Toxoflavin Produced by *Burkholderia glumae* Causing Rice Grain Rot Is Responsible for Inducing Bacterial Wilt in Many Field Crops

Yeonhwa Jeong, Jinwoo Kim, Suhyun Kim, and Yongsung Kang, School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea; Tomohisa Nagamatsu, Faculty of Pharmaceutical Sciences, Okayama University, Tsushima, Okayama 700-8530, Japan; and Ingyu Hwang, School of Agricultural Biotechnology, Seoul National University, Suwon 441-744, Korea

ABSTRACT

Jeong, Y., Kim, J., Kim, S., Kang, Y., Nagamatsu, T., and Hwang, I. 2003. Toxoflavin produced by *Burkholderia glumae* causing rice grain rot is responsible for inducing bacterial wilt in many field crops. Plant Dis. 87:890-895.

Severe wilt symptoms similar to bacterial wilt caused by *Ralstonia solanacearum* were observed in tomato, hot pepper, eggplant, potato, perilla, sesame, and sunflower in 2000 and 2001 in Korea. From diseased crops at 65 different locations, we obtained 106 isolates that produced green pigment on CPG medium; 36 were isolated from discolored rice panicles. The causal pathogen was identified as *Burkholderia glumae* based on its biochemical characteristics, fatty acid methyl ester analysis, and 16S rRNA gene sequence. Nine representative isolates produced toxoflavin, as determined by electrospray ionization mass spectrometry using a direct inlet system and TLC analyses, and caused bacterial wilt on tomato, sesame, perilla, eggplant, and hot pepper. However, BGR12, a wild-type isolate lacking toxoflavin production and toxoflavin-deficient mutants generated by Tn5lacZ failed to cause bacterial wilt on those five field crops. Cells of *B. glumae* and synthetic toxoflavin caused wilt symptoms on field crops, demonstrating a lack of host specificity. Synthetic toxoflavin caused wilt symptoms on tomato, sesame, perilla, eggplant, and hot pepper at 10 µg/ml concentration 1 day after treatment. This is the first report of bacterial wilt on various crops caused by *B. glumae*, and our results clearly demonstrate that toxoflavin is a key factor in wilt symptom development.

Tomato (*Lycopersicon esculentum* Mill.), hot pepper (*Capsicum annuum* L.), eggplant (*Solanum melongena* L.), and potato (*Solanum tuberosum* L.) are economically important solanaceous crops. Of the various bacterial diseases affecting these crops, bacterial wilt caused by *Ralstonia solanacearum* is the most serious in tropical, subtropical, and warm temperate regions of the world (9,10). Bacterial diseases affecting these crops include wilt diseases caused by *Clavibacter michiganense* subsp. *sepedonicum*, *C. michiganense* subsp. *michiganense*, *Curtobacterium flaccumfaciens*, *Erwinia tracheiphila*, *Pantoea stewartii* subsp. *stewartii*, and *Xanthomonas campestris* pv. *campestris* (1).

Bacterial wilt differs from fungal wilt in that fungi remain in the vascular tissues until plant death, whereas bacteria often destroy parts of the cell wall of xylem

vessels. In bacterial wilt, the vascular tissues of diseased stems and roots turn brown, and bacterial ooze flows in cross-sections (1,29). *R. solanacearum* produces exopolysaccharides (EPS) that plug the xylem vessels of plants, which leads to wilt symptoms (7,11,12,30).

Recently, we observed severe bacterial wilt on hot pepper, tomato, potato, eggplant, sesame (*Sesamum indicum* L.), perilla (*Perilla frutescens* var. *japonica* Hara), and sunflower (*Helianthus annuus* L.) in Korea. As expected, we isolated *R. solanacearum* from many of the plants with typical wilt symptoms. In many cases, however, *R. solanacearum* was not present in the infected plant tissues; surprisingly, we isolated *Burkholderia glumae* from the infected plant tissues. *B. glumae* is reported to cause seedling rot in rice nursery boxes and rice grain rot in paddy fields (5,6,26). Rice grain rot occurs at the flowering stage, when temperature and moisture are high, and causes yield losses up to 34% (27,32). In Korea, the occurrence of rice grain rot is becoming more common, because recent summers in the southern Korean peninsula have been very hot and humid. The bacterium produces toxoflavin {1,6-dimethylpyrimidino[5,4-c]-1,2,4-tria-

zine-5,7(1H,6H)-dione: molecular weight = 193}, a 7-azapteridine antibiotic that is essential in the pathogenicity of rice seedling rot and grain rot (8,13,18,21,26).

Here, we report for the first time that *B. glumae* causes bacterial wilt that is symptomatically indistinguishable from the bacterial wilt caused by *R. solanacearum* in many field crops. We found that all *B. glumae* isolates from rice panicles and other field crops caused rice grain rot and wilting symptoms in tomato, sesame, perilla, eggplant, and hot pepper. In addition, we determined that toxoflavin is involved in inducing the wilting symptoms in the field crops tested.

MATERIALS AND METHODS

Isolation of the causative agent. Infected stem tissues of tomato, hot pepper, eggplant, potato, sesame, sunflower, and perilla plants were cut into small pieces (5 × 5 mm), surface-sterilized (1% sodium hypochlorite, 1 min), and then placed in sterile Eppendorf tubes containing 1 ml of sterile distilled water. The resulting suspensions were spread on CPG agar media (1 g casein hydrolysate, 10 g peptone, 5 g glucose, and 15 g agar per liter) and incubated at 28°C. Discolored rice panicles were surface-sterilized, ground with a mortar, and added to 1 ml of sterile distilled water. The suspensions were spread on Luria-Bertani (LB) broth agar plates (10 g tryptone, 5 g yeast extract, and 5 g NaCl per liter) and incubated at 37°C. After incubation for 2 to 3 days, single colonies were isolated and further purified by streaking them on CPG or LB agar plates. We named the isolates after the initial host plants: BGR, rice isolates; PW, hot pepper isolates; PERW, perilla isolates; POW, potato isolates; TW, tomato isolates; EW, eggplant isolates; SW, sunflower isolate; and SEW, sesame isolate.

Identification of the pathogen. Colony morphology was determined by growth on CPG, TZC (CPG medium containing 50 mg of triphenyl tetrazolium chloride per liter) (14), or KB (20 g protease peptone, 1.5 g K₂HPO₄, 1.5 g MgSO₄·7H₂O, 15 ml glycerol, and 15 g agar per liter) agar plates. Gram staining was performed as

Corresponding author: Ingyu Hwang
E-mail: ingyuh@snu.ac.kr

Accepted for publication 11 February 2003.

Publication no. D-2003-0519-02R
© 2003 The American Phytopathological Society

described by Schaad (22). To identify the bacterial isolates, nine isolates (BGR1, BGR12, PW30, PERW5, POW17, TW34, EW12, SW2, and SEW5) representative of the 142 isolates from different host plants were subjected to the Biolog system, fatty acid methyl ester analysis (GC-FAME), and 16S rRNA gene sequencing analysis.

The carbon source utilization profiles of the nine representative isolates were compared three times on Biolog microplates, as described by the manufacturer (Biolog GN MicroPlate; Biolog, Hayward, CA). After a 24- to 48-h incubation, the plates were read using the MicroLog 3-Automated Microstation system (Biolog). The bacteria were identified by comparison with the Microlog Gram-negative database (Version 4.0).

For fatty acid methyl ester analysis, the nine representative isolates were cultured on Trypticase soy broth (Becton Dickinson and Co., Franklin Lakes, NJ) agar at 28°C for 48 h, and the fatty acid methyl esters were extracted using a standard method (20). The fatty acids were analyzed with the Sherlock Microbial Identification System Version 2.11 (MIDI Inc., Newark, DE). The fatty acid methyl ester analyses of nine representative isolates were repeated three times.

16S rRNA gene sequencing was performed as follows. The 16S rDNA genes of nine representative isolates were amplified by the polymerase chain reaction (PCR) in 50- μ l reaction volumes containing 5 μ l of 10 $\times$ PCR buffer (Takara Bio Inc., Otsu, Japan), 5 μ l of each dNTP (2.5 mM, Takara), 1 μ l of each primer (100 pmol, 27mF: 5'-AGAGTTTGATCMTGGCTCAG-3', 1492mR: 5'-GGYTACCTTGTTACGA CTT-3'), 0.5 μ l of *Taq* polymerase (250 U/ μ l, Takara), and 2 μ l of the bacterial suspension (A_{600nm} = 0.1). PCR amplifications were performed in an automated thermal cycler (model PTC-150, Perkin-Elmer Cetus, Norwalk, CT) with an initial denaturing at 94°C for 5 min, followed by 29 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min, with a final extension at 72°C for 10 min. Amplified DNA was cloned into the *Sma*I site of pBluescript II (SK+) (Stratagene, Cedar Creek, TX) as described by Sambrook et al. (19). DNA sequencing was accomplished using an ABI3700 automated DNA sequencer (Applied Biosystems Inc., Foster City, CA). The DNA sequence data were analyzed using the BLAST program at the National Center for Biotechnology Institute (2).

Pathogenicity and host range tests.

Seeds of tomato (cv. Kwangsoo), hot pepper (cv. Nokkwang), eggplant (cv. Chugyang), perilla (cv. Gupo), and sesame (cv. Hwangbaek) were sown in 50-cell plastic trays (Hunngong Seeds, Seoul, Korea) filled with commercial peat mix soil (Baroco, Seoul Agriculture Materials Co., Seoul, Korea). The seedlings were grown in a greenhouse until they reached the five-

or six-leaf stage. To prepare an inoculum of the isolates and *R. solanacearum*, the bacteria were grown on CPG agar medium for 24 h at 37°C and 28°C, respectively, suspended in sterile distilled water, and adjusted to 10⁸ to 10⁹ CFU/ml. The roots of these plants were cut to 3 to 5 cm with sterile scissors and dipped in the bacterial suspension for 2 h. As a control, plants were treated with sterile water in the same way as those treated with bacterial cells. Inoculated plants were planted in plastic pots (15 cm) and kept in the greenhouse. All the plants were grown in a greenhouse

at 25 to 35°C under natural lighting throughout the growing season.

To fulfill Koch's postulates, the pathogen was isolated from the diseased parts as described above. Symptom development was recorded every day for 10 days using the following scale: no symptoms, 25% wilt symptoms, 50% wilt symptoms, 75% wilt symptoms, and 100% wilt symptoms.

Rice (*Oryza sativa* cv. Milyang 23) was grown in a greenhouse and inoculated at the flowering stage with bacterial suspension (10⁸ to 10⁹ CFU/ml) using an atomizer (Binks Wren airbrush; Binks, Glendale

Fig. 1. Bacterial wilt symptoms caused by *Ralstonia solanacearum* (A to D) and *Burkholderia glumae* (G to J) on hot pepper plants in a greenhouse and colony morphologies on different media. A, G, symptoms 5 days after inoculation; B, H, internal symptoms 5 days after inoculation; C, I, symptoms 14 and 7 days after inoculation, respectively; D, J, internal symptoms 14 and 7 days after inoculation, respectively; E, *R. solanacearum* colonies on CPG agar plates after a 3-day incubation at 28°C; F, *R. solanacearum* colonies on TZC agar plates after a 3-day incubation at 28°C; K, *B. glumae* colonies on CPG agar plates after a 3-day incubation at 28°C; L, *B. glumae* colonies on TZC agar plates after a 3-day incubation at 28°C (a, control; b, inoculated plants).

Fig. 2. Disease progression curves of bacterial wilt in hot pepper after inoculation of *Burkholderia glumae* isolates. Disease severity is the mean of wilt rating from 0 to 100%. The values plotted are means of three replications per experiment pooled over three experiments. Vertical bars indicate standard deviations.

Heights, IL). Inoculated rice plants were kept in the greenhouse, and when symptoms developed on the panicles, the pathogen was isolated as described above to complete Koch's postulates. The degree of disease of the tested rice plants was evaluated daily for 10 days, as described by Iiyama et al. (13), using the following scale: 0 = healthy panicle, 1 = panicle 0 to 20% discolored, 2 = panicle 20 to 40% discolored, 3 = panicle 40 to 60% discolored, 4 = panicle 60 to 80% discolored, 5 =

panicle 80 to 100% discolored. The degree of disease was determined using: Disease degree = $\Sigma(\text{number of samples per score} \times \text{score}) / \text{total number of panicles}$. The pathogenicity tests were repeated three times with three replications.

To investigate the host range of the bacteria, 22 isolates (BGR1, BGR7, BGR8, BGR12, PW20, PW27, PW30, PW45, PERW1, PERW5, PERW7, POW17, POW36, POW59, TW34, TW45, TW72, EW10, EW12, EW13, SW2, and SEW5)

Table 1. Fatty acid compositions of total membrane lipid extracts from *Burkholderia glumae* isolates and *Ralstonia solanacearum*

Fatty acid	Fatty acids in total membrane lipid extract (%)		
	Range of nine isolates ^a	<i>B. glumae</i> ^b	<i>R. solanacearum</i> ^b
14:0	2.91 - 3.72	3.2	4.5
16:0	15.53 - 22.64	16.4	25.1
17:0 cyclo	12.40 - 14.55	8.7	4.5
16:1 2-OH	0.44 - 0.80	1.0	3.6
16:0 2-OH	0.49 - 1.56	0.9	0.7
16:0 3-OH	4.12 - 4.41	3.7	
18:0	0.71 - 1.38	0.8	0.5
18:1 2-OH	3.90 - 3.93	3.5	4.6

^a Isolates: BGR1, BGR12, PW30, PERW5, POW17, TW34, EW12, SW2, and SEW5.

^b Described by Stead (25).

Fig. 3. Bacterial wilt and rice grain rot caused by *Burkholderia glumae* isolates on various crops: **A**, hot pepper; **B**, tomato; **C**, eggplant; **D**, perilla; **E**, sesame; **F**, rice. (a, control; b, BGR1; c, LT389-2; d, PW30; e, LT479). All plants tested were grown in a greenhouse at 25 to 35°C under natural lighting. Photographs were taken 7 days after inoculation.

from different hosts were inoculated on hot pepper, tomato, eggplant, sesame, perilla, and rice plants, as described above. Disease severity was evaluated 10 days after inoculation, as described above. Synthetic toxoflavin was dissolved in distilled water, diluted to 25, 10, 5, and 2.5 µg/ml, and used to treat hot pepper, tomato, eggplant, sesame, and perilla roots cut to 3 to 5 cm with sterile scissors by dipping for 2 h. As a control, plants were treated with sterile water in the same way as those treated with toxoflavin. Disease severity was evaluated daily for 7 days after inoculation, as described above.

Identification of toxoflavin. The nine representative isolates were precultured in a test tube containing 10 ml of LB broth at 37°C for 1 day. Then the bacterial suspension was inoculated into 1 liter of LB broth and bacteria were grown at 37°C for 36 h with shaking. Culture supernatants were extracted three times with a half volume of chloroform. The chloroform extracts were concentrated *in vacuo* using a rotary evaporator at less than 40°C. The residue was dissolved in a small volume of methanol, and then developed on a silica gel 60 TLC plate (MERCK, Darmstadt, Germany) with chloroform:methanol (95:5 vol/vol). The silica-gel powder was collected from the dark orange area [$R(f) = 0.25$ to 0.35] and eluted with methanol. The eluate was concentrated and subjected to TLC with ethylacetate:chloroform:acetic acid (20:8:1 vol/vol/vol). Then the orange zone was scraped from the plate and eluted with methanol. The eluate was filtered through a 0.2-µm PVDF syringe filter (Whatman, Kent, UK) to remove any ash. After storage at -20°C for 10 days, crystals formed in the cold methanol. The crystals were analyzed with electrospray ionization mass spectrometry (ESIMS) (model JMS-LC mate, JEOL Ltd., Tokyo, Japan) using a direct inlet system. The spectra obtained were compared with that of synthetic toxoflavin synthesized previously (18,31).

Mutagenesis of BGR1 and PW30. The BGR1 and PW30 strains were mutagenized with Tn5lacZ to isolate toxoflavin-deficient mutants as described by Simon et al. (23). Mutagenized colonies were screened for the ability to produce toxoflavin on CaPG medium (potato decoction 1 liter: 200 g of peeled, sliced potatoes in 1 liter of distilled water, 5 g peptone, 5 g glucose, 1.4 g CaCl₂·2H₂O, and 20 g agar, pH 6.8) as determined by production of the yellow pigment characteristic of toxoflavin (18). The toxoflavin-deficient phenotype was confirmed by TLC analysis, as described above.

RESULTS

Isolation of bacteria from diseased specimens. Severe symptoms of bacterial wilt were observed on plants in many different locations in Korea in 2000 and 2001. Typical wilt symptoms were indistinguish-

able from the bacterial wilt caused by *R. solanacearum* when inoculated in the greenhouse (Fig. 1). In both cases, the vascular tissues in the stems appeared healthy in the initial stage of symptom development, but turned light brown as the symptoms progressed (Fig. 1). In most diseased specimens, bacterial ooze leaked when the vascular tissues were cut. The *B. glumae* colonies on CPG plates were circular, entire, raised, and creamy. Although some of the bacterial colonies appeared similar to those of *R. solanacearum*, they were very mucoid and produced green pigment (Fig. 1). We observed typical colonies of *R. solanacearum* on TZC plates; these were white with a light red area at the center of the colony.

We obtained 106 isolates that produced green pigment on CPG plates from symptomatic plants from 65 different locations: 17 isolates from hot pepper, 34 from tomato, 37 from potato, 8 from eggplant, 7 from perilla, and 1 each from sesame, sunflower, and paprika. The plants were found in seven provinces in Korea: Kyonggi, Chungbuk, Chungnam, Jeonbuk, Jeonnam, Gyeongbuk, and Gyeongnam. Rice grains with symptoms of grain rot were collected from throughout Korea during the rice-growing season in 2001. The 36 rice isolates were isolated from panicles collected from 26 different locations. These rice panicles exhibited a range of symptoms, varying from slightly discolored to brown and blotchy.

Identification of the bacteria. All the isolates were gram negative and did not produce fluorescent pigment on KB agar plates. All the isolates except BGR12 produced green pigment on CPG or TZC agar plates (Fig. 1). The nine representative isolates were oxidase positive and induced hypersensitivity on tobacco plants (data not shown). L-arabinose, D-arabitol, D-fructose, L-fucose, D-glucose, D-mannitol, D-sorbitol, D-trehalose, mono-methyl succinate, formic acid, quinic acid, succinic acid, L-alanine, and L-serine were utilized as carbon sources. The isolates could not utilize dextrin, lactose, D-raffinose, L-rhamnose, sucrose, or thymidine. These characteristics were very similar to those of *B. glumae* (15). The Biolog database placed the isolates closest to *B. glumae* with similarity from 83 to 99%. The fatty acids of the isolates were composed of 14:1, 16:0, 17:0 cyclo, 16:1 2-OH, 16:0 2-OH, 16:0 3-OH, 18:0, and 18:1 2-OH, and the fatty acid composition of the strains was closely related to that of *B. glumae* (Table 1). The 16S rRNA sequences showed the highest homology (99%) to the 16S rRNA sequences of *B. glumae* (GenBank accession no. AY224131). Based on these results, the isolates that we tested were identified as *B. glumae*.

Pathogenicity and host range. When the 22 isolates were inoculated on tomato, hot pepper, eggplant, perilla, and sesame

plants, wilt symptoms first appeared on the lowest leaves 3 days after inoculation, except with BGR12. Five days after inoculation, symptoms included wilting to one side, and the nodes of the stems of inoculated plants were bent. The disease severity was 50 to 75%. One of the disease progress curves from the various crops is shown in Figure 2. The symptoms were identical to

those observed on diseased plants in the field. The plants wilted completely, and the vascular tissues of the stem turned brown 7 days after inoculation (Fig. 3). When the isolates were inoculated into rice, panicle discoloration appeared after 3 days (disease index: 1.2). The number of diseased panicles increased gradually (disease index: 3.4), and most of the panicles of the

Fig. 4. Toxoflavin analysis produced by *Burkholderia glumae* isolates. **A**, TLC analysis of toxoflavin produced by *B. glumae* isolates (a, synthetic toxoflavin; b, BGR1; c, BGR12; d, PW30; e, PERW5; f, POW17; g, TW34; h, EW12; i, SW2; j, SEW5; k, LT389-2; l, LT479). Arrow indicates location of toxoflavin. **B**, Electrospray ionization mass spectrometry spectra of synthetic (a) and purified (b) toxoflavin produced by the BGR1 isolate. The toxin was analyzed using a direct inlet system and gave M+1 ion at m/z 194 and fragment ions at m/z 169, 137, and 109.

rice plants tested were discolored after 10 days (disease index: 4.6) (Fig. 3). Toxoflavin-deficient mutants of BGR1 and PW30 did not cause bacterial wilt symptoms on any plants (Fig. 3). Isolate BGR12 induced weak grain rot symptoms on rice (data not shown). Figure 3 shows representative results of our pathogenicity assays; all isolates, regardless of their origin, were pathogenic on tomato, sesame, perilla, eggplant, hot pepper, and rice. This indicates that the *B. glumae* isolates tested were highly pathogenic and had no host specificity. Control plants treated with sterile water did not show any symptoms. Similar results were seen in three repeated experiments.

Identification of toxoflavin and its role in pathogenicity. After mutagenesis of BGR1 and PW30 with Tn5lacZ, toxoflavin-deficient mutants that did not produce green pigment on the CaPG plates were isolated. Toxoflavin-deficient mutants LT389-2 and LT479 were chosen for further analysis as BGR1 and PW30 derivatives, respectively. The mutants were phototropic, and colony morphology was identical to that of the parental isolate (data not shown). Isolate BGR12, mutant LT389-2, and LT479 did not produce toxoflavin, whereas the nine representative

isolates produced the yellow compound analyzed by TLC. The synthetic toxoflavin migrated to the same position as the yellow compound [$R(f) = 0.3$] on the TLC plates (Fig. 4A). The mass spectrum of the yellow compound revealed a molecular ion at m/z 194 ($M+1$) and characteristic fragment ions at m/z 169, 137, and 109 in the ESIMS spectrum using a direct inlet system. Synthetic toxoflavin possessed the same ESIMS fragmentation patterns (Fig. 4B). These results demonstrated that the yellow compound produced by the nine representative isolates was toxoflavin.

When tomato, sesame, perilla, eggplant, and hot pepper plants were treated with toxoflavin, concentrations of 10 and 25 $\mu\text{g/ml}$ produced wilt symptoms 1 day after inoculation (disease severity: 25 to 50%). Three days after inoculation, the plants wilted completely (disease severity: 75 to 100%) (Fig. 5). At 5 $\mu\text{g/ml}$, wilt appeared 3 days after inoculation and the inoculated plants died 7 days after inoculation. Weak wilt symptoms appeared at 2.5 $\mu\text{g/ml}$ 5 days after inoculation and the disease severity was 25% 7 days after inoculation.

DISCUSSION

Bacterial wilt caused by *R. solanacearum* occurs in Korea most years and dam-

ages important solanaceous crops when the summers are hot and humid (16,17,28). Therefore, it is natural for farmers and plant bacteriologists to believe that *R. solanacearum* is responsible for all bacterial wilt in Korea. However, this work shows that other causes of bacterial wilt are frequently overlooked. From wilted plants, we isolated bacterial colonies unlike typical *R. solanacearum* colonies and identified the bacterial pathogen as *B. glumae*, which causes rice grain rot. Recently, we have observed bacterial grain rot in rice in Korea (4,24). However, it was not known that *B. glumae* causes bacterial wilt in other plants, and this is the first report that *B. glumae* causes bacterial wilt in tomato, sesame, perilla, eggplant, and hot pepper. The fact that we isolated *B. glumae* from various crops in many locations throughout Korea indicates that the bacterium is widespread.

It is very difficult to diagnose bacterial wilt caused by *B. glumae* in the field because the symptoms are very similar to those of the bacterial wilt caused by *R. solanacearum*. The easiest, most practical way to distinguish whether bacterial wilt is caused by *R. solanacearum* or *B. glumae* is to isolate the causal bacteria and grow them on CPG plates. Green pigment is produced around colonies of *B. glumae*, and the colonies are very mucoid, whereas *R. solanacearum* colonies do not produce this pigment and are creamy.

A major factor involved in bacterial wilt caused by *R. solanacearum* and *P. stewartii* subsp. *stewartii* is the EPS produced by the pathogens (3,7,12,30). The fact that toxoflavin-deficient mutants lost the ability to cause wilt in tomato, sesame, perilla, eggplant, and hot pepper, while synthetic toxoflavin caused wilt at 10 $\mu\text{g/ml}$ concentration 1 day after inoculation, is consistent with toxoflavin being the factor causing wilt symptoms in hot pepper, tomato, sesame, perilla, and eggplant infected by *B. glumae*. To our knowledge, no bacterial toxins involved in inducing wilt symptoms in various field crops have been reported. Toxoflavin has a relatively broad host range as indicated by our tests on tomato, sesame, perilla, eggplant, and hot pepper. Toxoflavin has antibacterial, antifungal, and herbicidal activities (18). Its mechanism of herbicidal activity is unclear; however, it may cause wilt symptoms via a common mode of action. Toxoflavin is also toxic to mice, causing hematuria, diarrhea, and lachrymation, with an intravenous LD_{50} of 1.7 mg/kg and an oral LD_{50} of 8.4 mg/kg (18). However, the phytotoxic mechanism by which toxoflavin causes wilt symptoms needs to be determined.

Since the optimum growth of the bacterium occurs at 37°C, the weather conditions during summer in Korea are very favorable for bacterial multiplication. Therefore, we believe that this disease has the potential to occur epidemically in Ko-

Fig. 5. Bacterial wilt symptoms caused by toxoflavin on various crops. **A**, hot pepper; **B**, tomato; **C**, eggplant; **D**, perilla; **E**, sesame plants. (a, control; b, toxoflavin (25 $\mu\text{g/ml}$); c, toxoflavin (10 $\mu\text{g/ml}$); d, toxoflavin (5 $\mu\text{g/ml}$); e, toxoflavin (2.5 $\mu\text{g/ml}$). All plants tested were grown in a greenhouse at 25 to 35°C under natural lighting. Plants were photographed 4 days after inoculation.

rea, especially when field crops and rice are cultivated in close proximity in the same growing period. We do not have any direct evidence that rice panicles infected by *B. glumae* can serve as an inoculum for bacterial wilt in field crops or vice versa. However, it seems likely that cross infection between field crops and rice occurs in the field when many different crops are grown near paddy fields during the summer. Since this disease is highly dependent on weather conditions, the ecological aspects of its occurrence need to be studied in order to manage it effectively.

ACKNOWLEDGMENTS

This work was supported by grant no. CG1411 from the Crop Functional Genomics Center of the 21st Century Frontier Research Program, which is funded by the Ministry of Science and Technology of the Republic of Korea. Y. J., J. K., S. K., and Y. K. are recipients of graduate fellowships from the Ministry of Education as part of the Brain Korea 21 Project.

LITERATURE CITED

- Agrios, G. N. 1997. Plant Pathology 4th ed. Academic Press, San Diego, CA.
- Altschul, S. F., Gish, W., Miller, W., Myerw, E. W., and Lipman, D. J. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403-410.
- Braun, E. J. 1990. Colonization of resistant and susceptible maize plants by *Erwinia stewartii* strains differing in exopolysaccharide production. *Physiol. Mol. Plant Pathol.* 36:363-379.
- Cha, K. H. 1995. Occurrence of *Pseudomonas glumae* and its control. *Plant Dis. Agric.* 1:14-18.
- Cottyn, B., Cerez, M. T., Van Outryve, M. F., Barroga, J., Swings, J., and Mew, T. W. 1996. Bacterial diseases of rice. I. Pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the Philippines. *Plant Dis.* 80:429-437.
- Cottyn, B., Van Outryve, M. F., Cerez, M. T., De Cleene, M., Swings, J., and Mew, T. W. 1996. Bacterial diseases of rice. II. Characterization of pathogenic bacteria associated with sheath rot complex and grain discoloration of rice in the Philippines. *Plant Dis.* 80:438-445.
- Denny, T. P. 1995. Involvement of bacterial polysaccharides in plant pathogenesis. *Annu. Rev. Phytopathol.* 33:173-197.
- Furuya, N., Iiyama, K., Shiozaki, N., and Matsuyama, N. 1997. Phytotoxin produced by *Burkholderia gladioli*. *J. Fac. Agric. Kyushu Univ.* 42:33-37.
- Graham, J., Jones, D. A., and Lloyd, A. B. 1979. Survival of *Pseudomonas solanacearum* race 3 in plant debris and in latently infected potato tubers. *Phytopathology* 69:1100-1103.
- Hayward, A. C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.* 29:65-87.
- Hendrick, C. A., and Sequeira, L. 1984. Lipopolysaccharide-defective mutants of the wilt pathogen *Pseudomonas solanacearum*. *Appl. Environ. Microbiol.* 48:94-101.
- Huang, Q., and Allen, C. 2000. Polygalacturonases are required for rapid colonization and full virulence of *Ralstonia solanacearum* on tomato plants. *Physiol. Mol. Plant Pathol.* 57:77-83.
- Iiyama, K., Furuya, N., Takanami, Y., and Matsuyama, N. 1995. A role of phytotoxin in virulence of *Pseudomonas glumae*. *Ann. Phytopathol. Soc. Jpn.* 61:470-476.
- Kelman, A. 1954. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetraxolium medium. *Phytopathology* 44:693-695.
- Krieg, N. R., and Holt, J. G., eds. 1984. *Bergey's Manual of Systematic Bacteriology*. 9th ed. Williams and Wilkins Co., Baltimore.
- Lim, Y. S., and Kim, B. S. 1994. Resistance to bacterial wilt in pepper (*Capsicum annuum* L.). *Korean J. Plant Pathol.* 10:73-77.
- Mew, T. W., and Ho, W. C. 1977. Effect of soil temperature on resistance of tomato cultivars to bacterial wilt. *Phytopathology* 67:909-911.
- Nagamatsu, T. 2001. Syntheses, transformation, and biological activities of 7-azapteridine antibiotics: Toxoflavin, fervenu-lin, reumycin and their analogs. *Recent Res. Devel. Org. Bioorg. Chem.* 4:97-121.
- Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. *Molecular Cloning: A Laboratory Manual*. 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- Sasser, M. 1997. Identification of bacteria by gas chromatography of cellular fatty acids. Technical note #101. MIDI, Newark, DE.
- Sato, Z., Koiso, Y., Iwasaki, S., and Matsuda, I. 1989. Toxins produced by *Pseudomonas glumae*. *Ann. Phytopathol. Soc. Jpn.* 55:356-359.
- Schaad, N. W., ed. 1988. *Laboratory Guide for Identification of Plant Pathogenic Bacteria*. American Phytopathological Society, St. Paul, MN.
- Simon, R., Priefer, V., and Puhler, A. 1983. A broad host range mobilization system for in vivo genetic engineering: Transposon mutagenesis in Gram negative bacteria. *Biol. Technol.* 1:784-791.
- Song, W., and Kim, H. 1999. Current status of bacterial grain rot of rice in Korea. *Plant Dis. Agric.* 5:1-7.
- Stead, D. E. 1992. Grouping of plant-pathogenic and some other *Pseudomonas* spp. by using cellular fatty acid profiles. *J. Syst. Bacteriol.* 42:281-295.
- Suzuki, F., Zhu, Y., Sawada, H., and Matsuda, I. 1998. Identification of proteins involved in toxin production by *Pseudomonas glumae*. *Ann. Phytopathol. Soc. Jpn.* 64:75-79.
- Tsushima, S., Naito, H., and Koitabashi, M. 1996. Population dynamics of *Pseudomonas glumae*, the causal agent of bacterial grain rot of rice, on leaf sheaths of rice plants in relation to disease development in the field. *Ann. Phytopathol. Soc. Jpn.* 62:108-113.
- Tung, P. X., Rasco, E., Zaag, P. V., and Schmiediche, P. 1990. Resistance to *Pseudomonas solanacearum* in the potato: II. Aspects of host-pathogen-environment interaction. *Euphytica* 45:211-215.
- Vasse, J., Frey, P., and Trigalet, A. 1995. Microscopic studies of intercellular infection and protoxylem invasion of tomato roots by *Pseudomonas solanacearum*. *Mol. Plant-Microbe Interact.* 8:241-251.
- Xu, P., Iwata, M., Leong, S., and Sequeira, L. 1990. Highly virulent strains of *Pseudomonas solanacearum* that are defective in extracellular polysaccharide production. *J. Bacteriol.* 172:3946-3951.
- Yoneda, F., and Nagamatsu, T. 1975. A convenient synthesis of toxoflavins, toxoflavin 4-oxides and 1-demethyltoxoflavins. *Chem. Pharm. Bull.* 23:2001-2009.
- Yoshimura, D., Yoshida, K., and Otofujii, M. 1987. Effect of meteorological factors on the occurrence of rice bacterial grain rot. *Proc. Assoc. Plant Prot. Kyushu* 33:9-12.