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The Potency of *Bacillus siamensis* LDR as Biocontrol Agent Against Fungal Phytopathogen

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ABSTRACT
Bacillus spp. was recognized as biocontrol agent because could produce antifungal towards many phytopathogenic fungi. In relation to study the potency of *B. siamensis* LDR as biocontrol, antagonistic activity of the bacilli was evaluated against *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228. The antagonist assay was carried out by dual culture method using streak and pour plate technique. Result of antagonistic activity assay with streak technique showed that growth inhibition rate of *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228 were 36.83%, 55.20%, and 33.78%, respectively. Antagonist assay using pour plate technique showed that percentage growth of inhibition for *Fusarium* sp. (93.96%), *Ganoderma* sp. (100%), and *Chaetomium globosum* InaCC F228 (76.64%) were higher than the results from streak method. Furthermore, results from antibiosis assay showed that growth inhibition rate of *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228 were 10.85%, 73.31%, and 30.32% respectively.

Keywords: *Antifungal activity, Bacillus siamensis LDR, dual culture*

1. INTRODUCTION

Fungal phytopathogen was most devastating problem in agriculture industry. It could reduce the productivity and also quality of the plant products and further more can affect its economic value. Some of the common fungal phytopathogen which usually infect plant agriculture were *Fusarium* sp., *Ganoderma* sp. and *Chaetomium* sp. Infection of *Fusarium* sp. on agricultural plant causes vascular wilts, root rots, and yellowing disease [1]. *Ganoderma* sp. can infect stem of plantation plant, such as oil palm (*Elaeis guineensis*) and coconut plant (*Cocos nucifera*) cause rot of the plant [2]. Susanto et al. [3] stated infection of *Ganoderma boninense* cause basal stem rot disease which is most destructive disease due to more than 50% productive plant have died. Another fungal phytopathogen, *Chaetomium* sp. was known firstly cause leaf spot on *Hevea brasilliensis* [4] and *Punica granatum* by Guo et al. [5].

Therefore, infection of those fungal phytopathogen should be managed and controlled. Nowadays, biocontrol agent was more common to be used to overcome plant fungal infection. It is because the biocontrol agent is more eco-friendly compare to chemical pesticide.

The genus *Bacillus* has been known as one of potential biocontrol agent. The *Bacillus* spp. have more advantages such as rapid growth, can grow in simple nutrition, have thick peptidoglycan, and produce endospore. The thick peptidogliycan and endospore will make cells are more resistant to the environmental stress [6]. *Bacillus* are reported to produced several bioactive compounds including antifungal. *Bacillus siamensis* JFL15 was reported can produce lipopeptide antifungal compound which inhibit *Colletotrichum gloeosporioides* [7]. *Bacillus siamensis* LDR has been isolated from cocopeat [8]. Putri et al. [9] reported B. siamensis LDR could inhibit growth of Aspergillus niger ABP and ART. Moreover, Pertiwi et al. [10] reported *B. siamensis* LDR have antagonist activity against *A. clavatus, A. fumigatus*, and *A. tamarii*. The aim of this research is to explore the potency of antifungal activity from *B. siamensis* LDR against fungal phytopathogen, *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228.

2. MATERIALS AND METHODS

2.1. Microorganisms

The microorganisms used in this research were *Bacillus siamensis* LDR and fungal phytopathogen such as *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228. *Bacillus siamensis* LDR, *Fusarium* sp. and *Ganoderma* sp. were provided by Microbiology Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. *Chaetomium globosum* InaCC F228 was obtained from LIPI, Cibinong, Bogor. The micoorganisms were purified using quadrant streak method on Potato Dextrose Agar (PDA) medium. The single colony of each isolate was maintained in PDA medium slant.

2.2. Antagonistic Activity Assay

The antagonistic activity was done using dual culture method with streak [11,12] and pour plate technique [13,14] on PDA medium. In streak technique, *B. siamensis* LDR was streaked on PDA medium then the fungal phytopathogen, *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228 were inoculated 4 cm beside the bacteria. The pour plate technique was perfomed by inoculating cells suspension of *B. simaensis* LDR (200 µl) into melted PDA medium, then the medium was poured into petri dish. After the medium has solidified, cell suspension of fungal phytopathogen was inoculated in to paper disc and then was placed on the center of medium in petri dishes. Control was prepared same as treatment, but the PDA medium was not inoculated with bacterial cells.

The result of antagonist assay was observed after 5 days incubation at 28°C. The radius of fungal colony was measured from center of inoculated fungal colony up to the edge colony which is facing the bacterial colony. Meanwhile for pour plate technique the diameter of fungal colony was measured. The inhibition growth then calculated to obtained Growth Inhibition Rate (GIR) of fungal phytopathogen [11].

$$GIR\ (\%) = \frac{(C - T)}{T} \times 100\% \quad (1)$$

C= width mycelial of control fungal
T= width mycelial of treatment fungal

2.3. Antibiosis Activity Assay

Antibiosis assay was performed with filtrate fermentation of *B. siamensis* LDR against fungal phytopathogen according to Putri et al. [9]. The Potato Dextrose Broth (PDB) medium (200 mL) was inoculated by suspension cell of *B. siamensis* LDR (1% v/v, 10^7 CFU/mL). The medium was incubated for 14 days at 28°C with still culture method. After fermentation, the growth medium was centrifuged at 3000 g for 20 minutes to obtain the filtrate free of cells. The filtrate was used to dissolved PDA medium. As a control PDA was dissolved using aquadest. Cell suspension of fungal phytopathogen, *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228 were inoculated into paper disc (10 µL). The inoculated paper discs, then were placed on the center of PDA filtrate and PDA control.

The antibiosis activity was presented by inhibition growth of fungal phytopathogen after 7 days incubation at 28°C. The diameter of fungal phyopahtogen was measured using caliper and the data were represented as growth inhibition rate which were calculated according the formula of Li et al. [11].

3. RESULT AND DISCUSSION

3.1. Antagonistic Activity Assay

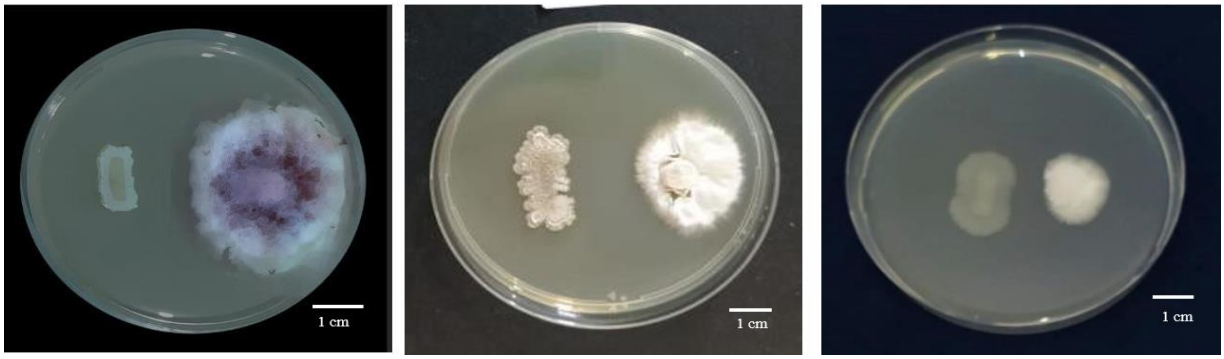
Antagonistic activity assay showed *Bacillus siamensis* LDR can inhibit fungal phytopathogen, *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228. The inhbition was represented as percentage of growth inhibition rate. Growth inhibition of fungal phytopathogen can be seen in streak and pour plate technique.

Result of antagonistic assay using streak technique showed in Table 1. *Bacillus siamensis* LDR can inhibit 36.83%, 55.20%, and 33.78% of *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228, respectively. Growth inhibition of fungal phytopathogen caused by *B. siamensis* LDR using streak technique were showed in Figure 1.

Antagonistic activity assay using pour plate technique also showed *B. siamensis* LDR have antagonistic activity against fungal phytopathogen (Table 2).

Table 1. Result of antagonist assay using streak technique of *Bacillus siamensis* LDR against fungal phytopathogen

Fungal phytopathogen	Repetition	Width of control (mm)	Width of treatment (mm)	GIR (%)
Fusarium sp.	1	**37.45±0.04**	22.99	38.61
	2		23.62	36.93
	3		24.36	34.95
	average		**23.66±0.69**	**36.83±1.83**
Ganoderma sp.	1	**25.12±1.15**	11.47	54.34
	2		11.04	56.05
	3		11.33	54.90
	average		**11.26±0.30**	**55.20±0.87**
Chaetomium globosum InaCC F228	1	**17.25±0.08**	11.61	32.70
	2		11.20	35.07
	3		11.46	33.57
	average		**11.42±0.21**	**33.78±1.20**



a **b** **c**

Figure 1. Growth inhibition of fungal phytopathogen fungi caused by *Bacillus siamensis* LDR using streak techique: **(a)** *Fusarium* sp., **(b)** *Ganoderma* sp., and **(c)** *Chaetomium globosum* InaCC F228

In pour plate technique, growth inhibition of fungal phytopathogen were higher compared to antagonist assay using streak technique. *Bacillus siamensis* LDR can inhibited *Fusarium* sp. (93.96%), *Ganoderma* sp. (100%), and *Chaetomium globosum* InaCC F228 (76.64%). The inhibition growth of fungal phytopathogen showed in Figure 2.

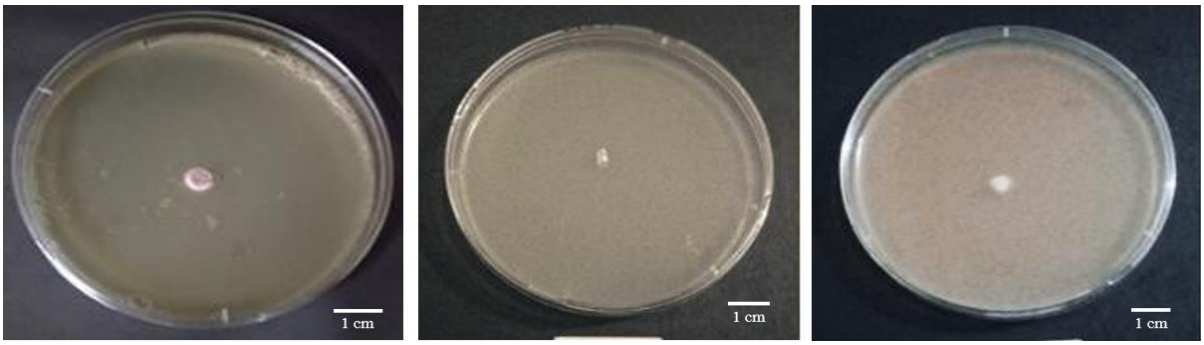
The *Bacillus* genus has been well known as potential biocontrol agent. *Bacillus* sp. can inhibit 54.2% growth of *Ganoderma boninense* [3]. Yuliar and Suciatmih [15] reported *B. amyloliquefaciens* EB13 have antagonistic activity against *Ganoderma boninense*. Furthermore, Khan et al. [1] stated *Bacillus* species was potential to become potential biocontrol against *Fusarium*. *Bacillus simplex* 30N-5 and *Bacillus subtilis* 30VD-1 can inhibit growth of *Fusarium* spp. Chen et al. [16] reported *B. velezensis* FJAT-46737 as potential biocontrol agent for *Fusarium oxysporum*.

3.2. Antibiosis activity assay

The result of antibiosis activity assay showed *Bacillus siamesis* LDR can inhibit all fungal phytopathogen tested (Table 3). The inhibition also respresented as percentage inhibition growth of fungal phytopathogen. *Bacillus siamensis* LDR can inhibit *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228 with percentage value 10.85%, 73.31%, and 30.32% respectively. Nevertheless, the percentage inhibtion of fungal phytopathogen were lowest than antagonist assay either in streak or pour plate technique. Growth inhibition of fungal phytopathogen can be seen in Figure 3.

Table 2. Result of antagonistic assay using pour plate technique of *Bacillus siamensis* LDR against fungal phytopathogen

Fungal phytopathogen	Repetition	Width of control (mm)	Width of treatment (mm)	GIR (%)
Fusarium sp.	1	**74.74±0.01**	4.87	93.48
	2		4.38	94.14
	3		4.29	94.26
	average		**4.51±0.31**	**93.96±0.42**
Ganoderma sp.	1	**30.95±3.04**	0.00	100
	2		0.00	100
	3		0.00	100
	average		**0.00**	**100±0.00**
Chaetomium globosum InaCC F228	1	**24.90±0.95**	5.59	77.55
	2		5.58	77.59
	3		6.28	74.78
	average		**5.82±0.40**	**76.64±1.60**



a b c

Figure 2. Growth inhibition of fungal phytopathogen fungi caused by *Bacillus siamensis* LDR using pour plate techique: (a) *Fusarium* sp., (b) *Ganoderma* sp., and (c) *Chaetomium globosum* InaCC F228

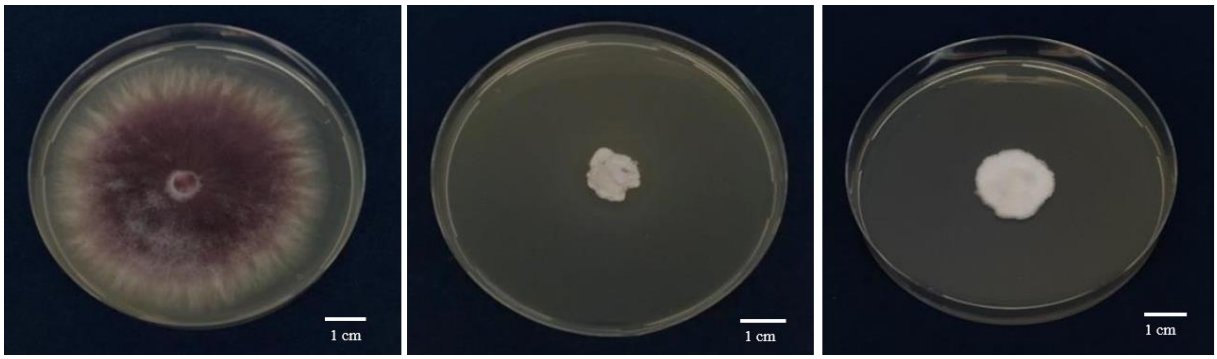
3.3. Discussion

Bacillus siamensis LDR has antagonistic and antibiosis activity against fungal phytopathogen such as *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228. The antagonistic activity was assumed that *B. siamensis* LDR produces antifungal compound which inhibit growth of the fungi tested. Result of antagonistic activity with pour plate technique showed in Table 2 was higher than streak technique in Table 1. In pour plate technique, the bacterial cell will spread over the medium and will dominate the space and nutrient uptake besides produce antifungal compound. The double effect of *B. siamensis* LDR will give more suppression to the growth of all fungal tested.

Result of antibiosis assay exhibited lower inhibition percentage value compared to antagonist assay. It indicated that the hyphae of *Fusarium* sp. were more resistant to antifungal produces by *B. siamensis* LDR. Probably the most essential antagonist effect was due to the fast growth of *B. siamensis* LDR cells. This will compete with the fungal cell, dominate the space and nutrient uptake as can be observed in pour plate technique. Alabouvette et al. [17] stated tnhat one of the mechanisms of antagonistic activity is through the competition of the nutrient.

Table 3. Result of antibiosis assay of *Bacillus siamensis* LDR against fungal phytopathogen

Fungal phytopathogen	Repetition	Width of control (mm)	Width of treatment (mm)	GIR (%)
Fusarium sp.	1	**87.49±0.06**	76.86	12.15
	2		79.93	8.64
	3		77.21	11.75
	average		**78.00±1.68**	**10.85±1.92**
Ganoderma sp.	1	**51.65±0.86**	13.38	74.09
	2		13.06	74.71
	3		14.92	71.11
	average		**13.79±0.81**	**73.31±1.92**
Chaetomium globosum InaCC F228	1	**37.97±0.96**	26.23	30.92
	2		27.54	27.47
	3		25.60	32.58
	average		**26.46±0.81**	**30.32±2.61**



a **b** **c**

Figure 3. Growth inhibition of fungal phytopathogen fungi in antibiosis assay: (a) *Fusarium* sp., (b) *Ganoderma* sp., and (c) *Chaetomium globosum* InaCC F228

4. CONCLUSION

In conclusion, *Bacillus siamensis* LDR can inhibited growth of fungal phytopathogen, *Fusarium* sp., *Ganoderma* sp., and *Chaetomium globosum* InaCC F228. The inhibition growth showed in result of antagonist and antibiosis assay. The antagonistic activity assay using pour plate technique showed highest inhibition of fungal phytopathogen compared to streak technique and antibiosis assay.

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