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Role of Phytotoxins in the Pathogenesis of *Burkholderia* Species

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Relationship between phytotoxin production and pathogenicity in *Burkholderia* spp. was investigated. Tropolone productive strains of *B. plantarii* and *B. vandii* were pathogenic to rice seedlings, gladiolus leaves and onion bulbs. Similarly, toxoflavin productivity correlated with pathogenicity of bacterial isolates to all plants used *i.e.* rice seedlings, dendrobium, cymbidium, gladiolus leaves, potato tubers and onion bulbs in *B. glumae* and *B. gladioli*. Treatment of these plants with tropolone caused damages on rice seedlings and onion bulbs, whereas no damage was observed on gladiolus leaves. Toxoflavin treatment gave damages on rice seedlings, leaves of orchidaceous plants, gladiolus and onion bulbs, but no distinct damage was shown on potato tuber. Some unknown factor(s) other than phytotoxin will also be associated with virulence in *Burkholderia* spp.

INTRODUCTION

Phytotoxin production has been known to be an important element of virulence in many plant pathogens (Gross *et al.*, 1985; Bender, 1995). *Burkholderia* (synonym, *Pseudomonas*) *glumae* and *B. plantarii*, the causal agents of rice diseases, produce phytotoxic toxoflavin (Sato *et al.*, 1988; Iiyama *et al.*, 1994) and tropolone (Azegami *et al.* 1985), respectively. These phytotoxins are closely related to virulence to rice plants (Iiyama *et al.*, 1995, Azegami *et al.*, 1985). Moreover, it was found that some strains of *B. gladioli*, which is closely related to *B. glumae*, *B. plantarii* and *B. vandii* for bacteriological characteristics, also produce toxoflavin (Matsuda *et al.*, 1990; Furuya *et al.*, 1997). Although these bacterial pathogens of rice plant are known to be pathogenic to various plants such as gladiolus and orchidaceous plants, a limited amount of information exists on the role of phytotoxin in pathogenicity. In this paper, relationship between phytotoxin production and pathogenicity in *Burkholderia* spp. is discussed.

MATERIALS AND METHODS

Bacterial strains

Strains of plant pathogenic *Burkholderia* spp. used in this experiment were originally donated or purchased from various institutes and were maintained in our laboratory. Details of the strains are presented in Table 1.

Table 1. Relationship between phytotoxin productivity and pathogenicity to various plants.

Burkholderia spp.	Origin[1]	Source	Productivity[2] of toxoflavin (μg/ml)	Productivity[2] of tropolone (μg/ml)	Pathogenicity[3] to rice	dendrobium	cymbidium	gladiolus	potato	onion
B. gladioli pv. *gladioli*										
ATCC10248^T	ATCC	Gladiolus	1.9	0.0	+	+	+	+	−	+
MAFF301580	MAFF	Dendrobium	6.9	0.0	+	+	+	+	+	+
MAFF302515	MAFF	Tulip	3.7	0.0	+	+	+	+	+	+
MAFF302537	MAFF	Onion	15.3	0.0	+	+	+	+	+	+
MAFF302544	MAFF	Rice	16.8	0.0	+	+	+	+	+	+
MAFF301064	MAFF	Freesia	<0.01	0.0	+	−	−	−	−	−
NIAES1065	NIAES	Freesia	0.0	0.0	−	−	−	−	−	−
MAFF301728	MAFF	Vanda	0.0	302.5	+	−	−	+	−	+
B. gladioli pv. *alliicola*										
ATCC19302	ATCC	Onion	1.0	0.0	±	+	+	+	−	+
B. gladioli pv. unidentified										
MAFF302409	MAFF	Adzuki bean	9.9	0.0	+	+	+	+	+	+
MAFF302418	MAFF	Green gram	11.8	0.0	+	+	+	+	+	+
MAFF302424	MAFF	Cymbidium	13.5	0.0	+	+	+	+	+	+
B. glumae										
MAFF301169^T	MAFF	Rice	10.8	0.0	+	+	+	+	+	+
N7501	NIAS	Rice	1.0	0.0	+	+	+	+	+	+
2	KNAES	Rice	9.2	0.0	+	+	+	+	+	+
Ku8112	KU	Rice	4.3	0.0	+	+	+	+	+	+
Ku8104	KU	Rice	0.0	0.0	−	−	−	−	−	−
N7503	NIAS	Rice	0.0	0.0	−	−	−	−	−	−
N7504	NIAS	Rice	0.0	0.0	−	−	−	−	−	−
B. plantarii										
AZ8201^T	NIAS	Rice	0.0	101.8	+	−	−	+	−	+
MAFF302387	MAFF	Rice	0.0	221.8	+	−	−	+	−	+
MAFF302484	MAFF	Rice	0.0	94.6	+	−	−	+	−	+
B. vandii										
JCM7957^T	JCM	Vanda	0.0	194.6	+	−	−	+	−	+

1) Abbreviations of culture collections: ATCC, American Type Culture Collection; MAFF, Ministry of Agriculture, Forestry and Fisheries; NIAES, National Institute of Agro-Environmental Sciences; NIAS, National Institute of Agricultural Sciences; KNAES, Kyushu National Agricultural Experiment Station; KU, Kyushu University; JCM, Japan Collection of Microorganisms.

2) Concentrations of toxoflavin and tropolone were estimated by measuring absorbance of organic extracts from each culture at 260 and 320 nm, respectively.

3) Each strain was inoculated to rice seedlings, leaves of dendrobium, cymbidium, gladiolus, onion bulbs and potato tubers. Details are given in the text (Materials and Methods).

Phytotoxin productivity

To ascertain the productivity of toxoflavin, each bacterium was inoculated on a slant of yeast peptone dextrose agar (YPDA; peptone 0.6 g, yeast extract 3.0 g, D-glucose 3.0 g, agar 15 g in 1,000 ml distilled water, pH 7.2), and cultured at 30 °C for 3 days. Toxoflavin secreted in the slant was extracted with chloroform for three times. The chloroform extract was evaporated *in vacuo*, and the residue was dissolved in 80% aqueous methanol and absorbance at 260 nm (A_{260}) was measured. Concentration of toxoflavin

was determined using a following formula: Concentration (μg/ml) = $4.1 \times A_{260}$.

Tropolone productivity of each strain was estimated according to the method of Azegami *et al* (1994). Briefly, each strain was shake-cultured at 30°C for 3 days in Ayers broth supplemented with D-glucose (AG; $NH_4H_2PO_4$ 1.0 g, KCl 0.2 g, $MgSO_4 \cdot 7H_2O$ 0.2 g, D-glucose 10.0 g in 1,000 ml of distilled water, pH 7.0). Tropolone was extracted with ethylacetate and absorbance at 320 nm (A_{320}) was measured. Concentration of tropolone was decided using a following formula: Concentration (μg/ml) = $100 \times A_{320}/15.6$.

Pathogenicity tests

Each bacterium was tested for pathogenicity to rice seedlings (*Oryza sativa*, cv. "Asominori"), gladiolus (*Gladiolus gandavensis*, cultivar unknown), cymbidium (*Cymbidium* sp., cv. "Pine Clash Moon Venus"), dendrobium (*Dendrobium* sp., cv. "Yukidaruma Queen"), slices of potato tuber (*Solanum tuberosum*, cv. "May Queen") and scaly bulbs of onion (*Allium cepa*, cultivar unknown). Inocula were prepared by suspending bacterial cells, which were cultured at 30°C for 2 days on YPDA, in sterilized distilled water so as to be a concentration of 10^8cfu/ml. Inoculation to leaves of gladiolus, cymbidium and dendrobium was performed by pricking with a pin dipped in the inocula. The inoculated plants were placed in an inoculation chamber under high humidity and dark conditions at 30°C for 48 h, then transferred to an air-conditioned greenhouse at 25–30°C. Slices of potato tuber and scaly bulbs of onion were inoculated and placed in Petri dishes at 30°C to test soft rotting potential of bacterium. The degree of rotting was evaluated periodically for 3 days. Rice seedlings were inoculated as previously reported (Iiyama *et al.*, 1995), and the pathogenicity was determined at 10 th day after inoculation.

The damages of plants by phytotoxin treatment

Damages on dendrobium, gladiolus and cymbidium leaves by treatments with toxoflavin and tropolone were tested. Solutions of biosynthetic toxoflavin and tropolone (Tokyo Kasei Chemical Co.) at different concentrations were prepared with 50% aqueous methanol. Detached leaves were pricked with a pin and 3μl each of the phytotoxin solution was dropped. The leaves were kept on moistened filter paper in Petri dishes. After incubation at 30°C for 2 days, the damages of leaves were examined.

RESULTS AND DISCUSSION

The relationship between phytotoxin production by *Burkholderia* spp. and their pathogenicity to plants was investigated. As shown in Table 1, all strains of *B. plantarii* and *B. vandii* produced tropolone. One strain (MAFF301728) which was originally identified as *B. gladioli* pv. *gladioli* produced tropolone, but this strain should be transferred to *B. vandii*, a novel species proposed by Urakami *et al.* in 1994 (Dr. Azegami's personal communication and our data). Toxoflavin was produced by most strains of *B. glumae* and *B. gladioli*.

In *B. glumae* and *B. plantarii*, a close correlation between phytotoxin productivity and their pathogenicity to rice plant was confirmed (Iiyama *et al.*, 1995, Azegami *et al.* 1994). As can be seen in Table 1, tropolone productive strain of *B. vandii* as well as *B. plantarii* also showed virulence to rice seedlings, gladiolus leaves and scaly bulbs of

Table 2. Damage of various plants by phytotoxin (tropolone of toxoflavin) treatment[1].

Phytotoxin	Concentration (μg/ml)	Damage of					
		rice	dendrobium	cymbidium	gladiolus	potato	onion
Tropolone	1	−[2]	−	−	−	−	−
	10	+	−	−	−	−	−
	100	+	−	−	−	−	+
Toxoflavin	1	−	+	+	+	−	−
	10	+	+	+	+	−	+
	100	+	+	+	+	−	+

1) Details are given in the text (Materials and Methods).
2) Index: +, Visible damage induced by phytotoxin treatment; −, Not induced.

onion. Similarly, toxoflavin productivity and pathogenicity to all plants tested correlated highly in *B. gladioli* except for the case of *B. gladioli* pv. *gladioli* MAFF301064 which was originally isolated from freesia. This isolate produced little toxoflavin on YPDA medium and was pathogenic only to rice seedlings but not to the other plants tested. In this strain, toxoflavin productivity highly depended on nutrients (data not shown).

Azegami *et al.* (1985) demonstrated the role of tropolone in virulence of *B. plantarii* to rice plants. As shown in Table 2, tropolone gave damages to rice seedlings and onion bulbs. However, gladiolus leaves treated with 100μg/ml tropolone were not damaged, though tropolone productive strains were pathogenic to gladiolus. Similarly, in the previous study the authors showed that toxoflavin is a virulence determinant of *B. glumae* in rice plants (Iiyama *et al.*, 1995). However, a destructive effect of these phytotoxins on other plants besides rice has not yet been clarified. Dendrobium, cymbidium, gladiolus leaves and onion bulbs treated with toxoflavin were damaged, whereas potato tubers were almost intact.

As described above, all the symptoms observed in infected plants were not always reproduced by the phytotoxin alone. Unknown factor(s) together with phytotoxin will also be associated with full expression of pathogenesis by *Burkholderia* species. Further research on the unknown factor(s) will be required.

REFERENCES

Azegami, K. 1994 Studies on bacterial seedling blight of rice. *Bull. Natl. Inst. Agro-Environ. Sci.*, **11**: 1-80 (in Japanese)

Azegami, K., K. Nishiyama, Y. Watanabe, T. Suzuki, M. Yoshida, K. Nose and S. Toda 1985 Tropolone as a root growth-inhibitor produced by a plant pathogenic *Pseudomonas* sp. causing seedling blight of rice. *Ann. Phytopath. Soc. Jpn.*, **51**: 315-317

Bender, C. L. 1995 Phytotoxin production in *Pseudomonas syringae In* "Plant Microbe Interactions" vol. 3, ed. by G. Stacey and N. T. Keen, International Thomson Publishing. pp. 124-144

Furuya, N., K. Iiyama and N. Matsuyama 1997 Phytotoxin produced by *Burkholderia gladioli*. *J. Fac. Agri., Kyushu Univ.*, **42**: 33-37

Gross, D. C. and Y. S. Cody 1985 Mechanisms of plant pathogenesis by *Pseudomonas* species. *Can. J. Microbiol.*, **31**: 403-410

Iiyama, K., N. Furuya, K. Hara, N. Nakashima, Y. Takanami and N. Matsuyama 1994 Phytotoxin produced by *Pseudomonas glumae* Kurita *et* Tabei, a causal bacterium of the grain and seedling rot

of rice. *J. Fac. Agri., Kyushu Univ.*, **38**: 175-178

Iiyama, K., N. Furuya, Y. Takanami and N. Matsuyama 1995 A role of phytotoxin in virulence of *Pseudomonas glumae* Kurita et Tabei. *Ann. Phytopath. Soc. Jpn.*, **61**: 470-476

Matsuda, I. and Z. Sato 1990 Toxoflavin production by strains of *Pseudomonas gladioli* isolated from different plants (Author translation). *Ann. Phytopath. Soc. Jpn.*, **56**: 403 (Abst., in Japanese)

Sato, Z., Y. Koiso, S. Iwasaki, I. Matsuda and A. Shirata 1989 Toxins produced by *Pseudomonas glumae*. *Ann. Phytopath. Soc. Jpn.*, **55**: 353-356 (in Japanese)

Urakami, T., C. Ito-Yoshida, H. Araki, T. Kojima, K. Suzuki and K. Komagata 1994 Transfer of *Pseudomonas plantarii* and *Pseudomonas glumae* to *Burkholderia* as *Burkholderia* spp. and description of *Burkholderia vandii* sp. nov. *Int. J. Syste. Bacteriol.*, **44**: 235-245