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SUPPRESSION OF SHEATH BLIGHT OF RICE WITH ANTAGONISTIC BACTERIA

PENGHAMBATAN PENYAKIT BUSUK PELEPAH DAUN PADI OLEH BAKTERI ANTAGONIS

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INTISARI

Sebelas isolat bakteri diisolasi dari lahan sawah di daerah Wates, Kulon Progo. Hasil dari uji antagonisme pada media nutrient agar *(NA) menunjukkan bahwa hanya dua isolat (WKP-4 dan WKP-6) yang bersifat antagonis terhadap* Rhizoctonia solani. *Determinasi kedua isolat ini menunjukkan bahwa mereka termasuk dalam kelompok* Pseudomonas *yang bersifat fluoresen. Pada percobaan yang dilakukan di dalam rumah kaca, hanya isolat WKP-6 mampu menekan tinggi becak relatif busuk pelepah daun pada padi varietas IR 50.*

Kata kunci: Rhizoctonia solani, *busuk pelepah daun padi,* Pseudomonas *yang bersifat fluoresen*

ABSTRACT

Eleven bacterial isolates were isolated from wet rice field in Wates, Kulon Progo. The result of antagonism test on nutrient agar (NA) showed that only two isolates (WKP-4 and WKP-6) were antagonistic to *Rhizoctonia solani*. Determination on these two isolates showed that they were belong to the group of fluorescent pseudomonads. In the glass house trial, only WKP-6 suppressed the relative lesion height of sheath blight of rice variety IR 50.

Key words: *Rhizoctonia solani*, sheath bight of rice, fluorescent pseudomonads.

INTRODUCTION

Sheath blight caused by *Rhizoctonia solani* is an important disease of rice. It has been reported that the disease reduced 10% of total rice production each year in China and more than 50% in the other region in Asia. In Japan, 120,000 to 190,000 ha of wet rice field has been infected by *R. solani* and a loss of 24,000 to 38,000 ton of rice each year has been estimated (Ou, 1985).

Several methods have been applied to control sheath blight of rice. Fungicides has been used for several years in Japan to control this disease. Benomyl, iprodione and pentachlorophenol were reported to control sheath blight successfully. Antibiotics such as validamycin, polyoxin have also been reported to be effective to control sheath blight of rice (Ou, 1985).

Biological control is another alternative to control sheath blight of rice. This method controls the disease without causing any damaging effect to the environment (Sivamany & Gnanamanickam, 1988). Doutt, (1972) mentioned that biological control reduced the population of the pathogen. Mazzola (1999) observed that the increasing population of *Burkholderia cepacia* and *Pseudomonas putida* caused the reduction of *R. solani* AG 5 population in soils. Devi *et al.*, (1989) reported that bacterial isolates from wet rice field in India reduced the disease intensity of sheath blight. The bacteria antagonistic to *R.*

solani could reduce the fungal growth by inhibiting the sclerotium germination and mycelial growth.

This study was aimed to examine bacterial isolates originated from wet rice field in Wates Kulon Progo to reduce the development of sheath blight of rice caused by *R. solani.*

MATERIALS AND METHODS

Isolation of bacteria. Bacteria were isolated from soil, irrigation water, 40, 60, and 80 days old rice plants, and diseased rice plants. The methods of isolation from each sample were as follow: One gram of soil or 1 ml of irrigation water was suspended into 99 ml of sterile water (dilution $1:10^2$), shaken and the dilution was continued until $1:10^6$. Ten grams of healthy leaves or diseased sheath of rice plants were cut, suspended into 100 ml sterile water (dilution $1:10^2$), shaken and dilution was continued until $1:10^6$. Each suspension (0.1 ml) was dropped on a petri dish and poured with melted nutrient agar (NA) (Bacto) (45°C). The plates were incubated for a day at room temperature (Fernando *et al.*, 1994; Hebbar *et al.*, 1992). Bacteria that formed different morphological colonies were isolated, sub-cultured on NA slopes and recorded (Jutono, 1980).

In vitro antagonism test. A forty eight hours bacterial culture was suspended in sterile water and adjusted at 3×10^8 cfu/ml (Vinther, 1985). Forty μl of bacterial suspension was dropped on a 0.5 cm diameter sterile filter paper and put on a NA plate. As control the filter paper was dropped with 40 μl sterile water. Seven days old *R. solani* culture was put on the same plate at the opposite of the filter paper. The plate was incubated for 7 days at room temperature. The inhibition zone was measured between the bacterial and fungal colony (Hebbar *et al.*, 1992).

Determination of bacterial isolates. Antagonistic bacteria were determined for Gram reaction, colony morphology, fluorescent pigmentation, the growth on D-1 medium, anaerobic growth and starch hydrolysis (Schaad, 1988).

Glass house trials. Rice plants variety IR 50 was planted in a 25 diameter pot in a glass house. A complete randomized design with 3 replications was used in this experiment. A forty eight hours bacterial culture was suspended in sterile water and adjusted at 3×10^8 cfu/ml (Vinther, 1985). The bacterial suspension was prepared as bacterial inoculum. Bacterial inoculation was conducted before planting by soaking the rice seeds into bacterial inoculum for 2 hours. As control treatment rice seeds were soaked into sterile water. Fungal inoculum was prepared as follow: 3 g of rice bran, 100 g of sand, and 15 ml of water were sterilized with an autoclave at 121°C for 15 minutes. Seven days old *R. solani* culture was inoculated into rice bran-sand media and incubated for 2 weeks. Fungal inoculation was conducted a week before transplanting by mixing 25 g of fungal inoculum with soil in a 25 cm diameter pot (Sivamany & Gnanamanickam, 1988). The treatments were as follow:

I. Rice seeds were soaked in sterile water, no fungal inoculation
II. Rice seeds were soaked in sterile water, fungal inoculation
III. Rice seeds were soaked in bacterial suspension, no fungal inoculation
IV. Rice seeds were soaked in bacterial suspension, fungal inoculation

The development of sheath blight was observed weekly and the relative lesion height was measured. Relative lesion height was the proportion between the lesion height of sheath blight and the height of rice plant (Ahn *et al.*, 1986).

RESULTS AND DISCUSSION

There were 11 isolates of bacteria isolated from wet rice field in Wates Kulon Progo had different morphological colonies. On NA slopes, these isolates had white or yellow colonies. Most of them had thick growth, slimy, and formed filiform or beaded colonies (table 1). Not all of isolates could inhibit the growth of *R. solani* on NA plates. Only two isolates (WKP-4 and WKP-6) had inhibition zone varied from 5–10 mm (table 2). These 2 isolates were gram negative and formed blue fluorescent pigment under uv lamp. The result of bacterial determination showed that isolates WKP-4 and WKP-6 were belong to the group of fluorescent pesudomonads (table 3).

Fluorescent psudomanads are common microorganisms found in soil because of their aggressiveness to colonize plant root and their ability to produce chemical compounds that inhibit the growth of other soil microorganisms. Therefore they can compete with other soil microorganisms to obtain space or nutrients for their growth (Defago *et al.*, 1990).

Fluorescent pseudomonads have also been known to inhibited fungal growth on agar media. Arie *et al.*, (1987) showed that fluorescent pseudomonads inhibited the growth of *Fusarium oxysporum* f.sp. *lagenariae in vitro*. Antibiotics including phenazine, 2,4-diacetylphloroglucinol, pyoluteorin and pyrrolnitrin are produced in the biological control of fungal plant pathogens by specific strains of fluorescent pseudomonads (Mazzola *et al.*, 1995; Weller & Thomashow, 1993).

Of 2 isolates fluorescent pseudomonads WKP-4 and WKP-6, the relative lesion height was 20.5% when WKP-4 was applied to rice seeds which was not significantly different compared to fungal control treatment. The lesion height of these two treatments were the same and it showed that WKP-4 could not suppress the sheath blight disease. However, the relative lesion height was only 2.5% when WKP-6 was applied on rice seeds (table 4).

Although isolate WKP-4 and WKP-6 inhibited the growth of *R. solani* on NA plates, only isolate WKP-6 suppressed the relative lesion height of sheath blight of rice variety IR 50. Many bacterial strains suppressed fungal growth *in vitro* by the production of antifungal antibiotics. The antibiotics produced *in vitro* were generally assumed to be the compounds responsible for biological control *in vivo* (Leifert *et al.*, 1995).

Table 1. Morphological character of bacteria isolated from wet rice field on Nutrient Agar (NA) slopes

Bacterial isolates	Colony color	Colony form	Consistency	Growth
WKP-1	White	Filiform	Slimy	Thick
WKP-2	Yellow	Filiform	Butyrous	Thick
WKP-3	White	Effuse	Slimy	Thin
WKP-4	White	Echinulate	Slimy	Thick
WKP-5	White	Beaded	Slimy	Thick
WKP-6	Yellow	Filiform	Slimy	Thick
WKP-7	Yellow	Filiform	Slimy	Thick
WKP-8	White	Beaded	Slimy	Thick
WKP-9	Yellow	Filiform	Slimy	Thick
WKP-10	White	Beaded	Slimy	Thick
WKP-11	Yellow	Beaded	Slimy	Thick

Table 2. Inhibition zone of *R. solani* by bacterial isolates isolated from wet rice field

Inhibition zone (mm)	No. of isolates
< 5	9
5–10	2
> 10	0

Since no general relationship existed between the ability of various bacteria to inhibit growth of a plant pathogen *in vitro* and disease suppression caused by that pathogen *in vivo*, strains producing the largest zone of inhibition on agar media were not necessarily the best biocontrol agents (Weller, 1988). However *in vitro* production of antifungal antibiotics has frequently been used as a primary step in the selection of a potential biocontrol agent (Michereff *et al.*, 1994).

Inoculation of plant roots with fluorescent pseudomonads has also been reported to reduce the incidence of fungal diseases in the glass house trials (Gamliel & Katan, 1993). Harrison *et al.* (1993) showed that suppression of take-all disease caused by *Gaeumannomyces graminis* var. *tritici* of wheat by *Pseudomonas fluorescens* strain 2-79 and *P. chlororaphis* strain 30-84 was due to the production of the antibiotic phenazine-1-carbolic (PCA), whereas 2,4 diacetylphloroglucinol produced by *P. fluorescens* strain Q2-87 was also responsible for the suppression of this take-all pathogen (Vincent *et al.*, 1991).

Table 3. Some properties of present isolates

Characteristics	Present isolates		Genera (Schaad, 1988)				
	WKP-4	WKP-6	Er	Ps	Xn	Ag	Cr
Gram reaction	-	-	-	-	-	-	+
Yellow colony on YDC medium	-	-	-	-	+	-	-
Fluorescence pigment on King's B medium	+	+	-	+	-	-	-
Colony growth on D1 medium	-	-	-	-	-	+	-
Starch hydrolysis	-	-	-	-	+	-	-
Anaerobic growth	-	-	+	-	-	-	-

Notes: Er = *Erwinia*, Ps = *Pseudomonas*, Xn = *Xanthomonas*, Ag = *Agrobacterium*, Cr = *Corynebacterium.*

Table 4. Effect of bacterial isolates WKP-4 and WKP-6 on relative lesion height caused by *R. solani* on rice variety IR 50 eight weeks after inoculation

Treatment	Relative lesion height (%)	
	WKP-4	WKP-6
Rice seeds were soaked in sterile water, no fungal inoculation	0 a *	0 a
Rice seeds were soaked in sterile water, fungal inoculation	20.5 b	19.8 b
Rice seeds were soaked in bacterial suspension, no fungal inoculation	0 a	0 a
Rice seeds were soaked in bacterial suspension, fungal inoculation	20.5 b	2.5 a

Note: Numbers followed with same letters were not significantly different at 5% level of significant, using DMRT.

Strains RB425 and RB3292 of *P. cepacia*, isolated originally from roots of lettuce and which produce the antibiotics pyrrolnitrin and two pseudane compounds, were both suppressive of damping-off of radish caused by *R. solani* AG4. Following seed inoculation the incidence of damping-off was 71% to 74% less than for the uninoculated control plants (Homma & Suzui, 1989).

CONCLUSION

Of 11 isolates of bacteria isolated from wet rice field in Wates Kulon Progo, only two isolates (WKP-4 and WKP-6) inhibited the growth of *R. solani* on NA plates. These two isolates were belong to fluorescent pseudomonads. In the glass house trials only WKP-6 suppressed the relative lesion height of rice variety IR 50.

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