Antagonism of Bacterial Isolates from Local Microorganisms against *Rhizoctonia solani* and Their Effect on the Growth of Rice Seedlings

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Abstract

Local Microorganisms (MOL) are obtained from the liquid as a result of the fermentation of some organic material mixed in rice washing water or coconut water. Rice farmers usually use MOL in the cultivation of padi SRI (System of Rice Intensification).

The research was objected to obtain bacterial isolates from 6 different MOLs [the material in the mixture were daun cebreng (leaf of *Gliricidia sepium*), pucuk waluh (young leaves of *Sechium edule*), buah maja (fruit of *Aegle marmelos*), rebung (young bamboo shoots), bonggol pisang (banana hump), pisang (banana)], which are antagonistic to *Rhizoctonia solani* in vitro, and able to stimulate the growth of rice seedlings. The antagonism was tested by dual culture method on PDA. The experiment was arranged in the completely randomized design with 19 treatments (19 bacterial isolates) and a control. The experiment to study the stimulation effect of the isolates on the rice seedlings was carried out in the glasshouse. The isolates were applied by soaking the rice seeds in the bacterial suspensions for 24 hours.

From the 19 bacterial isolates obtained from the 6 MOLs, there were 6 isolates antagonistic to *Rhizoctonia solani*; 1 from maja, 1 from bonggol pisang, 3 from daun cebreng, and 1 from pucuk waluh. The isolates: MR1, MM4, MPW1, MM1, and MM3 were able to increase the number of germinated seeds which were 81%, 81%, 78%, 85%, and 98% respectedly, compared to control which was only 70%, and increase the growth of the seedlings.

Key words : Local Microorganisms, *Rhizoctonia solani*, antagonistic bacteria, PGPR

INTRODUCTION

Rice is a staple food crop for Indonesian. The government has attempted to increase the production of rice and managed to overcome some constraints such as the decrease of soil fertility, pests and diseases, excessive use of chemical fertilizers and pesticides, the in-optimal use of natural resources.

One of the attempts was the development of SRI (System of Rice Intensification) organic farming. In the SRI organic farming, organic material such as agricultural produce and crop residues are recycled and used as fertilizers and pesticides. It is common for the farmers to use organic fertilizer from rice straw, banana stem, and green

manures. Organic liquid fertilizers are also used. The liquid fertilizers are obtained from the fermentation of various organic materials available in the field and around the house of farmers such as kitchen waste. The organic materials are fermented in coconut water for 14-21 days. The result of the fermentation is called MOL (local microorganisms).

MOLs act as decomposer, fertilizer, and biological control agent, because of the bacteria which grow in the basic material. Kishore et al. (2005) reported that some bacteria obtained from the phylloplane and used in seed treatment, are able to induce the growth of peanut and increase the resistance to pathogen.

Leaf blight caused by *Rhizoctonia solani* is a major rice disease. The disease intensity on conventional rice is higher than the intensity on SRI organic rice farming (Hersanti et al., 2007). It was assumed that this was caused by the MOLs used in the SRI farming. The objective of this study was to isolate the bacteria from the MOLs which are able to control the pathogen and promote the growth of rice (act as Plant Growth Promoting Rhizobacteria).

METHODS

Inoculum of *Rhizoctonia solani*

The inoculum of *Rhizoctonia solani* was obtained from Balai Besar Penelitian Tanaman Padi, Sukamandi. It was cultured on potato dextrose agar.

Isolation and morphological characterization of MOL bacteria

Six MOLs were obtained from Yayasan Aliksa, Nagrak, Sukabumi; they were: daun cebreng (leaf of *Gliricidia sepium*), pucuk waluh (young leaves of *Sechium edule*), buah maja (fruit of *Aegle marmelos*), rebung (young bamboo shoots), bonggol pisang (banana hump), and pisang (banana).

The bacteria were isolated on nutrient agar by dilution method. The morphology of the colonies was characterized.

Antagonism test

The in vitro antagonism between MOL isolates and *Rhizoctonia solani* was tested by dual culture method on potato dextrose agar (PDA).
Agar disk of *Rhizoctonia solani* (0.7 cm) was placed on PDA in Petri dish 1.5 cm from the side; the bacterial suspension was streak 3 cm from the disk. The activity of the MOL bacteria is shown by the inhibition zone around the colony of *Rhizoctonia solani*.

The experiment was arranged in the completely randomized design, with 19 treatment of MOL bacterial isolates and 3 replications. The parameter observed was the percentage of the inhibition.

PGPR test

The potency of the MOL bacteria as PGPR was tested by soaking rice seeds in the bacterial suspension (10^7 cfu/ml) for 24 hours. The seeds were then spread on growth media which is the mixture of soil and manure. The parameters were the percentage of the seed germination and the length of the 8 days seedlings.

RESULTS AND DISCUSSION

Bacterial isolation

Nineteen isolates of bacteria were obtained from the six MOLs, i.e. 3 isolates from MOL daun cebreng, 1 from MOL pucuk waluh, 7 from MOL maja, 1 from MOL rebung, 3 from MOL bonggol pisang, and 4 from MOL pisang. The morphology of their colonies on nutrient agar were summarized in Table 1.

Table 1. Colony Morphology of MOL Isolates

Isolates	Colony Morphology			
	Color	Shape	Surface	Edge
MDC1	Cream	Round	flat	serrated
MDC2	white	Irregular	flat	serrated
MDC3	white	Round	raised	Round
MPW1	cream	Irregular	flat	undulated
MM1	yellow	Round	concave	Round
MM2	transparant	Round	concave	Round
MM3	orange	Round	concave	Round
MM4	cream	Irregular	flat	serrated
MM5	Dark yellow	Irregular	raised	undulated
MM6	yellow	Fibrous	raised	fibrous
MM7	cream	Irregular	flat	Round
MR1	transparant	Fibrous	flat	fibrous
MBP1	cream	Irregular	convex	Serrated
MBP2	yellow	Fibrous	concave	Fibrous
MBP3	cream	Round	raised	Undulated
MP1	yellow	Round	concave	Round
MP2	Orange	Round	Concave	Round
MP3	Transparant	Concentric Round	Concave	Undulated
MP4	White	Irregular	Raised	Serrated

Notes : MDC = MOL daun cebreng MBP = MOL bonggol pisang
MPW = MOL pucuk waluh MP = MOL pisang
MM = MOL maja MR = MOL rebung

Antagonism test

From the 19 bacterial isolates, 6 isolates were antagonistic to *Rhizoctonia solani*. The inhibition percentages are shown in Table 2.

Table 2. Inhibition of MOL isolates to *Rhizoctonia solani*

No.	Treatment	Inhibition (%)	
1.	MOL Maja 1	25,17	d
2.	MOL Maja 2	19,83	c
3.	MOL Maja 3	8,33	b
4.	MOL Maja 4	8,89	b
5.	MOL Maja 5	9,00	b
6.	MOL Maja 6	8,78	b
7.	MOL Maja 7	52,11	gh
8.	MOL bonggol pisang1	48,89	b
9.	MOL bonggol pisang 2	8,33	b
10.	MOL bonggol pisang 3	8,28	b
11.	MOL daun Cebreng 1	67,83	i
12.	MOL daun Cebreng 2	50,72	gh
13.	MOL daun Cebreng 3	44,78	e
14.	MOL pisang 1	9,78	b
15.	MOL pisang 2	9,56	b
16.	MOL pisang 3	7,56	b
17.	MOL pisang 4	5,94	b
18.	MOL pucuk waluh	54,44	h
19.	MOL rebung	8,94	b
20.	Kontrol	0	a

Some of the bacterial isolates were able to inhibit the growth of *Rhizoctonia solani* on PDA. Isolates from MOL daun cebreng caused the highest inhibition. It was assumed that the endophytic bacteria in the leaves of cebreng acted as the antagonistic bacteria.

Results of some laboratory tests showed that many strains of bacteria were able to protect rice from blas, sheath blight, sheath rot, and stem rot (IRRI, 2003). *Pseudomonas fluorescens* was reported as the biocontrol agent of rice blas (Vasudevan et al., 2002 *in* IRRI, 2003). The microorganisms which cause in vitro inhibition of the pathogen growth will also cause in vivo inhibition in a glass house trial (Broadbent *et al.*, 1971 *in* Hasanuddin, 2003). The antagonistic bacteria produce antibiotics and siderofor, and also act as the competitors.

The percentages of the germinating seeds were higher in those treated with the antagonistic isolates compare to control. MBP3, MM6, MM5, MP1, MP3, MP4, MDC3 and MM7 caused lower percentage of germinating seeds. Those isolates were probably pathogenic to rice.

MP2, MBP2, MBP1, and MM2 did not affect the seed germination. MM1, MM5, MM7, MBP1, and MDC2 had the potency to increase the germination of seeds and the growth of seedlings. It was assumed that these isolates acted as Plant Growth Promoting Rhizobacteria (PGPR).

Kishore et al. (2005) reported that some bacteria isolated from phylloplane and applied as seed treatment were able to promote the growth of peanut and induce the resistance to pathogens. It was also reported that the phylloplane bacteria had higher ability than the rhizoplane bacteria.

Table 3. Seed germination and seedling length

No.	Treatment	Germination (%)		Seedling length (cm)	
1.	MOL Maja 1	85.55	l	8.6	l
2.	MOL Maja 2	78.15	j	8.55	l
3.	MOL Maja 3	88.75	l	8	k
4.	MOL Maja 4	68.8	e	7.5	j
5.	MOL Maja 5	78.85	j	6.15	bc
6.	MOL Maja 6	71.05	f	6.3	bcd
7.	MOL Maja 7	97.95	l	7	hi
8.	MOL bonggol pisang1	81.05	k	6.55	def
9.	MOL bonggol pisang 2	58.9	bc	5.35	a
10.	MOL bonggol pisang 3	45.9	a	6	b
11.	MOL daun Cebreng 1	70	f	6.45	cde
12.	MOL daun Cebreng 2	81.4	k	8.3	l
13.	MOL daun Cebreng 3	75.9	i	6.85	fg
14.	MOL pisang 1	73.8	g	5.3	a
15.	MOL pisang 2	45.05	a	7.2	lj
16.	MOL pisang 3	58.2	b	7.2	lj
17.	MOL pisang 4	75.4	hi	5.25	a
18.	MOL pucuk waluh	60.65	d	6.7	efg
19.	MOL rebung	59.95	cd	6	b
20.	Kontrol	74.65	gh	6.075	b

SUMMARY

From the experiment it was obtained 2 bacterial isolates from MOL daun cebreng which were antagonistic to *R. solani* and able to promote the seed germination and the growth of seedlings (PGPR); and 3 bacterial isolates from MOL maja which were able to promote the growth of rice seedlings (PGPR).

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