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IHC 2010

Editors
I. Mourão
U. Aksoy

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PROCEEDINGS OF THE INTERNATIONAL SYMPOSIUM ON ORGANIC HORTICULTURE: PRODUCTIVITY AND SUSTAINABILITY

Co-Conveners

I. Mourão
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Protection of Eggplant and Chilli from Bacterial Wilt (*Ralstonia solanacearum*) with Antagonistic Bacteria

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Keywords: biological control, *Pseudomonas putida* Pf-20, *R. solanacearum* Rs-127

Abstract

Bacterial wilt caused by *Ralstonia solanacearum* is one of the limiting factors in eggplant and chilli production in Indonesia. Control of the disease is difficult with the available means. Biological control based on antagonism was therefore chosen as one of the control methods. A fluorescent pseudomonad (*Pseudomonas putida*, Pf-20 strain) isolated from the rhizosphere of *Mimosa invisa* proved successful in suppressing *R. solanacearum* in vitro, reducing disease intensity in greenhouse and field experiments. However, the degree of protection was low. The use of more than one species of microorganisms to control plant pathogens likely enhances the protection level. An avirulent-bacteriocin producing strain of *R. solanacearum* (Rs-127) was chosen as a companion of Pf-20 to control the disease. Rs-127 inhibited the growth of other *R. solanacearum* isolate, with an inhibition zone of 12 mm, and as much as 86% of other isolates were inhibited. Rs-127 did not inhibit the growth of Pf-20 in YPGA and CPG medium. However, Pf-20 inhibited the growth of Rs-127 in King's B medium with an inhibition zone of 15 mm, but did not work in YPGA and CPG medium. The greenhouse test showed that dipping the root system of eggplant and chilli in bacterial suspension of Rs-127 and Pf-20, both solely and in combination, were able to suppress the disease and prolong the incubation period. In the control plots, plants began wilting one week after transplanting. In the plots treated with Pf-20 and Rs-127, the diseased plants were first observed at two and five weeks after transplanting, with a disease index of 13 and 16, respectively, while in the control plots, the disease index at that time had already reached 56. When Pf-20 and Rs-127 were combined, the disease was first observed at 5 weeks after transplanting with a disease index of 6.

INTRODUCTION

Bacterial wilt caused by *Ralstonia solanacearum* is difficult to control with the available means, especially the one caused by Race 1 in lowland areas (Hayward, 2005). The great variability of the pathogens and the complexity of portal entrance into the host tissue make the disease and the pathogens difficult to control (Goto, 1994). Soil solarization is not effective and crop rotation is not feasible due to the high number of host plants that can be attacked by the pathogen. Resistant plants are difficult to obtain and could be broken by the pathogen. Until now there is no chemical that is effective to control the pathogen in the field. Biological control was therefore chosen as an alternative control method. Many authors report the use of antagonistic bacteria to control disease. However, most of them used a single antagonist instead of multi antagonists (Smith, 2005). The use of more than one antagonist to control plant pathogen likely gives better protection than single application. We report the use of two antagonistic bacteria against *R. solanacearum* in vitro.

MATERIALS AND METHODS

Strain Pf-20 of *Pseudomonas putida* was isolated from the rhizosphere of *Mimosa invisa* in 1996 (Arwiyanto, 1997). The bacteria was restored from stock kept under mineral oil by streaking on the King's B medium before use. *Ralstonia solanacearum* was isolated from several host plants on YPGA medium (yeast extract 5 g, peptone 10 g,

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glucose 10 g, distilled water 1000 ml, pH 6.8). Virulent colony type of *R. solanacearum* was transferred to YPGA slant medium.

Each isolate of *R. solanacearum* was tested against each other for antagonism on CPG medium (Arwiyanto et al., 1993). A selected avirulent strain of *R. solanacearum* producing bacteriocin was tested for its antagonistic activity against Pf-20 on CPG medium and the antagonistic activity of Pf-20 against *R. solanacearum* was tested on King's B medium (Arwiyanto and Nurcahyanti, 2007).

Local cultivars of eggplant ('Mustang') and chilli ('Lokal') were used. The seeds were sown in sterile soil and used at 35 days after sowing.

Seedling Treatment

The root systems of the seedlings were dipped in the water-bacterial suspension for 15 minutes. A final concentration of 100 ppm of Tween-20 was added into the suspension before treatments. As a control, root systems of the seedlings were dipped for 15 minutes in sterile distilled water with Tween-20 (100 ppm final concentration). After dipping, the seedlings were planted in pots filled with soil infected with virulent isolate of *R. solanacearum*. The treatments consisted of:

1. Root dipping in sterile distilled water followed by planting in soil infected with *R. solanacearum*.
2. Root dipping in a mixture of Pf-20 and Rs-127 water suspension with a 1:1ratio (v/v), with a final bacterial concentration of 10^8 cfu/ml.
3. Root dipping in a mixture of Pf-20 and Rs-127 water suspension with a 1:1 ratio (v/v), with a final bacterial concentration of 10^9 cfu/ml.
4. Root dipping in a water suspension of Rs-127 (10^8 cfu/ml) followed by planting in soil infected with *R. solanacearum*.
5. Root dipping in a water suspension of Rs-127 (10^9 cfu/ml) followed by planting in soil infected with *R. solanacearum*.
6. Root dipping in a water suspension of Pf-20 (10^8 cfu/ml) followed by in soil infected with *R. solanacearum*.
7. Root dipping in a water suspension of Pf-20 (10^9 cfu/ml) followed by planting in soil infected with *R. solanacearum*.

Disease development, expressed as disease index (Winstead and Kelman, 1952) was recorded for each treatment at specific times after the challenge inoculation.

RESULTS AND DISCUSSION

As much as 161 isolates of *R. solanacearum* from several host plants were tested against each other for their antagonistic activity on CPG medium. Isolate number 127 of *R. solanacearum* inhibited the growth 86% relative to other isolates tested with a zone of inhibition of 12.6 mm in average (Table 1). This isolate was therefore chosen for further experiments because of its ability to produce inhibition substance more successfully than other isolates.

The avirulent form of isolate Rs-127 (Rs-127 avir) was generated spontaneously in YPG broth medium after one week in still culture at room temperature. The avirulent form was obtained by streaking the culture on YPGA and incubating for 48 hours at room temperature.

The ability of Rs-127 avir to produce inhibition substance against the virulent isolate of *R. solanacearum* (Rs T) was the same as its parental wild type (Table 2). This means that the isolate could be used as a biological control agent.

Since the isolate of Rs-127 avir was used as a companion of Pf-20 for controlling the virulent form of *R. solanacearum*, the antagonistic activity of these control agents against each other was tested in vitro. Pf-20 inhibited the growth of Rs-127 avir in vitro with a resulting inhibition zone of 15 mm. The mechanism of inhibition was bacteriostatic. However, Rs-127 avir could not suppress the growth of Pf-20 in vitro (Table 3).

Rs-127 could suppress disease development caused by the virulent form of *R. solanacearum*. Disease development in the plot treated with Rs-127 was slower and the

disease index was constant around 10, from 3 weeks after inoculation onwards. There were slight differences between plots treated with Rs-127 at a concentration of 10^9 cfu/ml and at 10^8 cfu/ml. On the contrary, in the untreated plots, disease development was rapid and the disease index reached around 70 at the end of the experiment. Pf-20 also suppressed the development of eggplant bacterial wilt although the degree of protection was lower than the one offered by Rs-127 (Fig. 1).

The disease development in the plot treated with Pf-20 was constantly low until 5 weeks after inoculation. The disease index then moved progressively up until the end of experiments. However, it was still lower than in the untreated control plot.

When the antagonistic bacteria were combined to protect eggplant from bacterial wilt, significant protection was observed. The wilt symptoms in the treated plot were not observed until 2 weeks after inoculation. When the concentration of antagonistic bacteria was 10^9 cfu/ml, wilting symptoms went almost undetected until 5 weeks after inoculation. At the end of the experiment, the disease index was very low, i.e., at less than 10. On the contrary, disease index in the untreated control plot was more than 10 at 3 weeks after inoculation and rapidly increased to more than 70 at the end of the experiment. A similar result was obtained when the bacteria were used for controlling chilli bacterial wilt (Fig. 2).

The niche of Pf-20 is in the rhizosphere, while Rs-127 tends to invade the host plants through wounds. Therefore, when they are applied together as antagonistic bacteria, they do not reside in the same place, meaning antagonism between them could not occur. This hypothesis, however, needs clarification through further experiments.

CONCLUSIONS

P. putida strain Pf-20 and an avirulent bacteriocin producing strain (Rs-127) of *R. solanacearum* protected eggplant and chilli from bacterial wilt caused by virulent form of *R. solanacearum* in greenhouse conditions.

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Tables

Table 1. Selected isolates of *Ralstonia solanacearum* which inhibited other isolates in vitro.

No	Isolate number	Zone of inhibition (mm)*	Inhibition intensity (%)
1	20	12.27	62.12
2	59	11.04	55.22
3	66	12.57	47.69
4	70	10.76	76.12
5	104	10.28	79.10
6	108	10.66	69.70
7	114	10.22	66.67
8	115	10.48	78.79
9	116	10.68	78.79
10	117	11.29	81.82
11	119	11.27	82.81
12	120	9.47	81.25
13	122	11.13	82.81
14	124	13.51	81.25
15	125	11.09	85.94
16	126	12.13	81.25
17	127	12.62	86.15
18	129	7.34	78.46
19	131	10.10	72.31

* average of three replicates.

Table 2. Growth inhibition of a virulent *R. solanacearum* (Rs T) by antagonistic bacteria.

Medium	Inhibition zone (mm)*		
	Rs-127 against Rs T	Rs-127 avir against Rs T	Pf-20 against Rs T
King's B	0	0	12
CPG	7.75	7.00	0
YPGA	7.75	7.00	0

* average of three replicates.

Table 3. Antagonistic test between Pf-20 and Rs-127.

Medium	Inhibition zone (mm)*			
	Pf-20 against Rs-127	Rs-127 against Pf-20	Pf-20 against Rs-127 avir	Rs-127 avir against Pf-20
King's B	12	0	15	0
CPG	0	0	0	0
YPGA	0	0	0	0

* average of three replicates.

Figures

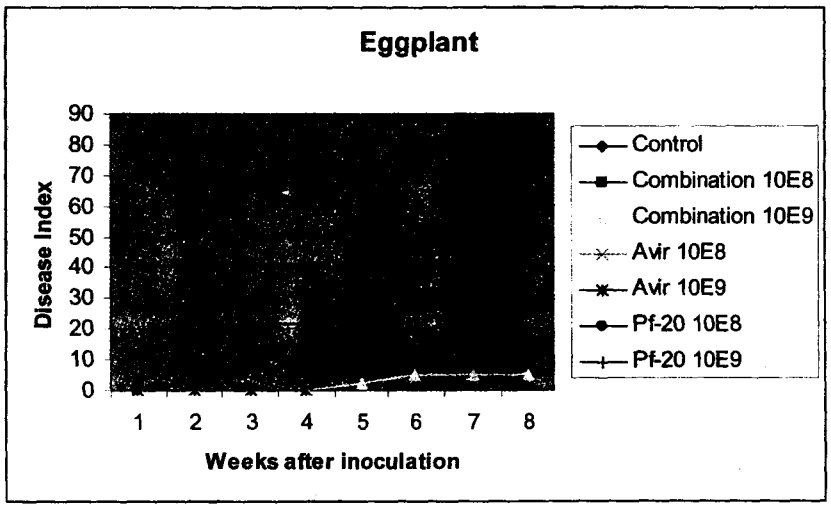

Fig. 1. Development of eggplant bacterial wilt treated with antagonistic bacteria.

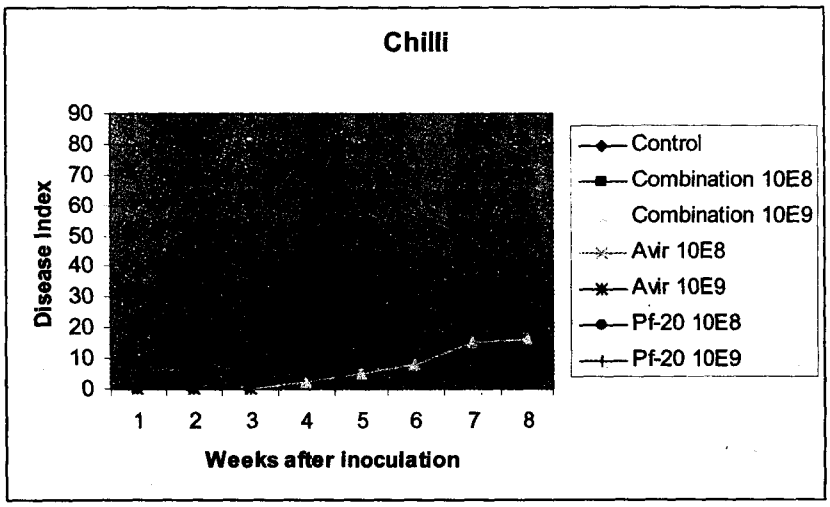

Fig. 2. Development of chilli bacterial wilt treated with antagonistic bacteria.