Available Online at ESci Journals
ESci Journal of Plant Pathology
ISSN: 2305-106X (Online), 2306-1650 (Print)
http://www.escijournals.net/EJPP

VIGOR OF PLANTLET FROM MICROPLANTLET TREATED BY FILTRATE AND CELL SUSPENSION OF SOME ISOLATES OF BACILLUS AND RESISTANCE TO BANANA WILT PATHOGEN AFTER ACCLIMATIZATION

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A B S T R A C T

Blood Disease Bacterium (BDB) and *Fusarium oxysporum* f.sp. *cubense* (FOC) is a couple wilt pathogen of banana. These pathogens are the most important constraint in cultivation of banana in Indonesia. In the integrated control strategy of the disease, the use of healthy seedlings produced from tissue culture technique is recommended. The seedling produced by tissue culture technique however leads to lower vigor and susceptibility to the disease due to the aseptic work *in vitro* causing the beneficial bacterial endophytic to be eliminated. Therefore, the utility of the beneficial endophytic bacteria should be studied for recovering the vigor and resistance of the seedling. Three isolates of endophytic Bacillus (B04, B05, B10) have been effective as growth promoter of microplantlet and antagonist of BDB and FOC *in vitro*. Here then, this article reports the study results of the vigor of the plantlet (treated microplantlet by filtrate or cell suspension of the Bacillus) after 3 months in acclimatization. The results were similar to the previous results on microplantlet *in vitro*, that Bacillus isolates B04, B05, and B10 were capable of promoting the growth and inducing the resistance to wilt pathogens on banana plantlets. The treatments with bacterial cell inoculums were more effective than those bacterial filtrate. Isolate B10 was most potential followed by B05 and B04 respectively.

Keywords: Banana, Blood Disease Bacterium, *Fusarium oxysporum* f.sp. *cubense*, Bacillus.

INTRODUCTION

Bacterial wilt caused by *Blood Disease Bacterium* (BDB) and fusarial wilt caused by *Fusarium oxysporum* f.sp. cubense (FOC) are two destructive diseases of banana. The diseases are the most important constraint in cultivation of banana in Indonesia. The earlier pathogen is most virulent on cooking banana group having genomic ABB i.e. Kepok by which in the fields, the disease incidence could reach over 80% (Mulyadi and Hernusa, 2001; Supeno, 2001; Sudirman & Supeno, 2001). Whereas the later pathogen is most virulent on table banana group having genomic AAA i.e. Ambon (Wibowo *et al*., 2007). Co-infection of the couple pathogen on single banana were most frequent in the field conditions causing severe disease than individual (Hadiwiyono *et al*., 2007).

One of the important components in integrated disease management is the use of healthy seedlings. The healthy seedlings of banana however are difficult to identify. In the evident, 20-30% of appeared healthy banana were infected by BDB (Hadiwiyono, 2010). Tissue culture technique is considerable to provide free seedlings-pathogens (Sunaryono, 2002; Nisa & Rodina, 2005). Smith *et al*. (2003) reported that banana seedling produced by tissue culture technique however was susceptible to wilt pathogen caused by the aseptic conditioning work throughout culture process *in vitro*. It is relevant to the result study on the community analysis of endophytic bacterium based on polymerase chain reaction-ribosomal integenic specer region analysis (PCR-RISA) showing that the symptomatic-less was more diverse and different from those symptomatic one (Hadiwiyono *et al*., 2009; Hadiwiyono, 2010).

A part of the beneficial genus of bacterial endophytic is *Bacillus* that live and associated with most of the plants, promoting plant growth, and inducing resistance to some important diseases. This is due to the capability of

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the bacterium to produce *indole acetic acid like substances* (IAAS), siderophore, antibiotic, and to dissolve phosphate (Hung & Annapurna, 2004; Compant *et al*., 2005; Lee *et al*., 2005; Wahyudi *et al*., 2011; Here et al., 2007; Zhao *et al*., 2011). Therefore, the use of endophytic Bacillus as the plant growth promoting and resistance inducers of banana wilt diseases is interesting to study. This research was aimed to study the effect of filtrate and inoculation of Bacillus to the vigor of the plantlet (treated microplantlet by filtrate or cell suspension of the Bacillus) after 2 months acclimatization and the resistance induction of the plantlet to BDB and FOC in screen house.

MATERIALS AND METHOD

Plantlet resulted from the microplanlet in the first year program was done to continue for acclimatization. It was aimed to evaluate the vigor of plantlet along acclimatization and resistance of the plantlet to banana wilt pathogen. The observation was lead to assess the percentage of live plantlet, the height of plant, the number of leaves, leaf area, root weight, and wilting intensity.

After acclimating for two months, the plantlets were inoculated by BDB and FOC to evaluate the effect of filtrate and cell of Bacillus as the agent of induced resistance to the pathogen on the polybag consisted of 1 kg soil infested by bacterial suspension of BDB 10^8 cfu/g of soil or spore suspension of FOC 10^5 spore/g of soil. The seedlings were incubated for 7 weeks after inoculation. The inoculums of BDB were prepared by growing the single colony BDB in CPB (*Casamino acid Peptone Broth*) incubated for 5 days. Whereas the inoculums of FOC were prepared by growing a bit of colony FOC on PDA incubated for 10 days. Spores of the pathogens were harvested by adding the culture with 10 ml sterile water and shaking gently to release the spore from their conidiophores. The conidiophore suspensions were collected and moved into Erlenmeyer tube (1L) as a suspension stock. Spore density of the suspension was determined by observing under microscope using haemocytometer.

All of the experiments were arrange by completely randomized design with 3 replications and each unit of treatment was an amount of 5 plantlets. The wilting intensity was assessed by ratting system from Winstead and Kelman (1952) modified by Hadiwiyono (2010).

$$I = \frac{\sum(n.v)}{100}.100\%$$

where *I*= wilting intensity (%); *n*= number of seedling with a certain score; *v*= scor of wilting (0, 1, 2, 3, or 4); *N*= total number of the seedling observed and *V*= highest score of wilting (4). Determination of the score was based on the rate of wilting as following : 0= no wilting leaf, 1= one of leaf-wilting, 2= two of leave-wilting, 3= three of leave-wilting, and 4= four or all of leave-wilting.

RESULTS AND DISCUSSION

The results showed that by 3 months after transplanting all of plantlets from microplantlet treated by filtrates and inoculation by cells suspension of Bacillus *in vitro* could grow a hundred percent. All of the treatments enhanced the growth of plantlet. It is clear from the results that all of the isolates of Bacillus (B04, B05, and B10) were potential as growth promoter of plantlet. The growth of treated plantlets were significantly different from untreated one (control treatment), the growth of treated plantlet were higher than those of untreated one (Table 1). It was showed by both the filtrate and inoculums of Bacillus. Statistically, between the isolates were not significantly different in capability of promoting the growth of plantlet. Descriptively however, the treatments using Bacillus cell inoculums tended to be more effective than those Bacillus filtrate. Bacillus isolate B10 was the most potential, followed by isolate B05 and B04 respectively. It was indicated consistently on most of the variables such height of plantlet, leaf area, and weigh of roots. Number of leaf was just in exception showing the same between the isolates.

The present results show that the isolates could promote the growth of pantlet. It is in lined with the previous test in the first years, the isolates promoted the growth of microplanlet *in vitro* (Hadiwiyono and Widono, 2012). Compant *et al*. (2005) and Zhao *et al.* (2011) explained that *Bacillus* spp. was including Plant Growth Promoting Rhizobacteria (PGPR) because of the capability of microbe to produce IAA, release Siderophore, and take a role as biological control agents through inducing systemic resistance of plant and release antimicrobial.

Until 7 weeks after inoculation, all of plantlets from microplantlet treated by filtrate and cell suspension of Bacillus showed the wilting intensity being significantly lower than the plantlet with no treatments (Table 2). Although the control treatment hasn't been wilting totally or at 54.33%, the wilting intensity of treated plantlet just reached at 25.00%. The treatment with inoculation on plantlet tends to generate lower wilting

than the other treatment. The results of inoculation FOC on plantlet by 7 weeks after inoculation showed all of treatment with no wilting and just on control treatment showed wilting with low intensity (6.33%). It indicates that all of isolate of Bacillus are antagonistic to BDB and FOC.

Table 1. Vigor of plantlet from microplantlet treated by Bacillus filtrates and cell suspension by 3 months along acclimatization.

Isolate of Bacillus	Growing plantlet (%)	Height of plantlet (cm)	Number of Leaf	Leaves Area (cm)	Weigh of Roots (gram)
Bacillus Filtrate					
B04	100	46.90±5.11 b	6.00±0.00 b	492.52±22.02 b	10.25±1.20 a
B05	100	47.50±5.40 b	6.00±0.00 b	503.35±21.00 b	10.05±1.22 a
B10	100	48.00±5.75 b	6.00±0.00 b	534.67±23.03 b	11.63±0.93 b
Bacillus Cell Inoculums					
B05	100	47.70±5.03 b	6.00±0.00 b	600.73±16.00 c	12.05±1.22 b
B10	100	48.00±5.21 b	6.00±0.00 b	680.26±13.03 d	12.63±0.93 b
No treatment	100	38.10±7.53 a	5.70±0.48 a	419.15±09.21 a	10.49±0.94 a

*the average marked by the same letter is not significantly different based on DMRT at 5%

Table 2. Resistance induction on microplantlet and plantlet to *Blood Disease Bacterium* and *F. oxysporum* f.sp. *cubence.*

Treatment and Isolate of Bacillus	Wilting Intensity (%)*	
	Blood Disease Bacterium	***F. oxysporum* f.sp. *cubence***
Filtrate on microplantlet		
B04	20.00±6.00 bc	0.00
B05	14.00±3.00 bc	0.00
B10	23.00±3.46 bc	0.00
Inoculation on microplantlet		
B04	13.67±3.05 a	0.00
B05	19.67±4.62 bcd	0.00
B10	25.00±3.00 d	0.00
Filtrate on plantlet		
B04	09.00±1.73 a	0.00
B05	07.43±1.50 a	0.00
B10	17.90±1.56 bcd	0.00
Inoculation on plantlet		
B04	08.33±2.05 ab	0.00
B05	05.43±1.89 a	0.00
B10	15.00±1.41 bc	0.00
No treatment	54.33±7.02 e	6.33±1.41

*the average followed by the same later are not significant different at level of 5 percent.

The present results are consistent with previous tests *in-vitro* that Bacillus isolates of B05, B05, and B10 were antagonistic to BDB and FOC. The isolates could produce antibiotic indicated by the zone of inhibition of the couple pathogen (Hadiwiyono & Widono, 2012). In accordance with previous evidents, some species of the genus of Bacillus were potential biocontrol agent of plant pathogen and plant growth promoting bacteria. Azevedo *et al*. (2000) reported that some endophytic bacteria from citrus were cultureable and Bacillus were included. Brooks *et al*. (1994) reported that 183 of 889 isolates of endophytic bacteria from *Oak* were antagonistic to wilt pathogen, *Ceratocystis fagacearum* in-vitro. Chen *et al*. (1998) also reported that 10 isolats of endophytic bacterium could suppress the disease severity of wilt cotton caused by *Fusarium oxysporum* f.

sp. *vasinfectum* through inoculating the seedling. Benhamou *et al*. (1998) added that *B. pumilus* race SE 34 could induce plant resistant of potato to wilt disease caused by *F.o.* f. sp*. radicis-lycopersici*.

Between the genus of Bacillus, some species have been reported to be having the role in promoting plant growth and disease control both caused by soil borne and air borne pathogens (Arwiyanto, 1997; Arwiyanto & Hartana, 1999; Cook & Baker, 1983; Nejad & Johnson, 2000; Bae *et al*., 2004; Compant *et al*. 2005; Wahyudi *et al*., 2011; Zhao *et al*., 2011). *B. cereus* could control take-all disease caused by *Gaeumannomyces graminis* on wheat of seedling (Cook & Baker, 1983). *B. subtilis* treatment on tuber could control bacterial wilt of potato and increase the yields of tuber reaching 160% (Sunaina *et al*., 2003). *B. subtilis* isolate A47 could activate biological control to Gram-negative bacterium due to by a metabolite compound the group of iturin being resistant to hydrolysis and thermo-stable (Ciampi *et al.*, 2003). *B. pumilus* strain INR7 and *B. subtilis* strain GB03 had capability of promoting the growth of plant and minimizing disease severity (Georg & Kloepper, 1998).

Endophytic bacterium such *Bacillus* spp. is promising biological agents of disease control and plant growth promoter than endophytic fungi. Bacillus is an endospore forming bacteria. Formulations of fungal spores are often environmental sensitive and have a shorter shelf-life compared to endospore forming bacteria, as endospores are highly resistant to heat and desiccation (Driks, 2004). In addition to the long-term viability of endospores, endospore-forming bacteria can often be successfully combined with agrochemicals (Jacobsen *et al*., 2004). Considering this background, endospore forming bacteria-based biological control agents offers a potentially superior alternative to fungal biological control agents, due to the resistant nature of the endospore. Some researchers conclude that *Bacillus mycoides* isolate BacJ (Bargabus *et al*., 2002) and *Bacillus pumilis* isolate 203-7 (Bargabus *et al*., 2004) suppressed Cercospora leaf spot in sugar beets. Suggesting the current results and discussion that Bacillus isolate B04, B05 and B10 should be studied further for biological control agents of BDB and FOC, in addition as plant growth promoting bacteria on the seedling of Banana producing with tissue culture techniques.

CONCLUSIONS

The present study on plantlet along acclimatization from the treated microplantlet until 3 months after transplanting showed that Bacillus isolate B04, B05, and B10 were potential as growth promoter and resistance bio-inducer agents to BDB and FOC. Both the bacterial filtrate and cell suspension could increase the growth of banana plantlet and reduce the wilting intensity. The bacterial cell suspensions however were more effective than those bacterial filtrates.

ACKNOWLEDGMENTS

This research was founded by *Hibah Bersaing* Project from the Directorate of Research and Social Services (DP2M), the Directorate-General of High Education, the Ministry of National Culture and Education of RI with Contract Number: 2339/UN27.16/PN/2012.

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