Antagonistic Potency of Bacteria Isolated from Local Microorganism of Banana Stump in Suppressing Blast Disease (*Pyricularia grisea* [Cooke] Sacc) on Rice

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ABSTRACT

Blast disease caused by *Pyricularia grisea* [Cooke] Sacc. is the most important disease on dry land rice. The use of antagonistic bacteria as bio-control agents is an alternative control method and friendly to environment.

The objective of the research was to isolate bacteria from Local Microorganism (MOL) of banana stump which were antagonistic to *P. grisea* in vitro and able to suppress blast on dry land rice var Cirata in glasshouse. The research was carried out in the Phytopathology Laboratory and Glasshouse of the Department of Plant Pests and Diseases, Faculty of Agriculture, Universitas Padjadjaran from December 2008 to March 2009.

There were two experiments i.e.: dual culture test on potato dextrose agar (PDA) and antagonism test on dry land rice var Cirata in the glasshouse. Both treatments were arranged in the completely randomized design consisted of 4 replications for in vitro and 3 replications for glasshouse test.

Among the fourteen bacterial isolates tested by dual culture, 7 isolates were antagonistic against *P. grisea* in vitro. The highest suppression was shown by B11BP isolate, the in vitro inhibition was 69,4%. However, the seven bacterial isolates were not effective in suppressing blast on dry land rice var Cirata in the glasshouse.

Key Word : Bacteria, Microorganisme Local, Blast, Rice

INTRODUCTION

One of the constraint factors of rice production in the world is blas disease (Agrios, 1997; Koga, 2001). This disease has lowered the the world's rice harvest around 50-90% (Agrios, 1997; Baker *et al.,* 1997). Blas disease's attacks could reach 12% of the total rice plantation area in Indonesia (Badan Pusat Statistik, 2004). Blas disease caused by the fungi *Pyricularia grisea* [Cooke] Sacc. *Pyricularia oryzae* Cavara synonyms.; teleomorph *Magnaporthe grisea* [Hebert] Barr., are important pathogenic fungi on rice plants (Rossman *et al.,* 1990 *in* Reflinur et al., 2006; Manandhar *et al.,* 1998; Suparyono *et al.,* 2003) .

Organic plantation system with SRI *(System of Rice Intensification)* using a mixture of natural materials found in the area around rice plantation as the effort to control organisms destructive plant. The material used is the bananas stump, coconut water, water, rice, maja fruit, bamboo sprout, papaya, bananas, water, sugar cane, squash leaf shoots, golden slug, and vegetables. Material's composition is adjusted to the available source material, and then fermented. Results of fermentation are known as the Local Microorganisms or MOL (Ekamaida, 2008; Hersanti & Djaya, 2008). MOL has been used by rice farmers, especially rice farmers using SRI planting methods (Hersanti & Djaya, 2008). According to Uphoff (2004) organic rice intensification system does not require chemical fertilizers and other chemicals, but will use the materials available in nature.

MOL usage besides as fertilizer and decomposer in the SRI component technology, MOL is also used as a natural pesticide to control plant's pest organisms (Anugrah, 2007; Ekamaida, 2008; Hersanti & DJAYA, 2008). Hersanti & Djaya research (2008) has been obtained 19 (Nineteen) isolated bacteria of different types of Mol which are from cebreng leaves' Mol, shoots squash's Mol, maja fruit's MOL, bamboo sprout's MOL, bananas' Mol, banana tuber's MOL. Test results of the *dual culture* of 19 (nineteen) isolated bacteria note 6 isolated bacteria have the antagonistic ability against fungus *Rhizoctonia oryzae* and 4 of isolated bacteria have the antagonistic ability against fungus *Cercospora oryzae.*

The availability of MOL raw materials needs to be put under consideration. The use of banana's stumps as the primary raw material in making of MOL has a strategic value, as there are many banana plantations found in Indonesia. In Asia, including Indonesia as the largest producer of bananas around 50% of banana production comes from Asia, Indonesia became fourth largest bananas production countries in the world. Almost all part of Indonesia is a regional producer of bananas (Astawan, 2008). Information of MOL usage with bananas stumps as the main raw material is expected to be one of the alternative controls of plant diseases. Antagonist ability of the bacteria that come from to control blas diseases is not yet known. Therefore, the research of utilizing antagonist isolated bacteria from bananas stump's Mol needs to be done, so it can be used as bio control agent in controlling the blas disease on rice.

RESEARCH METHOD

Research conducted in the Laboratorium Fitopatologi dan Rumah Kaca Jurusan Hama dan Penyakit Tumbuhan, Fakultas Pertanian, Universitas Padjadjaran, Jatinangor. Method used in this research is two stages of research scale which are *in vitro* test and In-vivo (greenhouse) test.

Testing the in-vitro

Testing potential antagonistic isolate bacteria of banana stump's MOL to prevent the growth of fungi *P. grisea* is done using the method *of dual culture test* with the Complete Random Design . Isolated bacteria obtained on the banana's stumps Mol are 14, then tested the treatment consisted of 14 treatments with 1 control.

Testing the in-vivo

Tests for antagonist isolated bacteria ability to press blas disease on rice are done using experiment method with the Complete Random Design (RAL). Treatment is based on the number of candidates tested antagonist bacteria, which is as much as 7 isolated antagonist bacteria obtained after the *in vitro* test-scale*,* and added a treatment of active isoprotiolan fungicide, positive control *(P. grisea* inoculation fungi), and negative control (without *P. grisea* inoculation fungi). Each treatment was repeated three times. Observation data analyzed statistically using the program SPSS version (16.0). Differences between treatments tested using the Duncan Double Distance Test in the 5% significant milestone.

RESULTS AND DISCUSSION

In-vitro test

In the banana stump MOL the in *Nutrient Agar* (NA) medium was found as much as 14 isolated bacteria. Isolated bacteria found are differentiated according to the color of the colony morphology, shape, surface, and the colony edge (Leung & Liu, 2002; Johnston, 2007). Characteristic colony morphology of 14 isolated bacteria can be seen in Table 1.

Table 1. Isolated bacterial colonies Morphology of banana stump's MOL on the *Nutrient Agar* (NA) medium

No	Isolat	Colony morphology			
		Color	Shape	Surface	Edge
1	B1BP	White	Circular	Raised	Curled
2	B2BP	White	Circular	Flat	Undulate
3	B3BP	Beige	Circular	Raised	Entire
4	B4BP	White	Circular	Umbonate	Entire
5	B5BP	White	Circular	Flat	Lobate
6	B6BP	White	Irregular	Flat	Entire
7	B7BP	Beige	Irregular	Convex	Entire
8	B8BP	Beige	Circular	Convex	Entire
9	B9BP	Beige	Circular	Convex	Undulate
10	B10BP	White	Filamentous	Flat	Filiform
11	B11BP	Yellow	Circular	Raised	Entire
12	B12BP	Yellow	Circular	Umbonate	Entire
13	B13BP	Beige	Irregular	Flat	Undulate
14	B14BP	White	Circular	Flat	Curled

Description: **B1BP - B14BP = bacteria of the 1st** Banana's stump, onwards.

Statistical results of the calculation the average percentage of deceleration in Table 2, indicates that bacteria with 8 isolate code B1BP, B2BP, B4BP, B5BP, B8BP, B10BP, B11BP and B12BP significantly different when compared with the control treatment.

Table 2. Persecution Percentage of 14 isolate bacteria of bananas stump's MOL against *P. grisea* dual culture test on 8th day.

No	Treatment	The average percentage of deceleraton (%)
1	B1BP	50.5 ab
2	B2BP	34.1 abc
3	B3BP	7.1 de
4	B4BP	44.1 abc
5	B5BP	43.5 abc
6	B6BP	13.1 de
7	B7BP	13.4 de
8	B8BP	29.5 bcd
9	B9BP	8.9 de
10	B10BP	50.5 ab
11	B11BP	69.4 a
12	B12BP	67.7 a
13	B13BP	2.9 e
14	B14BP	20.2 cde
15	Control	0.0 e

Description: The average value which followed by the same letter in the same column do not differ significantly according to Duncan Double Distance Test in 5% significant milestone

Observation results for activity deceleration are indicated by clear zone between the colony fungus *P. grisea* and the isolate bacterial in the medium. Antagonist bacteria produce the antibiotics, siderofor, and other secondary metabolite that does damage to the growth of microorganisms or prevent other activities (Fravel Hasanuddin *in* 1988, 2003). As presented by Howell and Stipanovic (1979) to Hasanuddin (2003), that the antibiotics bacteria *Pseudomonas fluorescens* effectively pressing the growth of *Rhizoctonia solani,* the pathogenic cause of topple shoot disease in the cotton sprout plants and also pyoluteorin antibiotics that can suppress *Pythium ultimum* growth.

Some bacteria have been widely used as bio control agents through the emphasis of antibiotics production to suppress the development of pathogen or disease (Mukerji & Garg, 1988 *in* Yulia et al. 2008). Microbes that produce antibiotics considered most appropriate to use as bio control agents compared with the other antagonism ways such as competition and parasitism (Yulia et al. 2008).

Other mechanism of the bio control agents is the consumption competition of food source or certain elements burly, so that constrain the growth room of other microorganisms (Weller Hasanuddin *in* 1988, 2003; Brock, 1966 *in* Sudadi, 2005).

In-vivo test

On the observation results of known incubation period of infectious blas diseases of rice plants has the different appearance on each bacteria treatment test. Results of Datnoff & Rodrigues' research (2005) of blas symptoms on leaf occurs the 4^{th} -5^{th} the day after inoculation. Symptoms appear as -small brown or black speck that appears after the first inoculation the fungus *P. grisea.*

Table 3 shows that the intensity of the blas disease in the 22^{nd} day after inoculation is not high. The intensity of the disease is decreased compared with the 15^{th} day after inoculation. This outcome occur because as the plants itself grow, also their ability to suppress disease become stronger (Sinaga, 2007).

Other factors that cause blas disease to have lower intensity may be due to the number of *P. grisea* fungi that have been inoculated too small. In addition, fungi *P. grisea* that have been growth on medium for inoculum's multiplication was suspected lowering their capabilities in infecting rice plant.

Table 3. Blas disease intensity on leaves of rice plants

No	Treatment	Blas disease incubation period (days)	Average intensity blas leaf disease (%)		
			8^{th} Day after inoculation	15^{th} Day after inoculation	22^{nd} Day after inoculation
1	B1BP	8	1.67 a	5.45 a	5.16 a
2	B2BP	9	0.00 a	5.58 a	5.11 a
3	B4BP	8	0.93 a	4.81 a	5.02 a
4	B5BP	9	0.00 a	6.67 a	5.29 a
5	B10BP	9	0.00 a	5.06 a	4.94 a
6	B11BP	11	0.00 a	4.94 a	4.76 a
7	B12BP	8	0.93 a	6.17 a	5.20 a
8	Fungicide (isoprotiolan)	9	0.00 a	5.55 a	4.93 a
9	Positive control *(P. grisea)*	8	0.93 a	5.31 a	5.82 a

Description: The average value which followed by the same letter in the same column do not differ significantly according to Duncan Double Distance Test in 5% significant milestone

Experiment in the greenhouse also experienced some technical obstacles, such as humidity and air temperature is hard to control so that some fluctuations occurred. Humidity and air temperature in the greenhouse for each observation were in the range of 56-91% and 23-28,5 ^{o} C, so that does not support the development of the fungus *P. grisea* to grow optimally. In general, the symptoms of blas leaves disease would appear and grow on about 90-92% humidity and temperature around 24 ^{o} C (Suparyono *et al.,* 2003; Rodrigues & Datnoff, 2005). The low blas disease intensity also caused by the variety of paddy gogo Cirata seed used which some what resistant to the disease blas (Balai Besar Penelitian Padi, 1996).

CONCLUSION

Results of banana stump's Local microorganisms (MOL) bacterial isolation obtained 14 isolate bacteria. Results of the *dual culture* test have 7 isolate bacteria antagonistic potential against fungi *Pyricularia grisea* [Cooke] Sacc. Treatment B11BP isolate bacteria showed the highest percentage of the 69.4%.

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