Control of Bacterial Seedling Rot of Rice by Avirulent Strains of *Pseudomonas glumae**

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Abstract

The treatment of rice seeds with avirulent strains of *Pseudomonas glumae* significantly suppressed the incidence of bacterial seedling rot of rice caused by virulent strains. The suppression varied depending on the combinations of the avirulent and virulent strains used. The remarkable suppression resulted by the use of high concentration (ca. 10^{10} cfu/ml) of avirulent strains for pre-treatment. The killed cells of avirulent strain of *P. glumae* or other bacteria belonging to the genus *Agrobacterium*, *Erwinia*, *Bacillus* and *Clavibacter* tested did not suppress disease incidence. Of five avirulent strains of *P. glumae* used, avirulent strain N7503 was the highest in the suppression to the disease incited by virulent strains. Avirulent strain N7503 may be useful agent for biocontrol of bacterial seedling rot of rice.

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Key words: *Pseudomonas glumae*, bacterial seedling rot of rice, avirulent strain, biological control.

INTRODUCTION

Pseudomonas glumae Kurita et Tabei 1967, the causal bacterium of grain rot of rice is widely distributed not only in Japan but also in Korea and China causing serious losses in rice production^{10,11}. The bacterium also incites bacterial seedling rot of rice in Japan where the seedlings are grown in nursery boxes under high humidity and temperature conditions. For control of the bacterial grain rot or seedling rot of rice, some measures such as seed or soil disinfection by chemicals have been recommended^{7,8,13}. Since the effect of these practices, however, was not enough and not economical, some feasible methods have been requested to be developed.

Recently, the studies on biological method to control bacterial diseases of plant have been emphasized. Biocontrol potential may offer clues to many persistent problems in agriculture including over-reliance on agricultural chemicals and pollutions caused by chemical application.

Actually, avirulent bacteriocin producing strains of *P. solanacearum* and *Agrobacterium tumefaciens* have been successfully applied to control bacterial wilt of solanaceous plants caused by *P. solanacearum*^{2,3,9} and crown gall of various dicotyledonous plants caused by *A. tumefaciens*^{5,6}, respectively. In this paper, we report the suppressiveness of avirulent *P. glumae* strains to bacterial seedling rot of rice and its mechanisms.

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MATERIALS AND METHODS

Bacterial strains. Strains of *P. glumae* used were described previously¹²⁾. Many strains of *P. glumae* collected from various localities of Japan and preserved in the Laboratory of Plant Pathology, Kyushu University, were considerably different in pathogenicity, i.e., some strains maintained strong pathogenicity, while others lost during preservation. The suppressiveness of avirulent strains of *P. glumae* to bacterial seedling rot of rice by seed treatment was tested. Six phytopathogenic and saprophytic bacteria, viz. *Agrobacterium tumefaciens* Ku7411, *Erwinia carotovora* subsp. *carotovora* N7129, *Clavibacter michiganensis* subsp. *michiganensis* N6601, *Bacillus subtilis* ATCC 6633, *P. syringae* pv. *syringae* I and *P. fluorescens* p-15 besides *P. glumae* strains were also tested for their suppressiveness. These bacteria had been preserved under freeze-dried conditions in the Laboratory of Plant Pathology, Kyushu University. All of the bacterial strains were preliminary cultured on the slants of YPDA medium (yeast peptone dextrose agar medium: yeast extract 3 g, peptone 0.6 g, dextrose 3 g, agar 15 g, distilled water 1 liter, pH 7.2) at 30°C for 48 hr. The bacterial cells were suspended in sterile distilled water. A ten ml of the suspension was added to 200 ml of YPD broth in Sakaguchi flask and shaken at 30°C for 48 hr. The bacterial cells were harvested by centrifugation at $6,000 \times g$ for 20 min, and resuspended in sterile distilled water. Viable cells were counted by plating on YPDA medium. The bacterial suspensions thus prepared were used for pre-treatment or as inoculum.

Killed bacterial cells. For preparing killed bacterial cells, heat-treatment, chloroform treatment and UV irradiation were applied. To kill the bacterial cells by heat, a 20 ml of avirulent strain N7503 (conc. 1.4×10^9 cfu/ml) was boiled at 100°C for 15 min in water bath. Chloroform treatment was carried out as follows: A 30 ml of the bacterial suspension in a 500-ml beaker was placed in a 2,000-ml beaker containing 100 ml of chloroform, which was covered with aluminum foil, and the suspension was exposed to chloroform vapor under gentle shaking for 4 hr. A five ml of the suspension was taken in petri dish (9 cm in diameter) and irradiated by UV (TOSHIBA GL 15) from the distance of 30 cm for 4 hr.

Rice Seeds. The seeds of rice cultivar Asominori were used. The seeds were disinfected with 3% hypochlorite solution (Antiformin) at room temperature for 90 min and washed with running tap water for 30 min.

Bacterization. Seeds were treated with bacteria as follows: Rice seeds were dipped in the suspension of avirulent strain containing 1.5% methylcellulose. The seeds used as check were similarly treated with a suspension of methylcellulose. The treated seeds were sown at the density of 60 seeds per seedling cup ($60 \times 60 \times 45$ mm) containing 80 g of the heat-sterilized soil (Kumiai), covered with a thin-layer of the same soil, and then a 10 ml of the bacterial suspension of virulent strain was poured as inoculum. The seedling cups were placed in an inoculation chamber at 32°C under high humidity for 48 hr, and moved to the growth chamber (28°C, 12 hr light/12 hr dark cycle) for greening. Soil moisture was always saturated by watering several times daily. Disease severity was evaluated with a rating scale ranging from 0–5, where 0=symptomless, 5=complete withering. Disease severity was calculated by the following formula; Σ (number of samples per rating $\times$ rating value)/60. Thus maximum disease severity = 5.

RESULTS

The application of avirulent strains of *P. glumae* to rice seeds prior to inoculation with virulent strains suppressed the seedling rot with various degree depending upon the combinations of both avirulent and virulent strains of *P. glumae* used (Table 1). The disease caused by strain Ku8111 was suppressed by avirulent strain N7503, YN7810 and 805, but not by avirulent strain N750 and YN7825. In the case of virulent strain So-1, all avirulent strains suppressed remark-

Table 1. Suppression of bacterial seedling rot of rice by pre-treatment with avirulent strains of *Pseudomonas glumae*

Pre-treatment ^{a)}	Inoculum ^{b)}	Disease severity
N7503	So-1	0.2
	2	0.0
	Kyu82-34-2	0.2
	Ku8111	0.9
N750	So-1	0.3
	2	4.2
	Kyu82-34-2	4.8
	Ku8111	3.4
YN7810	So-1	0.6
	2	4.6
	Kyu82-34-2	4.8
	Ku8111	1.2
YN7825	So-1	0.3
	2	4.7
	Kyu82-34-2	4.6
	Ku8111	4.4
805	So-1	0.6
	2	0.3
	Kyu82-34-2	4.1
	Ku8111	1.1
Check	So-1	4.9
	2	5.0
	Kyu82-34-2	4.9
	Ku8111	4.9

a) Concentration; N7503: 3.8×10^{10} , N750: 7.4×10^{10} , YN7810: 3.1×10^{10} , YN7825: 2.4×10^{10} , 805: 6.0×10^{10} , cfu/ml, respectively.

b) Concentration; So-1: 2.6×10^7 , 2: 0.9×10^7 , Kyu82-34-2: 1.2×10^7 , Ku8111: 1.3×10^7 , cfu/g of soil, respectively.

Table 2. Suppression of bacterial seedling rot of rice at different concentrations of avirulent and virulent bacterial suspensions used for pre-treatment and inoculation, respectively

Avirulent strain N7503 (cfu/ml)	Virulent strain So-1 (cfu/g of soil)	Disease severity
10^6	10^5	4.5
10^6	10^7	5.0
10^6	10^9	5.0
10^8	10^5	3.8
10^8	10^7	4.5
10^8	10^9	4.8
10^{10}	10^5	0.0
10^{10}	10^7	0.1
10^{10}	10^9	0.2
—	10^5	4.7
—	10^7	5.0
—	10^9	5.0

ably. When virulent strain 2 was used, disease severity was significantly reduced by avirulent strains N7503 and 805 except N750, YN7810 and YN7825. In the case of virulent strain Kyu-82-34-2, only N7503 suppressed, except other four strains. The disease suppressiveness of avirulent strains was also depended upon bacterial concentrations. When rice seeds were dip-

Table 3. Comparison between biological and chemical methods in the suppression of bacterial seedling rot of rice^{a)}

Treatment	Concentration	Disease severity
Seeds were coated with avirulent strain N7503	10 ¹⁰ cfu/ml	0.5
Soil was mixed with Kasugamycin • captan WP	0.4 mg/g of soil	0.0
Untreated	—	4.2

a) Inoculum: *Pseudomonas glumae* virulent strain So-1 at the concentration of 1.9×10^7 cfu/g of soil.

Table 4. Effect of avirulent strain N7503 on the suppression of bacterial seedling rot of rice incited by virulent strains of *Pseudomonas glumae*

Seed treatment	Virulent strains ^{a)} and disease severity						
	Ku8106	Ku8121	III	8001	8017	I	Ku8105
N7503 ^{b)}	0.1	0.1	0.1	0.3	0.2	0.3	0.0
1.5% methylcellulose	4.6	4.2	3.8	3.6	4.1	3.9	3.9

a) Concentration; Ku8106: 1.9×10^7 , Ku8121: 7.4×10^6 , III: 2.1×10^7 , 8001: 6.1×10^6 , 8017: 1.6×10^7 , I: 6.5×10^7 , Ku8105: 6.4×10^6 cfu/g of soil, respectively.

b) Concentration; N7503: 4.1×10^{10} cfu/ml.

Table 5. Suppression of bacterial seedling rot of rice^{a)} by various bacteria

Bacterium	Disease severity
<i>Agrobacterium tumefaciens</i> Ku7411	5.0
<i>Erwinia carotovora</i> subsp. <i>carotovora</i> N7129	5.0
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> N6601	5.0
<i>Bacillus subtilis</i> ATCC 6633	5.0
<i>Pseudomonas syringae</i> pv. <i>syringae</i> I	5.0
<i>Pseudomonas fluorescens</i> p-15	5.0
<i>Pseudomonas glumae</i> N7503	0.4
Check (1.5% methylcellulose)	5.0

a) Virulent strain So-1 was added to the oven-dried soil at the concentration of 3.5×10^7 cfu/g of soil.

Table 6. Suppression of bacterial seedling rot of rice^{a)} by pre-treatment with living and killed cells of avirulent *Pseudomonas glumae*

Strain	Seed treatment ^{b)}	Disease severity
N7503 ^{c)}	Living bacteria	0.0
	Heated bacteria ^{d)}	4.8
	UV irradiated bacteria ^{e)}	4.4
	Chloroform treated bacteria ^{f)}	4.9
Control	1.5% methylcellulose	5.0

a) Soil was incorporated with So-1 (3.5×10^6 cfu/g of soil).

b) Rice seeds were dipped in each suspension for 24 hr prior to sowing.

c) Concentration: 1.4×10^9 cfu/ml.

d) Heat treatment at 100°C for 15 min.

e) UV irradiated with TOSHIBA GL 15 at the distance of 30 cm for 4 hr.

f) Chloroform vapor treatment for 4 hr.

ped in the suspension of avirulent strain N7503 at various concentrations (10^6 , 10^8 , 10^{10} cfu/ml) before sowing and then inoculated with virulent strain So-1 at various concentrations (10^5 , 10^7 , 10^9 cfu/g of oven-dried soil), the highest disease suppression was obtained with the highest concentration of avirulent strain (Table 2). The disease suppression in this case was almost the same as that obtained by soil-treatment with Kasugamycin-captan WP at the dosage of 0.4 mg/g dried soil (Table 3). Avirulent strain N7503 showed remarkable suppressiveness to the disease caused by virulent strains (Table 4).

Other bacteria belonging to the genus *Agrobacterium*, *Erwinia*, *Clavibacter* and *Bacillus* did not suppress bacterial seedling rot of rice (Table 5). The cells of avirulent strain N7503 killed by heat, UV irradiation or chloroform treatment also did not show any suppressiveness (Table 6).

DISCUSSION

In the previous paper⁴⁾, we have reported on the suppression of the bacterial wilt of tomato by pre-treatment of the seedlings with *P. glumae*. It was exhibited not only by living cells but also by killed cells. The mechanism of suppression was suspected to be the competition of infection sites and induced resistance. It was confirmed in this experiment that the bacterial seedling rot of rice was also suppressed by pre-treatment with avirulent strains of *P. glumae*. However, the suppression in this case was extremely varied depending upon combinations of avirulent and virulent strains used. Furthermore, the suppression was exhibited only with living avirulent cells. Therefore, the mechanisms of the suppression of bacterial seedling rot by avirulent *P. glumae* seem to be quite different from those of bacterial wilt of tomato. The competition for infection sites and nutrients seem to be involved in the mechanisms.

It has been reported that some pathogens lost their pathogenicity by mutation maintained without losing their ability to colonize the niche, and invade to multiply in host tissues similarly to virulent strains^{1,9)}. If such an avirulent strain was applied first, the virulent strains will be prevented from effective access to infection sites. Other bacteria belonging to the genus *Agrobacterium*, *Erwinia*, *Bacillus* and *Clavibacter* did not show suppression at all.

Some strains of *P. glumae* produce bacteriocins which inhibit growth of other strains of *P. glumae*. However, no correlation was observed between suppressiveness and bacteriocin productivity of avirulent strain or bacteriocin sensitivity of virulent strains (data not shown). Therefore, the mechanism of the difference in the suppressiveness depending upon combination of the avirulent and virulent strains is not clear at present. Supposedly, it might be due to the difference in compatibility of each avirulent and virulent strain to rice plants.

Of the five avirulent strains used, the strain N7503 constantly showed highest suppressive effect against all virulent strains. Avirulent strain N7503 may be a rationally useful agent for biocontrol of bacterial seedling rot of rice. However, before practical application of this avirulent strain, those should be confirmed that the strain is strictly non-pathogenic to all host plants as well as to rice plant and that reverse mutation does not occur in natural conditions.

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和 文 摘 要

古屋成人・岡本知子・郡 裕一・松山宣明・脇本 哲：非病原性イネもみ枯細菌病菌によるイネ幼苗腐敗症の発病抑制効果

非病原性のイネもみ枯細菌病菌株でイネ種子を処理することによりイネ幼苗腐敗症の発病が抑制された。この発病抑制効果は、供試した非病原性菌株と病原性菌株の組合せにより異なり、また非病原性菌株の細菌濃度が高いほど顕著であった。発病抑制効果は、イネもみ枯細菌病菌の生菌でのみ認められ、それ以外の菌、すなわち、*Agrobacterium*, *Erwinia*, *Bacillus*, *Clavibacter* 等ではまったく認められなかった。供試した非病原性5菌株のうち、N7503は供試したすべての病原性菌株による発病を抑制し、その防除効果も高く、イネ幼苗腐敗症に対する生物的防除材としての利用の可能性が示唆された。