) medium at 37 °C. Ampicillin (50 μg/ml) was added as necessary to maintain selection of the resistance marker in the pGEM®-T Easy vectors. Double-stranded DNA sequencing was performed using a 373A automated DNA sequencer and an ABI Prism DNA Sequencing kit (Applied Biosystems, Foster City, CA) with standard universal T7 and SP6 primers. Sequence data were compiled and analyzed using the computer programs of GCG (Genetics Computer Group, Madison, WI). PCR-amplified DNA fragments that were used as probes were labeled with digoxigenin-11-dUTP (DIG) using a PCR DIG probe synthesis kit (Boehringer Mannheim Biochemicals, Indianapolis, IN). Prehybridization, hybridization, and washing for Southern hybridization with DIG-labeled probes were performed at 68 °C according to the manufacturer's protocol.

PCR and PCR-RFLP analyses

The genomovar status of the finger-tip rot and onion decay pathogens in the *B. cepacia* complex was determined by PCR-RFLP pattern and sequence analysis of 16S rDNA and *recA* as described by Mahenthiralingam et al. [9] 16S rDNA and *recA* gene fragments were amplified by PCR with UN12/UN15 and BCR1/BCR2 primer pairs,

Table 1 : Bacterial strains tested.

Banana finger tip rot pathogen[a]	
(*Burkholderia cepacia* genomovar III; *B. cenocepacia*)	
recA-IIIA: B5, B9, B10, B11; *recA*-IIIB: CT1, CA2, CB2. CC2	
Onion decay pathogen[a]	
(*B. cepacia* genomovar I; *B. cepacia*)	
OQ1-1; OQ1-2; OQ2-1; OQ2-2; OQ3-1, OQ3-2	
Other plant pathogenic bacteria[b]	
Erwinia species and strains	
E. carotovora subsp. carotovora Erc1, ErcE1; *E. chrysanthemi* S3-1. pB1.	
Xanthomonas species and pathovars	
X. axonopodis pv. citri XCI3-1, XW96; *X. axonopodis* pv. glycines XPS1, XPS3;	
X. axonopodis pv. phaseoli XP1, XP4; *X. campestris* pv. campestris XCC1-1, XCC33.	
Ralstonia solanacearum PS21, PS68, PSS4,	

[a] The bacteria were isolated from lesions of diseased banana fingers and onion bulbs in this study.

[b] The strains were isolated in our laboratory or obtained from Bioresources Collection and Research Center, Hsinchu, Taiwan.

respectively. Colonies of *B. cepacia* taken from the LA medium were suspended in 50 μl of sterile distilled water. The bacterial suspension was incubated for 10 min at 100 ℃ for cell lysis, and was maintained at 4℃ before use. PCR amplifications were performed with a GeneAmp® PCR system 2400 (Perkin-Elmer Corporation, Norway, CT) in a 50- μl reaction mixture containing 1 μl of cell lysate, 0.2 μM of each primer, 0.4 mM of each deoxynucleoside triphosphate, 1X reaction buffer (1.5 mM MgCl2, 50 mM KCl, 0.1% Triton X-100, 10 mM Tris-HCl; pH 8.8), 4% dimethyl sulfoxide (DMSO; J.T.Baker, Phillipsburg, NJ), and 4.0 U of *Taq* DNA polymerase (DyNAzyme II, Finnzymes Oy, Finland) by using the following program: 1 cycle of denaturation for 1 min at 94 ℃; 35 cycles consisting of denaturation at 94℃ for 30 s, annealing at 60℃ for 30 s, and extension at 72℃ for 45 s followed by a final extension (hold) at 72℃ for 2 min. Reactions were stored at 4℃ until they were used for analysis. Amplified DNA was detected by electrophoresis in 0.8% agarose (agarose I, Amresco Inc., Solon, OH) in 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH 8.0).

Two additional primer sets were designed for detection and differentiation of *B. cepacia* genomovar I and III. One primer set, bcscV-1F (5'-GACTG GCAGC GGTTG TTTTC CG-3') and CP11 (5'-GCCCT TCACG AACTT CATC-3') primers, was designed from the nucleotide sequence of *bcscV* (GenBank accession number AY028431), which is a type III secretion gene, and present in genomovar III but absent in genomovar I[10]. The bcscV-1F and CP11 primers delineated a 618-bp DNA fragment. The other primer set, Bx-UvrB-F (5'-CGATC AACGA GCACA TCGAG CAGA -3') and B-UvrB-R5 (5'-GCCGG CGCTC ACGCG CGCGT TG-3'), was designed from the nucleotide sequence of *uvrB* of *B. cepacia* (AY036068) for specific detection of genomovar I and III. The Bx-UvrB-F and B-UvrB-R5 primers delineated a 995-bp DNA fragment.

For restriction fragment length polymorphism (RFLP) analysis, PCR-amplified DNA fragments were recovered from agarose by using the GFX™ PCR DNA and gel band purification kit (Amersham Pharmacia Biotech. Inc., Piscataway, NJ). They were then digested with *DdeI* (New England Biolabs, Inc., Beverly, MA.) for 16S rDNA, *Hae*III or *Mnl*I for *recA*, and *Mlu*I for *uvrB* PCR fragments, and analyzed by 1.0-2.5% agarose gel electrophoresis.

RESULTS

Biochemical and physiological characterizations

All tested isolates of banana finger-tip rot and onion decay pathogens were gram-negative, aerobic, and positive for oxidase, lysine decarboxylase, and ornithine decarboxylase, but negative for arginine dihydrolase and

Table 2 : Phenotypic characteristics of banana finger tip rot and onion decay pathogens.

	Genomovar of *B. cepacia* complex		Banana finger-tip rot pathogen		Onion decay pathogen	
Characteristics	I[a]	III	B9	CA2	OQ1-1	OQ2-1
Gram stain	−[b]	−	−	−	−	−
Glucose fermentation	−	−	−	−	−	−
Oxidase	+	+	+	+	+	+
Pigment (yellow)	NR	NR	−	−	+	+
Growth at 42 ℃	−	+	+	+	−	−
β-galactosidase	+	+	+	+	+	+
Urease	V	V	−	−	−	−
Indole production	NR	−	−	−	−	−
Arginine dihydrolase	NR	−	−	−	−	−
Lysine decarboxylase	+	V	+	+	+	+
Ornithine decarboxylase	V	V	+	+	+	+
Gelatin liquefaction	V	V	−	−	−	−
Esculin hydrolysis	V	V	−	−	−	−
Acid production from						
Glucose	+	V	+	+	+	+
Lactose	+	V	+	+	+	+
Maltose	+	V	+	+	+	+
Mannitol	+	V	+	+	+	+

[a] Phenetic characteristics of *B. cepacia* genomovar I and III were based on Vandamme et al.[17] and Coenye et al.[3]

[b] +:positive reaction, -: negative reaction, v: variable (strain dependent), NR: Not determined.

indole production. They all hydrolyzed gelatin and esculin, and produced acids by utilizing glucose, lactose, maltose, and mannitol. However, the isolates of banana finger-tip rot pathogen were not pigmented and could grow at 42°C, but the isolates of onion decay pathogen were yellow-pigmented and could not grow at 42°C, indicating that two pathogens might belong to different genomovars. The representative results are shown in Table 2.

Determination of genomovar status of banana finger-tip rot and onion decay pathogenic bacteria

The genomovar status of the *B. cepacia* isolates from banana fingers and onion bulbs were determined by PCR-RFLP analysis of 16S rDNA and *recA* as described in Mahenthiralingam et al.[9] PCR amplification using UN12/UN15 and BCR1/BCR2 primers amplified a 1,020-bp 16S rDNA fragment and a 1,041-bp *recA* gene fragment, respectively, for the *B. cepacia* isolates tested. Restriction analysis of the amplified fragments was then performed using *Dde*I for 16S rDNA fragment, and *Hae*III and *Mnl*I for *recA* gene fragment. Only one 16S rDNA RFLP pattern was found for all the *B. cepacia* isolates tested.

Digestion of the *recA* amplicon with the endonucleases *Hae*III or *Mnl*I generated two distinct RFLP patterns for each restriction enzyme among the isolates from bananas, but only one pattern for the isolates of onion decay pathogen. The representative results are shown in Fig. 1. The *recA* RFLP patterns were compared with those from the previously published representative panel of the *B. cepacia* complex[15], where *Hae*III RFLP patterns designated A to J and *Mnl*I RFLP patterns a to i were reported. Two distinct *Hae*III RFLP patterns of banana finger-tip rot pathogen corresponded to patterns G and H, and the *Mnl*I RFLP patterns corresponded to patterns f and i (Table 1, Fig. 1). Isolates with pattern G and f belong to genomovar III-A, and those with patterns H and i belong to genomovar III-B of *B. cepacia* complex. The onion decay pathogen isolates obtained only one *Hae*III RFLP pattern, which corresponds to pattern D, and belong to genomovar I. However, the *Mnl*I RFLP pattern of onion decay pathogen in Taiwan revealed a novel pattern not reported by Mahenthiralingam et al.[9]. Thus, *recA* PCR-RFLP analyses were capable of separating the isolates of banana finger-tip rot and onion decay pathogens.

All isolates of banana finger-tip rot pathogen tested belong to *B. cepacia* genomovar III. *B. cepacia* genomovar III was proposed as a new species, *B. cenocepacia*[16]. Thus, banana finger-tip rot pathogen is *B. cenocepacia*. Isolates of onion decay pathogen belong to genomovar I and remain in *B. cepacia*.

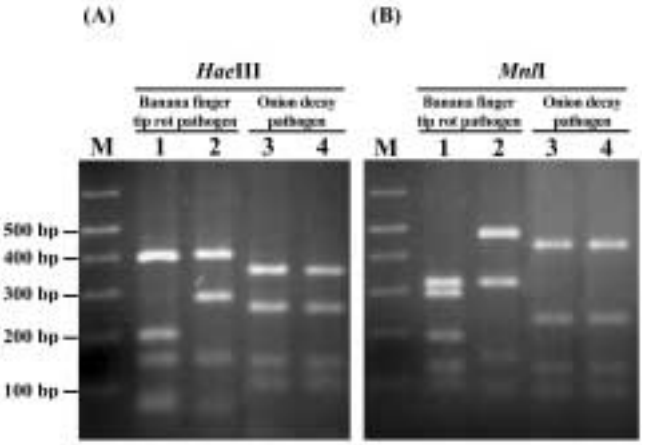

Fig. 1. PCR-RFLP analysis of the PCR-amplified *recA* fragments of strains of banana finger-tip rot and onion decay pathogens with (A) *Hae*III or (B) *Mnl*I digestion. Lanes: 1–2, banana finger-tip rot pathogen strains B9 and CA2; 3-4, onion decay pathogen strains OQ1-1, and OQ2-1. M, molecular size marker (1-kb plus DNA ladder, Gibco-BRL Life Technologies). The sizes of marker bands are indicated to the left.

Development of PCR detection methods of banana finger-tip rot and onion decay pathogens

The PCR amplification of *bcscV* using bcscV-1F and CP11 primers amplified a 618-bp DNA fragment only from isolates of banana finger-tip rot pathogen (*B. cenocepacia*), but not from those of onion decay pathogen (*B. cepacia*) (Fig. 2A). To determine the specificity of the primer sets, PCRs were also carried out with the other plant pathogenic bacteria listed in Table 1. No amplification using the primer set was observed with the other plant pathogenic bacteria.

The PCR amplification of *uvrB* using Bx-UvrB-F and B-UvrB-R5 primers amplified a 995-bp DNA fragment for all isolates of *B. cenocepacia* and *B. cepacia* tested (Fig. 2B). The amplified 995-bp *uvrB* DNA fragments of *B. cenocepacia* isolate B9 and *B. cepacia* isolate OQ1-1 were cloned, sequenced, and compared. Both share 98% identity on nucleotide sequence. Available restriction enzymes for PCR-RFLP analysis were assessed on the basis of the determined sequences of these strains by using the computer programs of GCG (Genetics Computer Group, Madison, WI). The sequence of *B. cepacia* isolate OQ1-1 contains a unique *Mlu*I restriction site, but that of *B. cenocepacia* does not. Thus, *Mlu*I was used for RFLP analysis of PCR products amplified with Bx-UvrB-F and B-UvrB-R5. As shown in Fig. 3, PCR products of onion decay pathogen isolates were cleaved by *Mlu*I resulting in two bands (528/467-bp) after agarose gel eletrophoresis, but *B. cenocepacia* isolates only showed a single band

(995-bp). The result indicated that *Mlu*I site of the *uvrB* sequence was present in the *B. cepacia* isolates tested. No amplification using the Bx-UvrB-F and B-UvrB-R5 primer set was observed with the other plant pathogenic bacteria in Table 1.

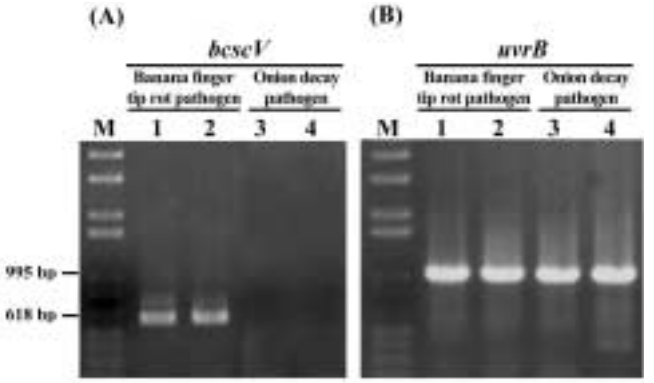

Fig. 2. Agarose gel electrophoresis of polymerase chain reaction (PCR) products from banana finger tip rot pathogen (*B. cenocepacia*) and onion decay pathogen (*B. cepacia*) isolates with (A) *bcscV*-1F/CP11 primer pairs for *bcscV* or with (B) Bx-UvrB-F/B-UvrB-R5 primer pairs for *uvrB*, respectively. Lanes: 1–2, *B. cenocepacia* isolates B9 and CA2; 3-4, *B. cepacia* isolates OQ1-1 and OQ2-1. M, molecular size marker (1-kb plus DNA ladder, Gibco-BRL Life Technologies); sizes of PCR products are indicated to the left.

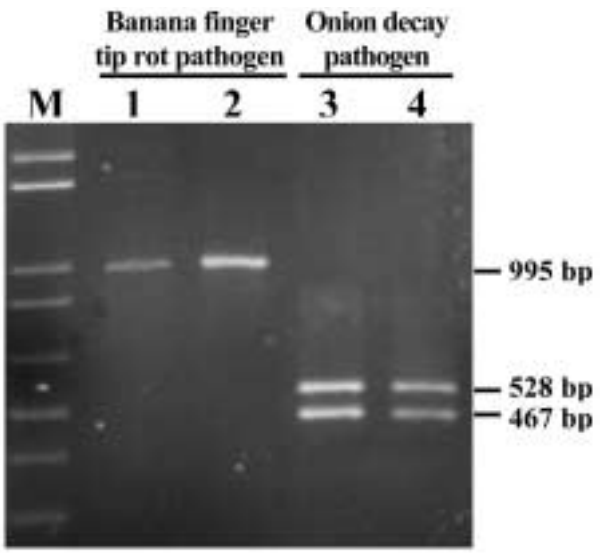

Fig. 3. PCR-RFLP analysis of the 995-bp PCR-amplified *uvrB* fragments of banana finger tip rot pathogen (*B. cenocepacia*) and onion decay pathogen (*B. cepacia*) isolates with MluI digestion. Lanes: 1–2, *B. cenocepacia* isolates B9 and CA2; 5-6, *B. cepacia* isolates OQ1-1 and OQ2-1. M, molecular size marker (1-kb plus DNA ladder, Gibco-BRL Life Technologies). The sizes of PCR products are indicated to the right.

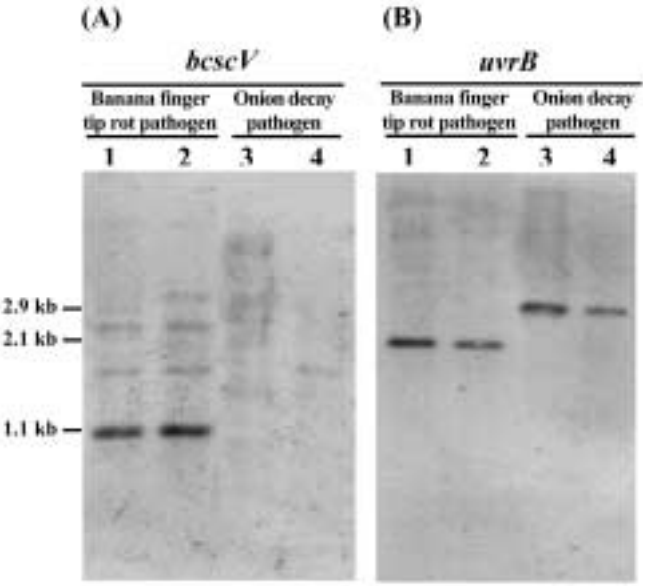

Fig. 4. Southern hybridization of *Sal*I- or *Eco* RI-digested total DNA from banana finger tip rot pathogen (*B. cenocepacia*) and onion decay pathogen (*B. cepacia*) isolates hybridized with the DIG-11-dUTP-labeled PCR-amplified (A) *bcscV* or (B) *uvrB* DNA fragments, respectively. Lanes: 1–2, *B. cenocepacia* isolates B9 and CA2; 3-4, *B. cepacia* isolates OQ1-1 and OQ2-1. The sizes of the hybridized bands are indicated on the left.

The PCR-amplified *bcscV* and *uvrB* DNA fragments were also used as probes to hybridize *Sal*I or *Eco*RI-digested total genomic DNAs from *B. cenocepacia* and *B. cepacia*, respectively. The hybridization patterns of *B. cenocepacia* were different from those of *B. cepacia*. The *bcscV* probe hybridized to 1.1-kb *Sal*I fragment of *B. cenocepacia* but not to *B. cepacia* (Fig. 4A). The *uvrB* probe could hybridize to both pathogens, a 2.1-kb *Eco*RI fragment of *B. cenocepacia* and a 2.9-kb *Eco*RI fragment of *B. cepacia* isolates tested (Fig. 4B).

To determine the sensitivity of detection of the PCR assay with Bx-UvrB-F and B-UvrB-R5, 2 μl samples of serial dilutions of *B. cenocepacia* or *B. cepacia* colonies grown on LA medium were used as template for the PCR. The tests were repeated six times, and the sensitivity of detection was determined as 10^5 cfu per ml of bacterial suspension on the ethidium bromide-stained gel. Since only 2 μl of bacterial suspension were used for PCR, this method was able to detect 200 cells of *B. cenocepacia* or *B. cepacia*.

DISCUSSION

The isolates of banana finger-tip rot pathogen had biochemical and physiological characteristics very similar to those of onion decay pathogen. There were only two

characteristics (pigmentation and the growth ability at 42 ℃) that could distinguish these two pathogens. If based on growth ability at 42 ℃, banana finger-tip rot pathogen belongs to genomovar III of *B. cepacia* complex, and onion decay pathogen belongs to genomovar I. However, only one characteristic is not enough to obtain this conclusion. Molecular analyses using PCR-RFLP were further conducted to determine their genomovar status. The *recA* PCR-RFLP analyses indicated that all tested isolates of banana finger-tip rot pathogen belonged to genomovar III, and that isolates of onion decay pathogen belonged to genomovar I. *B. cepacia* genomovar III was proposed as a new species, *B. cenocepacia*[16]. Accordingly, banana finger-tip rot and onion decay in Taiwan are caused by two separate pathogens. The banana finger-tip rot pathogen is *B. cenocepacia* and onion decay pathogen is *B. cepacia*.

The *recA* PCR-RFLP tests further showed the genetic variability of the *B. cenocepacia* isolates. The isolates contained *recA* IIIA and IIIB lineages. Nevertheless, all tested *B. cenocepacia* isolates obtained the same hybridization patterns when their genomic DNAs were hybridized with the PCR-amplified *bcscV* and *uvrB* DNA fragments, suggesting that the pathogen population might be originated from a single source and then branched into *recA* IIIA and IIIB lineages. The sample sizes used in this study were not adequate to draw definite conclusions about the genetic variability of *B. cenocepacia* populations in Taiwan, the results reported here emphasized that the *recA* PCR-RFLP tests were useful to determine the genomovar status of and to differentiate the banana finger-tip rot and onion decay pathogens. For understanding the genetic variability of *B. cenocepacia* in Taiwan, further investigations are needed, particularly comparison at molecular levels with *B. cenocepacia* isolates from various banana-growing areas in southern Taiwan, to obtain more data on genetic variability, population structure, and likely the origin.

The *recA* primers used in *recA* PCR-RFLP assay were for the determination of genomovar status of *B. cepacia* strains but not for the specific identification and detection of the pathogens. Therefore, before the *recA* PCR-RFLP assay, the identities of banana finger-tip rot and onion decay pathogens should be determined. In addition to biochemical and physiological characterizations, our strategy to rapidly determine the identity of *B. cenocepacia* was to examine the isolates by a PCR assay for *bcscV*. In the *B. cepacia* complex, *bcscV*, which is a type III secretion gene, is present in *B. cenocepacia* and absent in *B. cepacia*[10]. All tested isolates of banana finger-tip rot pathogen were PCR positive but the isolates of onion decay pathogen were negative for *bcscV*. In addition, *bcscV* probe hybridized only to *B. cenocepacia* but not to *B. cepacia*. The specificity of *bcscV* to *B. cenocepacia* made *bcscV* PCR assay as a useful detection method for future epidemiological studies.

Although banana finger-tip rot and onion decay pathogens in Taiwan were different, it does not rule out the possibility that two pathogens might co-exist in plants, irrigation water, soil, or even insect vectors. Since the *bcscV* PCR assay is only for *B. cenocepacia*, a detection method for both pathogens should be developed for the studies on distributions of the pathogens. The *recA* gene, which is involved in recombination and DNA repair, provided a useful way to taxonomically classify members of the *B. cepacia* complex[9], which led us to try to find another recombination and/or DNA repair gene for the development of specific detection method. UvrB is considered the central recognition protein in bacterial DNA repair as it interacts with all the components of the repair system: UvrA, UvrC, UvrD, DNA polymerase I and damaged DNA[14]. The *uvrB*-disrupted strains of *B. cepacia* were exceptionally susceptible to killing by trichloroethylene, which is a mutagen and has been used extensively as a fumigant and solvent for dry cleaning and in other commercial applications[20], and the *uvrB*-defective mutant of *Pseudomonas aeruginosa* is extremely sensitive to UV radiation[11]. Thus, *uvrB* should be very important to *B. cepacia* and *B. cenocepacia* for the survival on plant surfaces and soils. Since the nucleotide sequence of *uvrB* of *B. cepacia* is available in GenBank, the sequence was chosen to compare with those of *uvrB* from other bacteria. Two unique regions of the sequence of *B. cepacia* were used to design the specific PCR primers (Bx-UvrB-F and B-UvrB-R5). The PCR amplification using the primers amplified a 995-bp DNA fragment for all isolates of *B. cenocepacia* and *B. cepacia* tested, and no amplification was observed with other plant pathogenic bacteria. In addition, discovery of a unique *Mlu*I restriction site of *uvrB* of onion decay pathogen led to the development of a PCR-RFLP assay able to discriminate onion decay pathogen isolates from banana finger-tip rot pathogens. All tested onion decay pathogen isolates yielded PCR products with the *Mlu*I site. Accordingly, the *uvrB* PCR assay developed is effective for detection of both pathogens, and the *uvrB* PCR-RFLP, combined with the *bcscV* PCR test, could be used for further pathogen differentiation.

This study first assessed the physiological and molecular characteristics of banana finger-tip rot and onion decay pathogens in Taiwan. The pathogens were identified as genomovar III and I of *B. cepacia* complex, which are named as *B. cenocepacia* and *B. cepacia*, respectively. The members of *B. cepacia* complex may also cause a dramatic necrotizing pneumonia that results in

rapid death of the patient[4,5]. However, as there is considerable genetic variation among strains within *B. cepacia* complex[1], strains of banana finger-tip rot and onion decay pathogens may not be associated with clinical strains causing cepacia syndrome. Therefore, the epidemiological distribution and strain characterizations should be further considered. The *bcscV* and *uvrB* PCR and PCR-RFLP assays, combined with QY-TP semi-selective medium developed in a previous study[6], will be highly effective for the isolation, detection, and identification of *B. cenocepacia* and *B. cepacia* in future epidemiological studies.

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摘 要

李永安[1,3] 詹志文[1] 趙治平[2]. 2006. 台灣香蕉果指尖腐病原菌與洋蔥腐敗病原菌之生理及分子特性分析. 植病會刊 15 ：117-124 ([1] 輔仁大學生命科學系; [2] 台灣香蕉研究所; [3] 聯絡作者: 電子郵件: bio1007@ mails.fju.edu.tw; 傳眞: +886-2-29052193)

香蕉果指尖腐病原菌具有的生理生化特性與洋蔥腐敗病原菌極爲相似，不過洋蔥腐敗病原菌在 LA 洋菜培養基上呈黃色且無法在 42℃下生長。利用 *recA* PCR-RFLP 分析結果，發現香蕉果指尖腐病原菌的所有測試菌株均屬 *Burkholderia cepacia* complex 中的 genomovar III，而洋蔥腐敗病原菌的菌株則屬 genomovar I，由此結果可知香蕉果指尖腐病原菌應爲 *B. cenocepacia*，而洋蔥腐敗病原菌爲 *B. cepacia*，因此香蕉果指尖腐病原菌與洋蔥腐敗病原菌爲兩種不同的病原菌。此外，本報告亦研發出檢測及鑑定此兩種病原菌的 PCR 及 PCR-RFLP 方法。在 *bcscV*-PCR 測試，只有香蕉果指尖腐病原菌可得正反應，而洋蔥腐敗病原菌則爲負反應，並且 *bcscV* 探針只會與香蕉果指尖腐病原菌產生雜合反應，而不會與洋蔥腐敗病原菌產生反應。另一個 uvrB-PCR 檢測，則可對香蕉果指尖腐病原菌及洋蔥腐敗病原菌擴增出 995-bp 長的 DNA 片段，並且發現洋蔥腐敗病原菌的 *uvrB* DNA 片段中具有 *Mlu*I 限制酵素切點，因此可藉以研發出 PCR-RFLP 方法，以區分香蕉果指尖腐病原菌及洋蔥腐敗病原菌。其他測試的植物病原細菌在 *bcscV* 及 *uvrB*-PCR 測試中，均無 DNA 片段被擴增出來。因此本報告所研發出的 *bcscV* 及 *uvrB*-PCR 與 PCR-RFLP 的測試方法，將可在生態研究中，用於檢測及鑑定 *B. cenocepacia* 及 *B. cepacia* 兩種病原