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Research Article

Identification of *Pseudomonas corrugata*, the Causal Agent of Tomato Pith Necrosis, and Screening of Agrochemicals

Chia-Hsin Tsai[1], Shu-Ling Hwang[2], Mei-Ju Lin[3], Chiao-Yu Ku[3], and Ting-Hsuan Hung[4,*]

Abstract

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In 2014, tomato plants grown in a field located in the Waipu District, Taichung City, Taiwan, exhibited stunted growth and increased fruit abscission. Affected plants exhibited epidermal browning on the stems, and longitudinal sections of the stem revealed browning of the vascular bundles. Microscopic examination of the symptomatic stem tissues revealed abundant bacterial presence, indicating a bacterial infection. On nutrient agar, colonies of *Pectobacterium carotovorum* subsp. *carotovorum*, the known causal agent of bacterial soft rot, was isolated from symptomatic stem tissues. In addition, the unknown yellowish bacteria were also isolated. The yellowish bacteria were identified as *Pseudomonas corrugata* based on the Biolog identification system, physiological and biochemical characterizations, polymerase chain reaction (PCR) amplification using species-specific primer sets, and bacterial gene sequence analysis. Pathogenicity tests were conducted by inoculating the bacterial isolates into healthy tomato plants, which subsequently developed vascular bundle browning extending longitudinally along the stem, resembling the symptoms observed in the field. In cases of severe infection, pith necrosis was also observed. The same bacteria were successfully re-isolated from the symptomatic tissues, thereby fulfilling Koch's postulates. This is the first report of *P. corrugata* infection on tomato in Taiwan. To manage the disease, an *in vitro* screening of commercial agrochemicals was conducted. Streptomycin, streptomycin + tetracycline, and thiophanate methyl + streptomycin produced distinct inhibitory zones against bacterial growth. Afterwards, no further tomatoes were planted in the field, and the disease did not recur.

Key words: Tomato, Pith necrosis, *Pseudomonas corrugate*, Agrochemical screening.

INTRODUCTION

Tomato (*Solanum lycopersicum*) is an important horticultural crop in Taiwan, serving both as a fruit and a vegetable. It is widely consumed in fresh, cooked, or processed forms. The total tomato cultivation area in Taiwan in 2024 reached 3,637 ha, yielding an overall production of 27,298 Mg. The primary production regions are located in central and southern Taiwan. Chiayi County has the largest cultivation area, followed by Nantou County, according to the Agricultural Statistical Yearbook 2024 (https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx).

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* Corresponding author, e-mail: thhung@ntu.edu.tw
[1] Associate Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[2] Project Assistant, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[3] Assistant Research Fellows, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[4] Professor, Department of Plant Pathology and Microbiology, National Taiwan University, Taipei, Taiwan, ROC.

In 2014, some tomato plants showing stunted growth, premature fruit drop, and wilting were observed in a tomato flied located in the Waipu District, Taichung City (Fig. 1A). The epidermal surface of the stem exhibited brown-black patches (Fig. 1B), and longitudinal sections of symptomatic stems revealed vascular discoloration, characterized by browning and spread along the vascular bundles (Fig. 1C). Microscopic examination of the excised vascular tissues revealed abundant bacterial exudation, indicating a bacterial infection. A review of international literature indicates that various bacterial pathogens are capable of infecting vascular bundles in tomato such as *Ralstonia solanacearum* (causing bacterial wilt) (Phiri *et al*. 2024), *Pectobacterium carotovorum* (bacterial stem rot) (Caruso *et al*. 2016), *Clavibacter michiganensis* (bacterial canker) (Sen *et al*. 2015) and several bacterial species belonging to *Pseudomonas* genus including *Ps*. *viridiflava* (Aysan *et al*. 2004), *Ps*. *cichorii* (Ruan *et al*. 2019), *Ps*. *mediterranea* (Alippi & López 2010), *Ps*. *marginalis* (Bella & Catara 2010), *Ps*. *corrugata* (Moura *et al*. 2005; Molan & Ibrahim 2007), and *Ps*. *fluorescens* (Saygili *et al*. 2004; Molan & Ibrahim 2007), which are implicated in bacterial pith necrosis.

The field covered approximately 0.2 ha, with wilting symptoms observed in about 20% of the plants, resulting in significant economic losses. Therefore, this study aimed to identify the causal agents responsible for the wilt symptoms in tomato plants and to conduct *in vitro* screening of commercial agrochemicals. The results provide a reference for developing effective disease management strategies in tomato cultivation.

MATERIALS AND METHODS

The source of bacterial isolates

Samples of tomato plants exhibiting wilt and stem browning symptoms were randomly collected from a diseased field in the Waipu District, Taichung City. Infected stem tissues were excised and surface-sterilized by soaking in 1% sodium hypochlorite (NaOCl) for 30 s,

Fig. 1. Symptoms of diseased tomato plants in the field. Infected plants showed (A) wilting of lateral branches; (B) brown-black patches on the stem epidermis; and (C) vascular bundles browning.

followed by 3 rinses with sterile water. The symptomatic tissues were then finely chopped and placed in centrifuge tubes containing sterile water, shaken to release bacteria. A loop was used to collect the bacterial suspension, which was streaked onto nutrient agar (Difco Laboratories; Becton, Dickinson and Company, Le Pont-de-Claix, France) plates. The plates were incubated at 28℃ for 2–3 d until bacterial colonies appeared. A single bacterial colony was selected and streaked onto a new nutrient agar plate, and this purification process was repeated 3 times to obtain pure bacterial isolates for further experiments.

Biolog identification system test

To simultaneously analyze different types of bacterial isolates, the Biolog identification system (Biolog Inc., Hayward, CA, USA) was used to analyze 3 bacterial isolates of yellowish colonies, numbered TW01, TW02, and TW03, and 3 bacterial isolates of white colonies, numbered TW04, TW05, and TW06. First, the isolates were cultured on a 5.7% BUG™ agar (Biolog Universal Growth Agar, Biolog Inc., Hayward, CA, USA). After culturing at 30℃ for 16–24 h in an incubator, use a cotton swab to pick up bacterial colonies and inoculate them into IF-A inoculum (Biolog Inc., Hayward, CA, USA). The concentration of the inoculum was adjusted to 90–98% T (turbidity). The prepared inoculum was then added to the Biolog GENIII reaction plate (Biolog Inc., Hayward, CA, USA), and 100 µL was added to each well. The reaction plates were incubated for 25–35 h and measured with a spectrometer. The obtained data were analyzed using the Biolog MicroLog™ 3 ver. 5.2.2 system and Biolog GENIII database (version 2.6.1) to identify bacterial species.

Physiological and biochemical analysis

Physiological and biochemical characteristics of bacterial isolates TW01, TW02, and TW03 were determined following the methods of Schaad *et al*. (2001) and Catara *et al*. (2002), with all assays conducted in duplicate. Tests performed included Gram reaction, fluorescence pigment production on King's B medium, oxidative/fermentative utilization of glucose (O/F test), levan production, oxidase activity, potato soft rot assay, arginine dihydrolase activity, hypersensitive reaction on tobacco, gelatin liquefaction, and growth at 37℃. In addition, utilization of mannitol, benzoate, sorbitol, trehalose, sucrose, D-arabinose, L-rhamnose, histamine, meso-tartrate, and 2-ketogluconate was assessed. These assays were used to determine the taxonomic affiliation of the bacterial isolates tested.

16S ribosomal RNA (rRNA) gene and DNA gyrase subunit B (*gyrB*) gene sequence analysis

According to the Biolog identification system, TW01, TW02, and TW03 were identified as *Ps*. *corrugata*, a bacterium that has not yet been reported to infect tomatoes in Taiwan. To further identify the bacteria isolated in this study, sequence analysis of the 16S rRNA and *gyrB* genes was performed on the nucleic acids of bacterial isolates TW01, TW02, and TW03. Nucleic acid extraction was carried out using a slight modification of the simple extraction method described by Wang *et al*. (1993). The 3 tested bacterial isolates were first streaked onto nutrient agar plates and incubated until bacterial colonies formed. A single colony was picked using a sterile toothpick and suspended in 20 µL of sterile water. Then, 20 µL of 0.4 N NaOH was added, vortexed, and left to stand for 10 min. Next, 40 µL of 1 M Tris-HCl was added and thoroughly mixed, followed by a tenfold dilution with sterile water to serve as the polymerase chain reaction (PCR) templates. PCR amplifications were performed using the bacterial universal primer pair f8-27/r1510 for the 16S rRNA gene (Lipson & Schmidt 2004) and the primer pair UP-1/UP-2r (Yamamoto & Harayama 1995) for *gyrB* gene.

Amplified DNA products were analyzed by electrophoresis on a 1.4% agarose gel to confirm expected DNA fragment sizes. The PCR products were then cloned using the T&A™ cloning kit (Yeastern Biotech, Taipei, Taiwan). After bacterial

transformation, the bacteria carrying the cloned DNA fragments were propagated, and plasmids were extracted. The extracted plasmids were subjected to PCR and electrophoresis to verify the presence of the expected DNA fragments. The successfully transformed bacteria were subsequently sent to Tri-Biotech Inc. (Taipei, Taiwan) for DNA sequencing, and the resulting DNA sequence data were then compared against the National Center for Biotechnology Information (NCBI) gene database for identification.

Specific primer pair analysis

The bacterial isolates were initially identified as *Ps. corrugata* using the Biolog identification system. However, *Ps. corrugata* and *Ps. mediterranea* are highly similar, and *Ps. mediterranea* was previously regarded as a group within *Ps. corrugata* before being reclassified as a distinct species (Catara *et al*. 2002). To verify the taxonomic identity of the bacterial isolates, PCR assays were performed using specific primer sets. The bacterial isolates TW01, TW02, and TW03, along with the reference strains *Ps. mediterranea* (DSM 16733) and *Ps. corrugata* (DSM 7228), were subjected to PCR amplification using the primer pair PC5/1 and PC5/2 specific for *Ps. corrugata*, and PC1/1 and PC1/2 specific for *Ps. mediterranea* (Catara *et al*. 2000, 2002). The PCR conditions were slightly modified as follows: The reaction mixture had a total volume of 20 μL, consisting of 1× PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM dNTP, 1.25 pmol of each primer, 0.4 units of Taq DNA polymerase, and 2 μL of template DNA. The thermal cycling conditions were as follows: initial denaturation at 94℃ for 5 min; followed by 30 cycles of 94℃ for 1 min, 62℃ for 1 min, and 72℃ for 30 s; with a final extension at 72℃ for 5 min. The amplified DNA products were analyzed by electrophoresis on a 1.4% agarose gel to verify the presence of the expected DNA fragments.

Whole-genome DNA sequence analysis

The bacterial isolate TW01 was cultured in 5 mL of nutrient broth for 24 h. Total nucleic acids were extracted from the bacterial suspension using the nucleic acid extraction kit (EasyPure Stool Genomic DNA Kit). The extracted nucleic acids were submitted to a biotechnology company for Illumina NovaSeq paired-end sequencing. Short-read sequences were de novo assembled with Velvet assembly software (version 1.2.10) to generate a draft genome. The assembled draft genome of TW01 was subsequently used to estimate digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values for taxonomic identification. The dDDH analysis was performed using the Genome-to-Genome Distance Calculator (GGDC) with Formula 2 (Meier-Kolthoff *et al*. 2013). ANI comparisons against the *Ps. corrugata* type strains NCPPB 2445 (GenBank accession RBOJ00000000.1) and DSM7228 (GenBank accession LHVK00000000.1) were conducted using ortholog-based average nucleotide identity (OrthoANI) as described by Lee *et al*. (2016).

Koch's postulates test

To identify the causal agent of the disease, Koch's postulates were tested using 3 bacterial isolates (TW01, TW02, and TW03) obtained from the wilted tomato plant in the Waipu District. The tomato cv. 'Hong Fan' was used as the host plant. Seedlings were grown in pots until reaching approximately 60 cm in height before inoculation. Each bacterial isolate was first streaked onto nutrient agar and incubated at 28℃ for 24 h. Bacterial cells were then harvested and suspended in sterile distilled water. The concentration of each bacterial suspension was adjusted to an optical density (OD_{600}) of 0.3 (approximately 10^8 CFU mL^{-1}), as measured using a spectrophotometer (Bausch & Lomb, Bridgewater, NJ, USA), and used as the inoculum. For inoculation, one drop of the bacterial suspension was applied to the stem surface of each plant. A sterile toothpick was then used to create a small wound at the inoculation site to facilitate bacterial entry. Each plant was inoculated at 2 separate sites, and 3 plants were used for each bacterial isolate. Control plants were mock-inoculated

with sterile water. After inoculation, plants were enclosed in transparent plastic bags to maintain high humidity for 48 h, then transferred to a plant growth chamber maintained at 28℃. Symptom development was monitored daily. Upon the appearance of disease symptoms, bacteria were re-isolated from the infected stem tissues and identified using PCR with specific primer pairs to confirm that they were identical to the original bacterial isolates, thereby fulfilling Koch's postulates.

In vitro agrochemicals susceptibility test

The bacterial isolates TW01 and TW03 were selected for antimicrobial susceptibility testing. The paper disc diffusion method, as described by Adaskaveg & Hine (1985), was used to evaluate the sensitivity of the bacterial isolates to various commercially available agrochemicals at different concentrations. A total of 11 agrochemicals, known for their effectiveness against bacterial pathogens as listed in the database of the Plant Protection Information System (https://otserv2.acri.gov.tw/PPM/), were selected for testing. The concentration range for each agrochemical was based on the recommended dilution ratio on product labels, with additional tests conducted at concentrations increased and decreased by one-half to allow for comparative analysis. Four types of agrochemicals were used: (1) antibiotics, such as streptomycin (12.5%, soluble concentrate (SL), Great Victory Chemical Industry Co. Ltd., Taipei, Taiwan), streptomycin + tetracycline (10.0%, water soluble powder (SP), A11 Taiwan Pesticides Co. Ltd., Taipei, Taiwan), kasugamycin (2.0%, wettable powder (WP), Worldwide Agrochemical Co. Ltd., Taipei, Taiwan), oxolinic acid (20.0%, WP, Sumitomo Chemical Taiwan Co. Ltd., Taipei, Taiwan), and validamycin (20%, SL, Lih-Nung Chemical Co. Ltd., Yunlin, Taiwan) (2) copper-containing agrochemicals, such as copper hydroxide (53.8%, water dispersible granules (WG), Corteva Agriscience Taiwan Co., Ltd., Taipei, Taiwan) and tribasic copper sulfate (27.12%, suspension concentrate (SC), Taiwan Nissan Chemical Industries Corp., Taipei, Taiwan); (3) zinc-manganese-containing agrochemical such as mancozeb (80.0%, WP, Worldwide Agrochemical Co. Ltd., Taipei, Taiwan); (4) mixed agrochemical, such as thiophanate methyl + streptomycin (68.8%, WP, Jui Sui Corp., Taipei, Taiwan), kasugamycin + copper oxychloride (81.3%, WP, Worldwide Agrochemical Co. Ltd., Taipei, Taiwan), fosetyl-aluminium + isotianil (77%, WG, Bayer Taiwan Co. Ltd., Taipei, Taiwan). The antimicrobial activity of the test agrochemicals was evaluated using an agar diffusion assay. Bacterial cultures were first prepared as suspensions and adjusted to a final concentration of 10^8 CFU mL^{-1}. 0.1 mL of the bacterial suspension was mixed thoroughly with 6 mL of 0.8% water agar, which was then overlaid onto solidified nutrient agar plates to form a bacterial mixture layer. The tested agrochemicals were diluted to obtain a range of concentrations. A volume of 0.12 mL from each concentration was applied onto sterile filter paper discs (13 mm diameter; Whatman International Ltd., Leeds, UK). The impregnated discs were then placed on the agar plates previously overlaid with the bacterial mixture layer. Discs treated with sterile distilled water served as negative controls. Each treatment was performed in triplicate. The plates were incubated at 28℃ for 48 h. Following incubation, the diameters of the inhibition zones surrounding each disc were measured to compare bacterial growth inhibition at different agrochemical concentrations.

RESULTS AND DISCUSSION

Bacterial isolation

To investigate the causal agents of tomato wilt, 4 symptomatic tomato plants exhibiting wilting symptoms were randomly collected from a field. Bacterial isolation was performed from discolored vascular tissues of the stems. From one of the tomato samples, yellowish colonies were obtained on nutrient agar. Three representative yellowish isolates were selected and designated as TW01, TW02, and TW03. In contrast, white bacterial colonies were isolated

from the remaining 3 tomato samples. One white colony was selected from each sample, purified, and subsequently designated as TW04, TW05, and TW06 for further identification analyses.

Biolog identification system

The 6 tested bacterial isolates were analyzed using the Biolog identification system. The TW01, TW02, and TW03 isolates were cultured on Biolog GENIII Microplates to assess their physiological and biochemical characteristics. The experimental data were analyzed and compared with the Biolog MicroLog™ 3 ver. 5.2.2 Database. The results indicated that TW01, TW02, and TW03 were identified as *Ps. corrugata* with similarity values of 0.726, 0.682, and 0.667, respectively. TW04, TW05, and TW06 isolates were identified as *Pe. carotovorum* subsp. *carotovorum* with similarity values of 0.661, 0.626, and 0.625, respectively, all greater than the system's predefined threshold value of 0.5.

Pe. carotovorum subsp. *carotovorum* (formerly *Erwinia carotovorum* subsp. *carotovorum*) is an important phytopathogenic bacterium in Taiwan. It has a broad host range and infects various vegetable crops. In tomatoes, it causes bacterial stem rot, which leads to plant wilting (Hseu *et al*. 2003). This pathogen is already established and prevalent in Taiwan. However, Biolog analysis identified strains TW01, TW02, and TW03 as *Ps. corrugata*. According to international literature, *Ps. corrugata* was first reported in 1978 as the causal agent of tomato pith necrosis (Scarlett *et al*. 1978) and has since been reported worldwide, including in African countries such as Tanzania (Black *et al*. 1999); Asian countries such as India (Parvateesam & Shuchita Verma 1992; Trivedi *et al*. 2008) and Japan (Kaji *et al*. 1989; Morohoshi *et al*. 2025); North American countries such as the United States (Powell *et al*. 2013) and Mexico (Rodríguez-Alvarado *et al*. 2002); European countries such as Greece (Buonaurio *et al*. 1993) and Italy (Catara *et al*. 1997); Oceania, including New Zealand (Clark & Watson 1986); and South American countries such as Argentina (Alippi *et al*. 1993) and Brazil (Clark & Watson 1986). Nevertheless, there have been no reports of *Ps. corrugata* infecting crops in Taiwan. Therefore, this study aims to perform further nucleic acid analysis and physiological and biochemical tests on bacterial isolates TW01, TW02, and TW03 to clarify their taxonomic status.

Physiological and biochemical analysis

Physiological and biochemical characterization of the tested bacterial isolates, TW01, TW02, and TW03, was conducted in duplicate, and identical results were obtained. All 3 isolates were Gram-negative, oxidized glucose, and produced yellowish colonies with diffusible pale-yellow pigment on nutrient agar. No fluorescent pigment production was observed on King's B medium. When grown on crystal violet pectate (CVP) agar, the isolates did not form cavities. The isolates were capable of producing levan and were positive for oxidase and arginine dihydrolase activities. They did not cause soft rot on potato slices but elicited a hypersensitive reaction on tobacco leaves. Additionally, the isolates liquefied gelatin and grow at 37℃. Carbon utilization tests indicated that the isolates could metabolize mannitol, trehalose, sucrose, and D-arabinose, but not 2-ketogluconate, meso-tartrate, histamine, benzoate, sorbitol, and L-rhamnose, as summarized in Table 1.

The physiological and biochemical characteristics of the 3 tested bacterial isolates were largely consistent with those reported for *Ps. corrugata* by Schaad *et al*. (2001) and Catara *et al*. (2002), except for their ability to synthesize levan, indicating minor physiological and biochemical differences between the domestic and foreign isolates. Such variation in levan production has also been observed in other phytopathogenic bacteria belonging to the genus *Pseudomonas*, such as *Ps. viridiflava* and *Ps. savastanoi* (González *et al*. 2003; Marchi *et al*. 2005). Furthermore, *Ps. corrugata* was previously divided into 2 groups (Catara *et al*. 2000), one of which was later recognized as a distinct

Table 1. Physiological and biochemical analysis on tomato pith necrosis pathogen.

Characters	Strains from tomato	*Pseudomonas corrugata*[z]
Gram reaction	G (−)	G (−)[y]
O/F[y] test	O	O
Fluorescent pigment on KB[x]	−	−
Levan	+	−
Oxidase	+	+
Pectolytic activity	−	−
Arginine dihydrolase	+	+
Tobacco hypersensitivity reaction	+	V
Gelatin hydrolysis	+	+
Growth at 37℃	+	+
Utilization of:		
2-ketogluconate	−	−
Mannitol	+	+
Benzoate	−	−
Sorbitol	−	−
Trehalose	+	+
Sucrose	+	+
Meso-tartrate	−	−
D-arabinose	+	+
L-rhamnose	−	−
Histamine	−	−

[z] Data are from (Schaad *et al*. 2001; Catara *et al*. 2002).
[y] G (−): Gram negative; O: oxidative; F: fermentation; +: positive; −: negative; V: between 21–79% of strains positive.
[x] KB: King's B medium.

species, *Ps*. *mediterranea* (Catara *et al*. 2000, 2002). *Ps*. *mediterranea* has been reported to cause bacterial pith necrosis of tomato in Taiwan (Tsai *et al*. 2018), whereas no reports exist of *Ps*. *corrugata* infecting tomato in Taiwan. Although the 2 species share highly similar physiological and biochemical profiles, a major distinction lies in their utilization of 3 substrates: *Ps*. *mediterranea* can utilize meso-tartrate, histamine, and 2-ketogluconate, whereas *Ps*. *corrugata* cannot. In the present study, the tested bacterial isolates failed to utilize meso-tartrate, histamine, and 2-ketogluconate, indicating that their physiological and biochemical characteristics are more consistent with *Ps*. *corrugata* rather than *Ps*. *mediterranea*, which is already present in Taiwan.

16S rRNA and *gyrB* gene sequence analysis

To further ascertain the taxonomic position of the isolated bacteria, genomic DNA from the test bacterial isolates TW01, TW02, and TW03 was subjected to PCR amplification using the universal bacterial primer pair f8/r1510 for the 16S rRNA gene and UP1-1/UP-2r primer pair for the *gyrB* gene. All tested isolates yielded approximately 1500-bp DNA fragment of the 16S rRNA gene and 1200-bp DNA fragment of the *gyrB* gene. The fragments were cloned and sequenced, revealing that the 16S rRNA and *gyrB* gene sequences of TW01, TW02, and TW03 were identical, with 100% sequence similarity among the 3 bacterial isolates. Using TW01 as the representative bacterial isolate, the 16S rRNA and *gyrB* gene sequences were deposited in the GenBank database under accession numbers PX475021 and PX555835, respectively. Basic Local Alignment Search Tool (BLAST) analysis against the NCBI nucleotide database showed that the 16S rRNA gene sequence of TW01 shared 100% identity with *Ps*. *corrugata* strains (GenBank accession numbers LT629798.1, CP014262.1, CP102175.1), and *gyrB* gene sequences of TW01 also shared 99–100% identity with *Ps*. *corrugata* strains (GenBank accession numbers CP102177.1, OZ350621.1, LT629798.1) indicating a high degree of similarity between the tested bacterial isolates and *Ps*. *corrugata*.

Specific primer pair set analysis

To rapidly distinguish whether the bacterial isolates belonged to *Ps*. *corrugata* or to closely related species such as *Ps*. *mediterranea*, which was previously classified within the *Ps*. *corrugata* group, species-specific primer pairs PC5/1 and PC5/2 for *Ps*. *corrugata* and PC1/1 and PC1/2 for *Ps*. *mediterranea* were used for PCR amplification. Electrophoretic analysis of the PCR products revealed that the *Ps*. *corrugata*-specific primers PC5/1 and PC5/2 successfully amplified the expected ~1,100 bp DNA fragment from the 3 tested bacterial isolates

TW01, TW02, and TW03, as well as from the reference strain *Ps*. *corrugata* DSM 7228, but not from the reference strain *Ps*. *mediterranea* DSM 16733 (Fig. 2A). Conversely, PCR with the *Ps*. *mediterranea*-specific primers PC1/1 and PC1/2 yielded the expected ~600 bp DNA fragment from the reference strain *Ps*. *mediterranea* DSM 16733, but not from the 3 tested bacterial isolates and the *Ps*. *corrugata* reference strain DSM 7228 (Fig. 2B). These results indicate that the bacterial isolates obtained in this study are *Ps*. *corrugata*, rather than *Ps*. *mediterranea*, which has been previously reported in Taiwan.

Whole-genome DNA sequence analysis of the bacterial isolates

To confirm the taxonomic affiliation of bacterial isolate TW01, next-generation sequencing was performed, and the genome was assembled with Velvet assembly software. The resulting draft genome sequence of TW01 (GenBank accession number SUB15775757) was 6,358,395 bp in length, comprising 219 contigs, with an N50 of 68,745 bp and an average sequencing coverage of 334×. Comparative genomic analyses showed that TW01 shared dDDH values of 96.2% and 96.1% with the *Ps*. *corrugata* type strains NCPPB 2445 and DSM 7228, respectively. Similarly, the ANI values between TW01 and the type strains NCPPB 2445 and DSM 7228 were 99.46% and 99.45%, respectively. Because the dDDH and ANI values between TW01 and the *Ps*. *corrugata* type strains NCPPB 2445 and DSM7228 were higher than the accepted cut-off thresholds (70% for dDDH and 95–96% for ANI) for prokaryotic species delineation (Goris *et al*. 2007; Richter & Rosselló-Móra 2009; Meier-Kolthoff *et al*. 2013), TW01 was accordingly identified as *Ps*. *corrugata*.

Koch's postulates test

Tomato plants cv. 'Hong Fan' inoculated with bacterial isolates TW01, TW02, and TW03 by stem puncture developed wilting on lateral branches 14 d post-inoculation (Fig. 3B). Longitudinal stem sections revealed bacterial spread along the vascular bundles, resulting in vascular browning (Fig. 3C) and, in more severe cases, necrosis of the pith tissues (Fig. 3D). As the disease progressed, inoculated plants exhibited stunted growth, followed by chlorosis and decline within 2–3 mo. In contrast, sterile water-inoculated control plants remained symptomless (Fig. 3A). The inoculated bacteria were consistently re-isolated from symptomatic tissues, and molecular analysis confirmed they were identical to the original isolates, thereby fulfilling Koch's postulates.

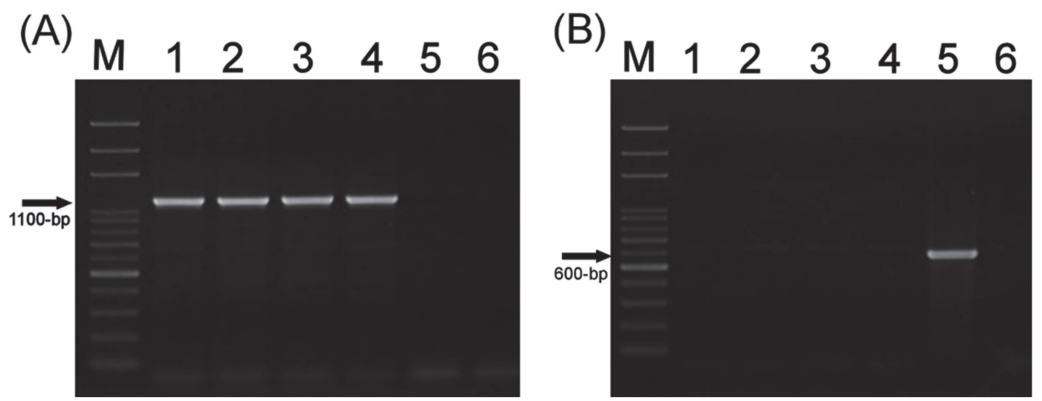

Fig. 2. Electrophoresis analysis of polymerase chain reaction (PCR) products for the identification of tomato pith necrosis pathogens. (A) PCR amplification with *Pseudomonas corrugata* specific primer PC5/1 and PC5/2; (B) PCR amplification with *Ps. mediterranea* PC1/1 and PC1/2. Lane M: 100 bp DNA marker; Lane 1–3: DNA from bacterial isolates TW01, TW02, and TW03; Lane 4: DNA from *Ps. corrugata* (DSM7228); Lane 5: DNA from *Ps. mediterranea* (DSM16733); Lane 6: no template control.

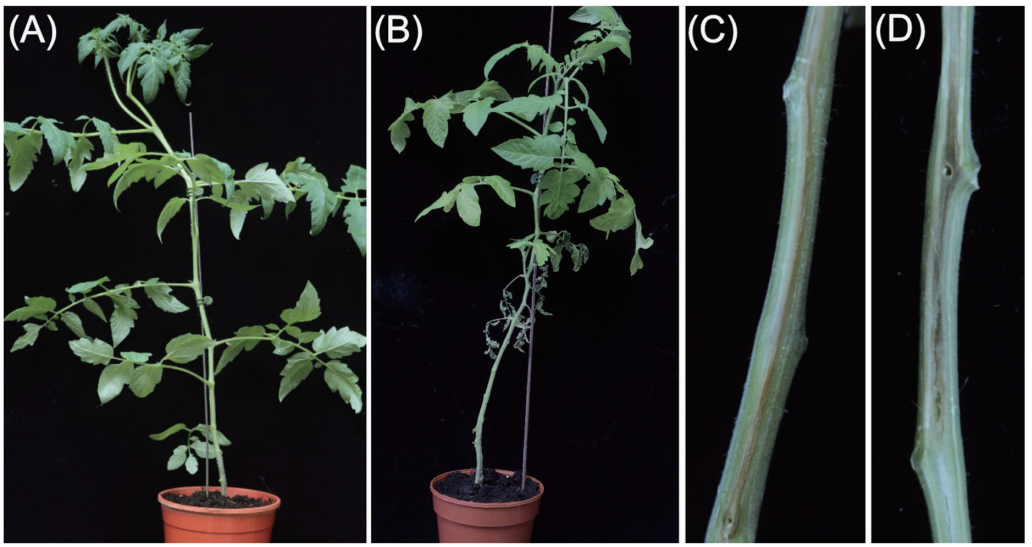

Fig. 3. Symptoms of the tomato plants 'Hong Fan' after inoculation with the isolated bacteria. (A) Tomato plants inoculated with sterile distilled water, serving as the control, showed no symptoms; (B) The lateral branches of tomato plants exhibited wilting near the inoculation sites; (C) Browning of stem vascular bundles in inoculated plants; (D) Necrosis of pith tissues in severely infected stems.

In vitro agrochemicals susceptibility test

The inhibitory effects of 11 commercial agrochemicals at 3 different concentrations against bacterial isolates TW01 and TW03 were evaluated on agar medium using the paper disc diffusion method. After 2 d of incubation, the diameters of inhibition zones (excluding the 13 mm diameter of the paper discs) were measured. Each was performed in duplicate, and the results were consistent; therefore, the data from one representative experiment are presented in Table 2. Among the tested agrochemicals, 4 produced inhibition zones against both bacterial isolates: streptomycin, streptomycin + tetracycline, thiophanate methyl + streptomycin, and kasugamycin. In contrast, the other 7 agrochemicals- validamycin, fosetyl-aluminium + isotianil, kasugamycin + copper oxychloride, copper hydroxide, tribasic copper sulfate, oxolinic acid, and mancozeb- showed no inhibitory zone at the tested concentrations. For bacterial isolate TW01, the largest inhibition zones were observed with streptomycin (500× dilution) and streptomycin + tetracycline (500× dilution), with diameters of 3.00–3.27 mm, followed by streptomycin (1,000× dilution) and thiophanate methyl + streptomycin (500× dilution), with diameters of 2.20–2.30 mm. For bacterial isolate TW03, the largest inhibition zones were observed with streptomycin (500× dilution), streptomycin + tetracycline (500× dilution), and thiophanate methyl + streptomycin (500× dilution) with diameters of 3.77–4.30 mm, followed by streptomycin (1,000× dilution), streptomycin + tetracycline (1,000× dilution), thiophanate methyl + streptomycin (1,000× dilution), and kasugamycin (125× dilution), with diameters of 2.50–3.03 mm.

Among the 4 agrochemicals that inhibited bacterial growth, 3 also produced inhibition zones against both bacterial isolates at the recommended product concentration, namely streptomycin (1,000× dilution), thiophanate methyl + streptomycin (1,000× dilution), and streptomycin + tetracycline (1,000× dilution). In contrast, copper-based agrochemicals failed to generate inhibition zones at any of the 3 tested concentrations, indicating that their antibacterial activities were ineffective in the range above and below 1/2 of the recommended concentration.

Table 2. Growth inhibition of *Pseudomonas courugata* TW01 and TW03 by various agrochemicals in different concentrations.

Chemical[z]	Dilution fold	Inhibition zone (mm in diameter)	
		TW01	TW03
Streptomycin (12.5% SL)	500	3.27 ± 0.10 a[y]	4.30 ± 0.38 a
	1,000	2.30 ± 0.15 b	2.90 ± 0.26 b
	1,500	0 e	1.67 ± 0.34 d
Streptomycin + Tetracycline (10% SP)	500	3.00 ± 0.10 a	4.07 ± 0.17 ab
	1,000	1.80 ± 0.06 c	2.80 ± 0.06 bc
	1,500	0.63 ± 0.13 e	1.57 ± 0.20 d
Thiophanate methyl + Streptomycin (68.8% WP)	500	2.20 ± 0.35 b	3.77 ± 0.18 ab
	1,000	1.33 ± 0.09 d	3.03 ± 0.03 b
	1,500	0.53 ± 0.27 e	2.13 ± 0.15 c
Kasugamycin (2.0%WP)	125	0.47 ± 0.47 e	2.50 ± 0.20 bc
	250	0 f	0 e
	375	0 f	0 e
Kasugamycin + Copper oxychloride (81.3% WP)	500	0 f	0 e
	1,000	0 f	0 e
	1,500	0 f	0 e
Validamycin (20%SL)	600	0 f	0 e
	1,200	0 f	0 e
	1,800	0 f	0 e
Fosetyl-aluminium + Isotianil (77%WG)	350	0 f	0 e
	700	0 f	0 e
	1,050	0 f	0 e
Oxolinic acid (20% WP)	500	0 f	0 e
	1,000	0 f	0 e
	1,500	0 f	0 e
Copper hydroxide (53.8% WG)	1,000	0 f	0 e
	2,000	0 f	0 e
	3,000	0 f	0 e
Tribasic copper sulfate (27.12% SC)	250	0 f	0 e
	500	0 f	0 e
	750	0 f	0 e
Mancozeb (80.0% WP)	250	0 f	0 e
	500	0 f	0 e
	750	0 f	0 e
LSD		0.34	0.35

[z] SL: soluble concentrate; SP: water soluble powder; WP: wettable powder; WG: water dispersible granules; SC: suspension concentrate; LSD: Fisher's least significant difference.

[y] Mean ± standard error (n = 3). Means within each column followed by the same letter(s) are not significantly different at 5% level by LSD test.

According to international literature on agrochemical assays of *Ps. corrugata*, this pathogen exhibits a relatively high tolerance to copper-based compounds (Alippi *et al.* 2003).

Consistently, in the present study, copper-based agrochemicals demonstrated limited inhibitory activity against the tested bacterial isolates at the applied concentrations, which is in agreement with the findings reported by Alippi *et al*. (2003). Since *Ps*. *corrugata* is newly reported in Taiwan and no local information on agrochemical control is available, this study provides an initial evaluation of agrochemicals against the pathogen. Four agrochemicals were identified as capable of producing inhibition zones and suppressing bacterial growth, suggesting their potential for disease management. These agrochemicals and the concentrations that produced inhibition zones *in vitro* may serve as preliminary references for future field trials or seed disinfection assays.

CONCLUSIONS

In 2014, tomato plants exhibiting wilting symptoms were observed in cultivation fields located in the Waipu District, Taichung City, Taiwan. From symptomatic plants, a yellowish bacterial isolate was obtained from one diseased sample. This bacterial isolate was identified as *Ps*. *corrugata* and subsequently confirmed as the causal agent by fulfilling Koch's postulates. Inoculated tomato plants developed vascular browning and pith necrosis, thereby verifying that the bacterium is the causal pathogen of tomato pith necrosis, as previously described by Scarlett *et al*. (1978).

Tomato pith necrosis is caused by several pathogenic species of the genus *Pseudomonas*. In Taiwan, *Ps*. *viridiflava* and *Ps*. *mediterranea* have previously been reported to infect tomato and induce pith necrosis (Tsai *et al*. 2016, 2018). In contrast, the pathogen isolated from symptomatic tomato plants in the Waipu District, Taichung City, was identified as *Ps*. *corrugata*, a species distinct from those previously reported in Taiwan. To date, *Ps*. *corrugata* has not been reported as a causal agent of tomato infection in Taiwan; this is the first report of tomato infection caused by *Ps*. *corrugata* in Taiwan. After the disease occurred in that field, tomatoes were no longer cultivated, and the disease has not reappeared since.

Regarding transmission, results from the inoculation trials indicate that *Ps*. *corrugata* can infect tomato plants through minor stem wounds. Thus, in the field, the pathogen may enter and colonize stems via pruning or other mechanical injuries. Previous reports have also shown that *Ps*. *corrugata* can survive in soil (Moura *et al*. 2012), infected fruits, and seeds (Cirvilleri *et al*. 2008). Therefore, effective management of this disease should include disinfecting pruning tools, protection of pruning wounds, selection of disease-free fields for planting, avoidance of irrigation using water from infected fields, timely removal of diseased plants, and use of pathogen-free seeds.

For agrochemical control, 4 commercially available agrochemicals were preliminarily screened *in vitro* in this study. Within the tested concentration ranges, these agrochemicals inhibited the growth of the pathogen on culture media. The identified agrochemicals and their effective concentrations may serve as references for future field trials or seed treatment experiments.

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番茄髓壞疽病之病原菌 *Pseudomonas corrugata* 之鑑定與藥劑篩選

蔡佳欣[1] 黃淑苓[2] 林玫珠[3] 古喬羽[3] 洪挺軒[4,*]

摘要

蔡佳欣、黃淑苓、林玫珠、古喬羽、洪挺軒。2026。番茄髓壞疽病之病原菌 *Pseudomonas corrugata* 之鑑定與藥劑篩選。台灣農業研究 75(2):293–306。

在 2014 年，臺中市外埔區 1 處番茄田植株部分植株出現生長緩慢，並且果實易有落果的情形，罹病植株莖部表皮黑褐化，縱切莖部發現維管束褐化且蔓延，將受害莖部組織在光學顯微鏡下鏡檢具有大量細菌湧出，疑似細菌性病害。從罹病株分離病原菌，在營養培養基分離出番茄細菌性莖腐細菌 *Pectobacterium carotovorum* subsp. *carotovorum* 之外，另外分離出 1 種未知的淡黃色細菌，該未知細菌，經 Biolog 鑑定系統分析、細菌生理生化特性分析、專一性引子對鑑定以及細菌基因序列分析，將該菌鑑定為 *Pseudomonas corrugata*，將其接種至番茄植株，在莖部出現維管束褐化蔓延病徵，與田間所見相同，嚴重感染之植株，髓部可觀察到壞疽病徵產生，並可再分離出該細菌，完成科霍氏法則。此為 *P. corrugata* 感染番茄在臺灣之首次報導。為防治此細菌，在培養基進行市售農藥篩選試驗，以鏈黴素、鏈四環黴素及多保鏈黴素具有明顯的抑制細菌生長效果，並形成明顯的抑制圈。該田區之後未再繼續種植番茄，未再發生該病害。

關鍵詞：番茄、髓壞疽病、*Pseudomonas corrugata*、藥劑篩選。

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* 通訊作者：thhung@ntu.edu.tw
[1] 農業部農業試驗所植物病理組副研究員。臺灣 臺中市。
[2] 農業部農業試驗所植物病理組計畫助理。臺灣 臺中市。
[3] 農業部農業試驗所植物病理組助理研究員。臺灣 臺中市。
[4] 國立臺灣大學植物病理與微生物學系教授。臺灣 臺北市。