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Effectivity of Anatagonistic Bacteria in Controlling of Fusarium Wilt Diseases of Banana (*Musa paradisiaca*) by in Vitro

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Abstract. *Fusarium* wilt disease caused by *Fusarium oxysporum* f sp. *Cubennse* (*Foc)* is a major disease on Banana plant which lost production more than 50 %. This patogen is a soil-born disease and persistence until five years in the soil. Biological control is one of stratetegic diseases control need to applied to inhibit development of Fusarium wilt disease of Banana. The purposes of this study are (a).to isolate antagonistic bacteria from banana plant rhizosphere which has a good potensial to inhibit *Foc* growth *by in vitro*;(b)to know antagonistic mechanisms of selected bacteria by secondary metabolite production by *in vitro.* This study was carried out in Biology Laboratory Universitas Negeri Makassar with method as follows:(a).Isolation and purification of antagonistic bacteria from Banana plant rhizoaphere;(2).Test of dual culture; (3) Test of secondary metabolite substance. The result of this study showed that (a) there are four selected bacteria which have a good potential in inhibiting *Foc* growth by *in vitro* such as : Isolate B_6, B_8, B_2 and B_1 with inhibitor capacity 80.47%, 80.17%, 78.78% and 77.74%, respectively. (b) Inhibitor capacity of selected bacteria by chitinaze enzyme, pectinase and high antibiotic substance.

1. Introduction

Fusarium oxysporum f.sp cubense (Foc) is a species of pathogenic fungi causing wilt diseases on Banana plant (Musa paradisiacal). The diseases reduced production both quality and quantity. It is difficult to be controlled because it has Clamydospore, a resistant structure which can survive for a long time as saprophyte even without host plant [1]. Initial symptom of diseases was characterized by chlorosis leaves and then old leaves change to yellow. For further attack of disease, Banana plant will be wilt and brown vascular bundle symptoms until two meters from the soil surface [2].

Farmers as the main perpetrators of agricultural activities tend to use excessive synthetic pesticides especially for difficult-to-control pathogens such as soil borne pathogens. The use of antagonistic bacteria as a biological control agent ideally uses the potential of local natural enemies in the hope that antagonistic bacteria will work more effectively and supported by appropriate environmental factors. In cultivated land if the microbial rhizosphere is added, it may also be resistant [3] because there will be abundance of microbes in the rhizosphere that directly protects the roots against wilting pathogens. Microorganisms can act as natural antagonists of various plant pathogens. Systemic antagonistic bacteria that can breed in plant tissue ensure successful control of the field.

The farmers usually used chemical pesticide a high dosage to control soil-born pathogen.Usage of antagonistic bacteria as a biological control agent ideally by using indigenously natural enemy more

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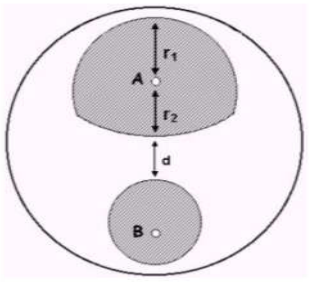
effectively and familiar with suitable environmental condition. Bioaugmentation of rhizosphere microbe will be possible resistant [3] because it will increase microbial population at rhizosphere area which prevent of roots from wilt pathogen. Microorganisms can be natural enemies for some plant pathogen. Antagonistic bacteria are systemic feature so they develop in plant tissues for successful treatment in the field control.

The effort to control of this plant pathogen by biological control agent has a high promising control because these agents are available exist in natural environment and their activity can be stimulated both environmental modification and host plant so that these microbes will grow well in rhizosphere area which prevent roots against wilt pathogen [4]. There were four microbial species which have a high potential in controlling F. oxyporum f.sp passiflorae by in vitro methods such as Bacillus sp (71.32 %), Trichoderma sp (86.11%), Gliocladium (78.54%) and Aspergillus sp (75.74%) [5]. Based on the background, It is important to do this research to investigate the effectivity of some species of antagonistic bacteria isolated from rhizosphere area of Banana plant to control Fusarium wilt disease of Banana plant.

2. Research Method

This research was conducted at Biology Laboratory State University of Makassar. Dilution soil samples from the rhizosphere of banana plants made from 10-1 to 10-6, the next was sample 10-6 dripped on the NA media as much 10-1ml. The ability of some bacteria in inhibiting pathogenic in vitro by using dual culture method direct opposition between bacteria of the rizosphere against pathogenic fungi Foc in PDA media.



Note :

A : Fusarium oxysporum f.sp Cubense (Foc)
d : Inhibition distance of microbial antagonistic to
B : Antagonistic bacterial species

$$P\ \frac{R1-R2}{R1}\ X\ 100\%$$

R1: Colonial diameter of fusarium (treatment) (cm)
R2 : Colonial diameter of fungi (control) (cm)
d : Percentage of growth inhibition (%)

Figure 1. Percentage of microbial inhibition test was calculated using the formula plant growth

Pathogenic growth was observed in 24 hours interval which started one day after application until ten days. Observation was stopped when the growth of pathogen closed to the edge of petri dish (control). The result of dual culture used in vitro technique will be obtained antagonistic isolates which have a good potential to control the growth of Foc. To understand mode of action of antagonistic bacteria were carried out by enzymatic and antibiotic tests. By qualitative analysis, It will be found out bacterial isolates which have a high potential in controlling Foc fungus.

Pure cultures of antagonistic bacterial isolates were identified based on [6], method namely. Their features were physiological and biochemical characteristics. Selected bacteria were quantitatively evaluated their potency in secreting extracellular enzymes. Qualitative analysis of enzymes were carried out on Czapek Dox Agar media (CDA) added with Commassssie Briliant Blue (CBB) with cellulose, chitin, and pectin substrates (0.1- 0.15 %) and pH 5.5. After inoculation of inoculum on CDA media. All inoculated petri dishes covered with paper then incubated for three days. Zone of color change on media occurred after 2- 3 days cultivation. Enzymatic activity levels were measured based on the change of color on media.

3. Results and Discussion

The results showed that bacterial isolates could inhibit *Foc* every two days observation significantly different with control (Table 1). *Foc* growth was unable to grow in the bacterial colonial zone. This inhibitory ability is due to the ability to compete for space. nutrients and oxygen so that antagonistic bacteria can grow quickly and inhibit the growth of *Foc* The capacity of inhibition caused by competition in space, nutrition and oxygen so that antagonistic bacteria can grow well and control growth of *Fusarium oxysporum*.

Tabel 1. Inhibition percentage of rhizoz sphere bacterial isolates from banana plants against *Foc* on PDA medium

	Average of inhibition activity (%)				
Isolates	**2 days**	**4 days**	**6 days**	**8 hari**	**10 days**
Control	0^{d}	0^{d}	0^{d}	0^{d}	0^{d}
Bacillus sp (B_1)	49.54^{c}	56.55^{d}	60.96^{abc}	65.58^{ab}	76.85^{b}
Bacillus sp (B_2)	58.42^{bc}	61.02^{cd}	62.25^{abc}	67.93^{a}	78.78^{b}
Bacillus sp (B_3)	54.12^{c}	55.85^{d}	64.18^{ab}	68.19^{a}	77.74^{b}
Bacillus sp ($B_{4)}$	56.19^{c}	55.08^{cd}	56.55^{bc}	70.13^{a}	74.63^{b}
Bacillus sp ($B_{5)}$	69.81^{ab}	73.37^{a}	72.69^{a}	73.08^{a}	74.64^{b}
Bacillus sp ($B_{6)}$	70.22^{ab}	69.74^{ab}	70.81^{ab}	76.14^{a}	80.46^{a}
Pantoea sp ($B_{7)}$	50.31^{c}	56.26^{a}	56.44^{bc}	54.36^{bc}	68.05^{bc}
Bacillus sp (B_8)	78.11^{a}	72.74^{a}	71.48^{ab}	70.67^{a}	80.17^{a}
Pantoea sp (B_9)	48.05^{c}	49.32^{c}	47.00^{c}	52.67^{c}	56.96^{c}
Pantoea sp (B_{10})	53.56^{c}	52.28^{c}	62.75^{b}	69.33^{b}	76.91^{b}

Isolates of banana rhizospher bacteria. The same letter means there was significant difference on Duncan test $\alpha = 0.05$.

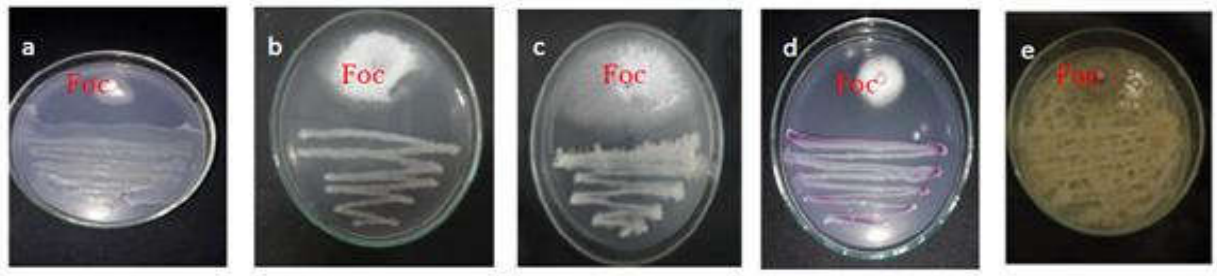


Figure 2. Inhibition activity of antagonistic bacterial isolates, *Bacillus* sp against Foc on PDA medium : a. *Bacillus* sp (B1); b. *Bacillus* sp (B3); c. *Bacillus* sp (B4); d. *Bacillus* sp (B5) ; and e. *Bacillus* sp (B8).

All antagonistic bacterial isolates had capability to inhibit *Foc* growth, but they have growth rate relatively different. There were two selected bacterial isolates which have the highest inhibition until 10 days on agar media such as B_6 and B_8 isolates. B_2 isolate showed the second highest inhibition but it was not significantly different with B_1, B_3, B_4 B_5 and B_{10} isolates. While the lowest inhibition level were treatment B_7, B_9 and B_{10}. Percentage of inhibition activity was B_6, B_8, B_2 and B_1; 80.4%, 80.17%, 78.78% and 77.74 %, respectively. Different inhibition zone occurred indicated that there were secondary metabolite secreted by bacteria. The higher secondary metabolite produced by bacteria, the more inhibition zone against *Foc* growth compared with control.

Tabel 4. Enzyme and Antibiotic produced by microbial isolates from the rhizosphre of Banana Plants by using in vitro method

Microbial Isolates				Antibiotik
	Cellulase	Chitin	Pectin	
Bacillus sp (B_1)	++	+++	++	++
Bacillus sp (B_2)	++	++	++	++
Bacillus sp (B_3)	++	+++	+++	+
Bacillus sp ($B_{4)}$	++	++	+++	++
Bacillus sp ($B_{5)}$	++	++	+++	++
Bacillus sp ($B_{6)}$	++	+++	++	+
Pantoea sp ($B_{7)}$	+	++	++	+
Bacillus sp (B_8)	++	+++	+++	++
Pantoea sp (B_9)	+	++	++	+
Pantoea sp (B_{10})	+	++	++	+

B = Bacterial isolates from Markisa Enzyme [- = not formed a clear zone ; ++++ = ≥ 75% clear zonae; +++ = > 50-75% clear zone; ++ 25-50% clear zone ; + < 25% clear zone]
Antibiotics [- = not formed clear zone ; ++++ = > 4,0 cm clear zone; +++ = > 2,0 cm – 4,0 cm clear zone; ++ = 0,5 – 2,0 cm clear zone; + < 0,5 cm clear zone zona clear .
+(weak), ++ (moderate), +++ (strong), ++++ (very strong).

Bacillus sp has the best ability to suppress the growth inhibition of *Fusarium* sp by using in vitro method in limited condition such as space and nutrition, The mechanisms of inhibition are antibiosis and nutrient competition with the highest inhibition percentage was 80.46%.. *Bacillus* sp produce enzymes and antibiotics which are the pathogens of inhibiting growth factors, the presence of secondary metabolites produced by *Bacillus* sp could be expected to suppress the growth of *Foc* and neutralize toxins released by *Foc*. According to [7] stated that *Bacillus* sp can to produce antibiotics that are toxic to the pathogen, but it also can produce spores and colonize the root so that It can survive in extremely environmental conditions that are potentially used in biological control.The existence of endospores is an advantage for the biotechnology application [8].

The work of antimicrobial substances inhibiting other microorganisms is to damage the cell wall, alter the permeability, damage the membrane of the nucleus so that it will lead to inhibition of cell growth or cell death resulting in cell damage [9]. Bacillus sp is capable of producing antibiotics that are toxic to pathogens because in very small amounts it is destructive or inhibits other microorganisms, it is also capable of producing endospores that can survive in extreme conditions so it is potentially used in biological control. Mode of action antimicrobial substances in controlling plant pathogen destroys cell walls, permeabiablity change, nuclear membrane so it can cause sel growth inhibition or cell death. *Bacillus* sp produces antibiotic which toxic to plant pathogen and control other microorganisms. It can produce endospore which resistant to extreme condition so it has high potential to be used as biological control agent [7].

Inhibitory capability of antagonistic bacteria caused is caused by they have a good characteristics in competing especially in space, nutrition and oxygen so antagonistic bacteria grow well and can suppress growth of *Foc* fungus. Disease control by using biological agent need to be developed because of cheap, easy to do and friendly environmental control strategy. Rhizosphere nicrobes are very suitable to be used as biological control agent because rhizosphere area can be colonized by some pathogenic microbes. Negative interaction will occure between antagonistic bacteria and plant pathogen when they infected the plant roots [10]. Most microbial activities are located in root tip area because they produce a lot of exudates substance which secreted by plant root. Bacterial population in the the root system normally spread and colonize of root and stability of microclmate around rhizosphere zone [8].

It can be concluded that bacillus sp is able to inhibit the growth of *Foc*., Bacterial isolation inhibition ability because it produces high enzyme and antibiotic substance. controlling using

biological agents is an option that needs to be developed because this control is relatively inexpensive, easy to do and environmentally friendly. *Bacillus* sp. can inhibit growth of *Foc* due to these bacteria produce both high enzyme and antibiotics substances. Usage of biological control agent is alternative control to be developed because of chief, easy to be done and environmentaly friendly control strategy.

4. Conclussion

There are four selected bacteria which have a good potential in inhibiting Foc growth by in vitro such as: Isolate B6, B8, B2 and B1 with inhibitor capacity 80.47%, 80.17%, 78.78% and 77.74%, respectively. Inhibitor capacity of selected bacteria by chitinaze enzyme, pectinase and high antibiotic substance.

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