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Reaction of Tobacco and Rice Leaf Tissue Infiltrated with *Burkholderia glumae* or *B. gladioli*

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Reaction of tobacco and rice leaf tissue infiltrated with either strains of *Burkholderia glumae* or *B. gladioli* was investigated. Visual alteration areas of tobacco leaf at sites infiltrated with high concentration (more than 10^7 cells/ml) of the bacteria were categorized into two types, one causing necrosis with hypersensitive-like reaction (HLR) within 24 hr of infiltration, and the other developing only yellowish discoloration. Living bacterial cells and the toxoflavin were effective for necrosis induction, whereas heat-killed cells, bacterial lipopolysaccharide (LPS) and extracellular polysaccharide (EPS) were ineffective. In the case of *B. glumae*, necrosis occurred with toxoflavin producing strains. No necrotic lesions developed with toxoflavin non-producing strains. All strains of *B. gladioli* induced necrosis with water soaking lesions in tobacco leaf. Infiltration of toxoflavin producing strains of *B. glumae* and *B. gladioli* to rice leaf caused necrosis or a chlorotic spot on the leaf. A toxoflavin dose of more than 100μg/ml induced necrosis, suggesting close relationship between virulence to rice and necrosis inductivity to an uncongenial plant such as tobacco. Thus, the principle which induces HLR can be regarded as an essential attribute for pathogenicity. In contrast to the reaction of rice leaf toward toxoflavin, necrosis in tobacco leaf could not simply be a reaction to toxoflavin but rather to additional unknown factors.

INTRODUCTION

The hypersensitive reaction (HR) of tissues infected with fungi and viruses occurs widely in plants. The ability of bacteria to elicit HR was discovered by Klement and coworkers in 1963. Since this first report on the pseudomonads-tobacco combination, HR has been confirmed for various host- or uncongenial plant-bacteria combinations. To date, however, there are no reports on HR for *Burkholderia glumae* (or *B. gladioli*) and rice varieties or uncongenial tobacco cultivars. In the present study, the reaction of tobacco and rice plants to strains of *B. glumae* and *B. gladioli*, recognized as closely related bacteria, were investigated by the leaf infiltration technique to characterize pathological properties of these bacterial strains.

MATERIALS AND METHODS

Bacterial strains

Eighteen strains of *B. glumae* and twelve strains of *B. gladioli* were used (Table 1). Each strain was grown on potato semi-synthetic agar (PSA) medium containing 5 g

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Table 1. Strain of *B. glumae* and *B. gladioli* used in this experiment.

Strain	Origin	Source[a)]	Strain	Origin	Source
B. glumae			*B. gladioli* pv. *gladioli*		
Ku8104	Rice grain	KU	MAFF301064	Freesia	MAFF
Ku8105	〃	〃	MAFF301580	Dendrobium	〃
Ku8112	〃	〃	MAFF302515	Tulip	〃
Ku8113	〃	〃	MAFF302537	Onion	〃
Ku8114	〃	〃	MAFF302544	Rice	〃
8012	〃	KNAES	NIAS1064	Freesia	NIAS
8015	〃	〃	NIAS1065	〃	〃
8020	〃	〃	E-14	Rice	KU
N7401	Rice seedling	NIAS	ATCC10248^T	Gladiolus	ATCC
N7501	〃	〃			
N7504	〃	〃			
N7505	〃	〃	*B. gladioli* pv. unidentified		
2	Rice grain	KNAES	MAFF302409	Adzuki bean	MAFF
Ⅲ	〃	〃	MAFF302418	Green gram	〃
AZ8224	〃	Dr. Azegami	MAFF302424	Cymbidium	〃
AZ84448	〃	〃			
805	unknown	NIAS			
MAFF301169^T	Rice	MAFF			

a) Abbreviations for culture collections: KU, Kyushu University; KNAES, Kyushu National Agricultural Experiment Station; NIAS, National Institute of Agricultural Sciences; MAFF, Ministry of Agriculture, Forestry and Fisheries; ATCC, American Type Culture Collection.

peptone, 15 g sucrose, 2 g $Na_2HPO_4 \cdot 12H_2O$, 0.5 g Ca $(NO_3)_2 \cdot 4H_2O$, 15 g agar in 1,000 ml of potato (300 g) decoction, pH 7.0, at 30 °C for 48 hr.

Test plants

Tobacco plants (*Nicotiana tabacum* L. cvs. Bright Yellow, White Burley, Xanthi, Xanthi-nc and Judy's Pride) were grown at around 25 °C under greenhouse conditions. At the 8th to 9th leaf stages, the fully expanded third and fourth leaves were served for the infiltration assay. Rice plants (*Oryza sativa* cvs. Chugoku No. 45, Asominori, Aichiasahi, Koshihikari, Nipponbare, Kinmaze, Norin No. 29, Kuju and Rantai Emas, *O. nivara*, *O. barthii*, *O. eichingeri*, *O. glaberrima* and *O. punctata*) were grown under normal greenhouse conditions and fully expanded leaves were used for the infiltration assay.

Preparation of inoculum and infiltration into the leaf tissue

Bacterial cells of each strain grown on PSA were suspended in sterilized distilled water so as to be 10^9 cfu/ml approximately. The leaf was pin-pricked at a site to be infiltrated. Subsequently, bacterial suspension was infiltrated with a hypodermic syringe into the intercellular spaces through the wound. Five leaves, at least, were infiltrated with each strain. In addition living whole bacterial cells, killed cells, extracellular polysaccharide (EPS), lipopolysaccharide (LPS) and toxoflavin from *B. glumae* and *B.*

gladioli were also infiltrated. To prepare killed bacterial cells, heat-treatment, chloroform treatment and UV irradiation were carried out. To kill the bacterial cells by heating, 20 ml of each bacterial suspeusion of *B. glumae* or *B. gladioli* (conc. ca. 10^9 cfu/ml) was autoclaved at 121 °C for 20 min. Chloroform treatment was conducted as follows: 30 ml of bacterial suspension in a 500-ml beaker was incubated in a 2,000-ml beaker moistened with 100 ml of chloroform, and wrapped with aluminum foil. The suspension was exposed to chloroform vapor under gentle shaking for 4 hr. Five ml of the suspension was placed in a Petri-dish (9 cm in diameter) and irradiated under UV light (TOSHIBA GL 15W) at a distance of 30 cm for 4 hr. EPS and LPS extraction was performed as previously described by Evans, L. R. and Linker, A. (1973) and Lucas, L. T. and Grogan, R. G. (1969a and 1969b), respectively. Toxoflavin produced by *B. glumae* or *B. gladioli* was extracted according to the method as described previously (Iiyama *et al.*, 1994). The reaction of the infiltrated leaves was observed periodically up to 96 hr after infiltration under the greenhouse conditions.

RESULTS

Reaction of tobacco leaf tissue to bacterial infiltration

Tobacco leaves infiltrated with ca. 10^9 cfu/ml suspensions of toxoflavin producing strains induced necrosis resembling HR within 24 hr (Fig. 1 and Table 2). In the case of

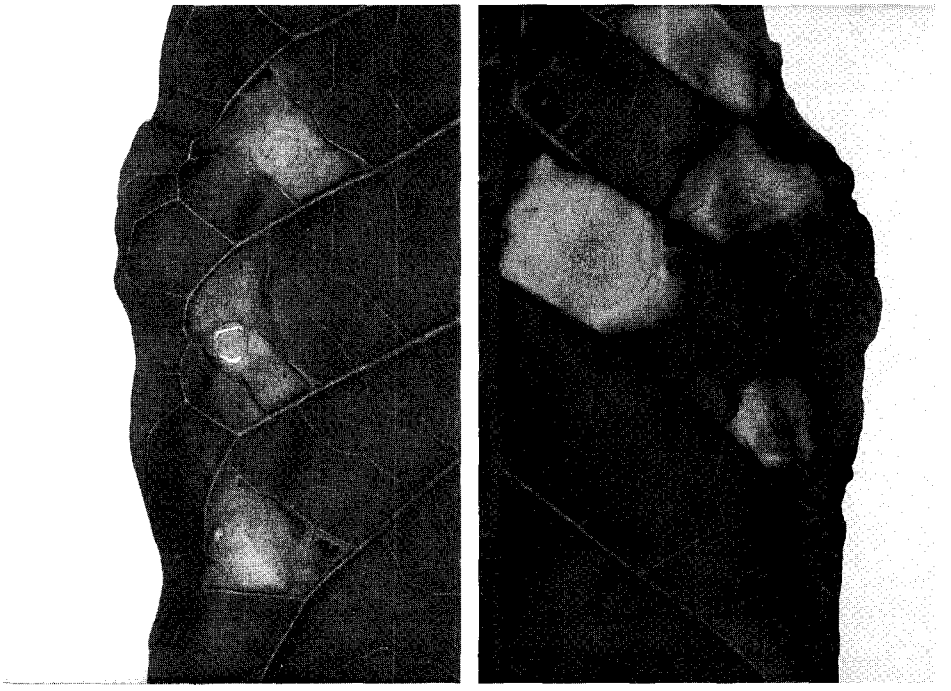

Fig. 1. Symptoms produced in tobacco leaves by *Burkholderia glumae* (left) and *B. gladioli* (right) at 24th hr after infiltration. Cell suspensions containing about 10^9 cfu/ml were infiltrated into each leaf segment.

B. gladioli, two toxoflavin non-producing strains (NIAS1065 and E-14) also induced necrosis in tobacco leaf tissue. On the other hand, yellowish discoloration appeared at areas infiltrated with toxoflavin non-producing strains of *B. glumae* up to 48th hr, but the same symptoms were not produced by toxoflavin producing strains. Infiltration of pure water did not cause the symptoms in any leaves.

Table 2. Relationship between toxoflavin production and reactions of tobacco leaf tissues (cv. Xanthi-nc) to infiltration with different strains of *B. glumae* or *B. gladioli*.

Strain[a]	Period after infiltration (hr)			Toxoflavin production[b]
	24	48	72	
B. glumae				
Ku8105	H[c]	B	B	+
Ku8112	B	B	B	+
Ku8113	B	B	B	+
Ku8114	B	B	B	+
8012	B	B	B	+
8015	B	B	B	+
N7401	B	B	B	+
N7505	B	B	B	+
N7501	B	B	B	+
2	B	B	B	+
Ⅲ	B	B	B	+
AZ8224	B	B	B	+
AZ84448	B	B	B	+
MAFF301169[T]	B	B	B	+
Ku8104	H	C	C	−
8020	H	C	C	−
N7504	H	C	C	−
805	H	C	C	−
B. gladioli				
MAFF302515	B	B	B	+
MAFF302537	B	B	B	+
MAFF302544	B	B	B	+
MAFF301580	B	B	B	+
MAFF302418	B	B	B	+
MAFF302424	B	B	B	+
MAFF302409	B	B	B	+
MAFF301064	B	B	B	+
NIAS1064	B	B	B	+
ATCC10248[T]	B	B	B	+
NIAS1065	B	B	B	−
E-14	B	B	B	−

a) Concentration was ca. 10^9 cfu/ml.

b) Toxoflavin production was investigated as follows: each bacterium was inoculated on slant of YPDA and incubated at 30 °C for 3 days. Toxoflavin secreted in slant was extracted with chloroform. The chloroform extract was evaporated *in vacuo* and the residue was dissolved in 80% aqueous methanol. The sample was subjected to visible ultraviolet spectorometory and TLC.

c) H: healthy (no visible reaction), C: yellowish discoloration, B: brown necrotic lesion.

Effect of concentrations of infiltrated bacteria on reactions

To determine the critical concentration of bacteria for necrosis inductivity, bacterial suspensions of both *B. glumae* and *B. gladioli* were serially diluted and infiltrated into tobacco leaf tissue, respectively. Distinct necrosis was induced at sites infiltrated with concentrated suspensions (higher than 10^8 cfu/ml) within 24 hr. However, when bacterial concentration was lower than 10^7 cfu/ml, infiltrated areas had yellowish discoloration but necrosis did not occur even the 3rd day after infiltration. No visible reaction was observed when bacterial concentration was below 10^6 cfu/ml (Table 3).

Table 3. Reactions of tobacco leaf tissues (cv. Xanthi-nc) to infiltration of various concentrations of bacterial suspension.

Strain (Initial concentration)	Serial dilution	Period after infiltration (hr)		
		24	48	72
B. glumae 2 (1.4×10^9 cfu/ml)	10^0	B[a)]	B	B
	10^{-1}	B	B	B
	10^{-2}	C	C	C
	10^{-3}	H	H	H
	10^{-4}	H	H	H
	10^{-5}	H	H	H
	10^{-6}	H	H	H
B. gladioli MAFF302544 (1.4×10^9 cfu/ml)	10^0	B	B	B
	10^{-1}	B	B	B
	10^{-2}	H	C	C
	10^{-3}	H	H	H
	10^{-4}	H	H	H
	10^{-5}	H	H	H
	10^{-6}	H	H	H

a) See Table 2.

Response of leaves of various tobacco cultivars to *B. glumae* or *B. gladioli*

As shown in Table 4, all toxoflavin productive strains except type strain of *B. gladioli* induced necrosis in all five tobacco cultivars tested. In the case of toxoflavin non-

Table 4. Reactions of tobacco-leaf tissues of various cultivars to infiltration with toxoflavin producing and non producing strains of *B. glumae* or *B. gladioli*.

Tobacco cultivar	Reactions of tobacco leaf tissue					
	B. glumae			*B. gladioli*		
	2 (tox$^+$)[a)]	Ku8104 (tox$^-$)	MAFF 301169^T (tox$^+$)	MAFF 302544 (tox$^+$)	NIAS 1065 (tox$^-$)	ATCC 10248^T (tox$^+$)
Xanthi	B[b)]	H	B	B	B	C
Xanthi-nc	B	H	B	B	B	B
White Burley	B	H	B	B	C	C
Bright Yellow	B	H	B	B	B	C
Judy's Pride	B	C	B	B	C	C

a) tox$^+$: toxoflavin productivity, tox$^-$: non-toxoflavin productivity.
b) See Table 2.

producing strains, *B. glumae* Ku8104 induced chorosis in Judy's Pride but not in four other cultivars. Furthermore, *B. gladioli* NIAS1065 induced chlorosis in two cultivars, White Burley and Judy's Pride but induced necrosis in three other cultivars, Xathi, Xanthi-nc and Bright Yellow. Thus, *B. glumae* and *B. gladioli* showed variable levels of necrosis (or chlorosis) depending on the cultivars.

Reaction of rice leaf to bacterial infiltration

When rice leaves were infiltrated with high concentrations (more than ca. 10^8 cfu/ml) of toxoflavin producing strains, brownish necrosis occurred within 24 hr. The browning gradually became distinct, but the size of the lesion remained constant. Subsequently, the central area of necrosis changed from brown to white over time (Fig. 2). No necrosis, however, occurred at infiltration of toxoflavin non-producing strains. Moreover, no significant differences were observed in the response among various species and cultivars of rice (Table 5).

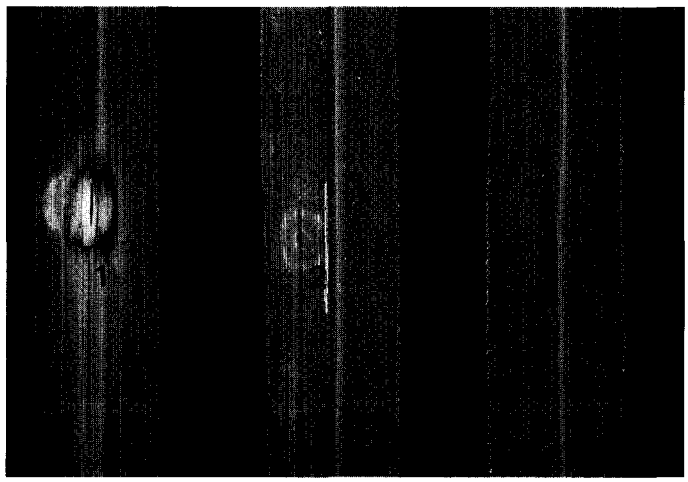

Fig. 2. Reactions of rice leaf tissues infiltrated with toxoflavin producing strain (1) and non-producing strain (2) at 48 hr after infiltration. (3): Control (Water).

Reaction of tobacco leaf tissue to infiltration with various inoculum preparations

To provide information concerning the roles of determinants in *B. glumae* and *B. gladioli* for induction of necrotic or yellow lesions in tobacco leaves, various inoculum preparations were infiltrated. Of preparations infiltrated, the fractions containing living cells and toxoflavin induced necrosis, but killed cells, EPS and LPS showed no critical effect on necrosis inductivity (Table 6).

Table 5. Reactions of rice leaf tissues to infiltration with toxoflavin producing and non-producing strains of *B. glumae* or *B. gladioli*.

Cultivar or species	Reactions of rice leaf tissue[a]			
	B. glumae		*B. gladioli*	
	2 (tox⁺)[b]	Ku8104 (tox⁻)	MAFF302544 (tox⁺)	NIAS1065 (tox⁻)
Oryza sativa				
Chugoku No.45	+	−	+	−
Asominori	+	−	+	−
Aichiasahi	+	−	+	−
Koshihikari	+	−	+	−
Nipponbare	+	−	+	−
Kinmaze	+	−	+	−
Norin No.29	+	−	+	−
Kuju	+	−	+	−
Rantai Emas	+	−	+	−
O. nivara	+	−	+	−
O. barthii	+	−	+	−
O. eichingeri	+	−	+	−
O. glaberrima	+	−	+	−
O. punctata	+	−	+	−

a) -: no visible reaction, +: albication or browning.
b) tox⁺: toxoflavin productivity, tox⁻: non-toxoflavin productivity.

Table 6. Reactions of tabacco leaf tissues (cv. Xanthi-nc) infiltrated with various preparations of strains[a] of *B. glumae* and *B. gladiloli*.

Preparation infiltrated[b]	Reactions of tabacco leaf tissues (5 days after infiltration)
Living bacteria (ca. 10^9 cfu/ml)	+[c]
Heated bacteria	−
UV irradiated bacteria	−
Chloroform treated bacteria	−
Extracellular polysaccharide solution (1,000 μg/ml)	−
Lipopolysaccharide solution (1,000 μg/ml)	−
Toxoflavin solution (1,000 μg/ml)	+

a) *B. glumae* strain 2 and MAFF301169^T, *B. gladiloli* strain MAFF302544 and ATCC10248^T were used.
b) See the text.
c) +: necrotic lesion, −: no visible reaction.

Reaction of tobacco and rice leaf tissue to infiltration of various concentrations of toxoflavin

As shown in Table 7 and Fig. 3, when the tobacco and rice leaves were infiltrated with high concentrations (more than 100 μg/ml) of purified toxoflavin, necrotic lesion was

induced. This reaction closely resembled symptoms caused by the living cells of toxoflavin producing strains. Thus, the concentration of toxoflavin affects the induction of the necrotic response.

Table 7. Reactions of tobacco and rice leaf tissues to infiltration with different concentrations of toxoflavin

Concentrations (μg/ml)	Reactions of leaf tissues	
	Tobacco (cv. Xanthi-nc)	Rice (cv. Asominori)
1000	B[a)]	B
100	B	B
10	H	B
1	H	H
0.1	H	H
Control	H	H

a) See Table 2.

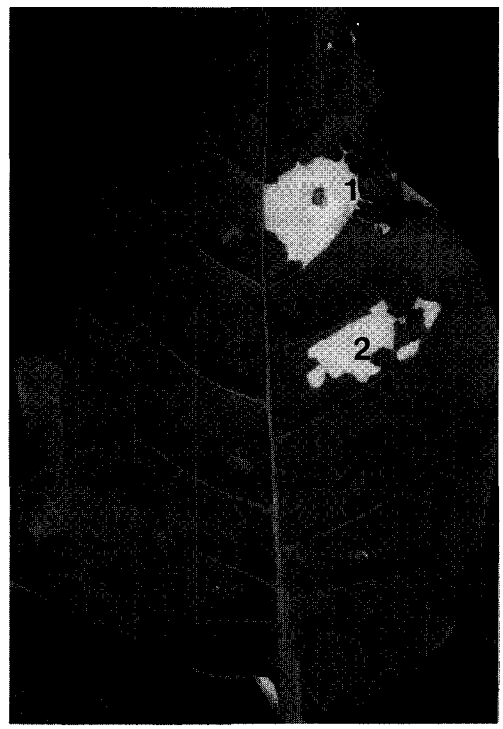

Fig. 3. Reactions of a tobacco leaf injected with different concentrations of toxoflavin. Toxoflavin was prepared as described in the text and infiltrated into tobacco leaf panels. Reactions were photographed 24 hr later. Panel 1: 1,000 μg/ml, 2: 100 μg/ml, 3: 10 μg/ml, 4: 1 μg/ml, 5: 0.1 μg/ml, 6: Water.

DISCUSSION

In our previous paper (Iiyama *et al.*, 1995), it was clearly demonstrated that the producing of toxoflavin by *B. glumae* is closely related to the virulence of the bacterium to rice plants. All toxoflavin producing strains were virulent to rice plants with a few exceptions, whereas all toxoflavin non-producing strains were avirulent.

In the present study, infiltration of virulent strains (toxoflavin producing strains) of *B. glumae* or *B. gladioli* to tobacco, an uncongenial test plant, at high concentrations (more than 10^8 cfu/ml), induced necrosis at the infiltrated leaf area within 24 hr of inoculation. This necrosis could be considered the result of HR which has been reported in other combinations of bacteria and incompatible plants.

Rapid necrosis was induced in tobacco leaves infiltrated with all virulent strains of *B. glumae* and not induced by avirulent strains. These results indicate the existence of a close relationship between virulence to rice and necrosis inductivity in uncongenial plants. It appears, therefore, that toxoflavin is essential for pathogenicity in host plants and induction of necrosis in non-hosts.

In the case of *B. gladioli*, two strains (NIAS1065 and E-14), which are toxoflavin non-producing, also induced rapid necrosis in tobacco leaf tissue. Elicitation of HLR in tobacco leaf by *B. gladioli* might not only be the result of toxoflavin but also of other unidentified factors.

REFERENCES

Evans, L. R. and A. Linker 1973 Production and characterization of the slime polysaccharide of *Pseudomonas aeruginosa*. *J. Bacteriol.*, **116**: 915-924

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. Agr., Kyushu Univ.*, **38**: 175-181

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

Klement, Z., G. L. Farkas and L. Lovrekovich 1963 Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology,* **54**: 474-477

Lucas, L. T. and R. G. Grogan 1969a Serological variation and identification of *Pseudomonas lachrymans* and other phytopathogenic *Pseudomonas* nomenspecies. *Phytopathology,* **59**: 1908-1912

Lucas, L. T. and R. G. Grogan 1969b Some properties of specific antigens of *Pseudomonas lachrymans* and other *Pseudomonas* nomenspecies. *Phytopathology*, **59**: 1913-1917