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Research Article

Inhibition of the phytopathogenic fungi *Curvularia lunata* BM and *Ganoderma* sp. TB4 by antifungal compounds produced by *Bacillus siamensis* LDR grown on hanjeli (*Coix lacryma-jobi* L.) starch

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Abstract

Bacillus siamensis LDR was tested for its potential as a biocontrol agent against the phytopathogenic fungi *Curvularia lunata* BM and *Ganoderma* sp. TB4. Fermentation of *B. siamensis* LDR for the production of antifungal compound was performed in modified Czapex-Dox broth using hanjeli (*Coix lacryma-jobi* L.) starch as carbon source. The *Bacillus siamensis* LDR inoculum was 10^5 CFU/ mL, and fermentation was conducted for up to 16 days. Antibiosis assay conducted to test the antifungal activity of filtrate medium. The results showed inhibition of *C. lunata* BM and *Ganoderma* sp. TB4 were 47.08% and 85.99%, respectively on 14th day of fermentation. Antifungal assay of the crude extract from filtrate medium revealed growth inhibition of *C. lunata* BM (60.70%) and *Ganoderma* sp. TB4 (65.25%). Thin layer chromatography of the crude extract revealed pink-colored spots indicative of lipopeptide compounds. Analysis of the crude extract by ultraperformance liquid chromatography-mass spectrometry was tentatively identified as iturin A, bacillomycin F, and surfactin.

Keywords

antifungal compound, *Bacillus siamensis* LDR, biocontrol agent, phytopathogenic fungi

Introduction

Agriculture is one of the most important national economic sectors in Indonesia. Palm oil and coffee are important agricultural commodities that contribute to Indonesian's foreign exchange (FAO 2017). Nevertheless, the agricultural sector is facing many problems, especially plant disease caused by infections of pathogens, such as fungi. Fungal infections can adversely affect agricultural products and lower their economic

value (Almeida et al. 2019). The most devastating phytopathogenic fungi affecting palm oil plantations are *Ganoderma* sp. (Hushiarian et al. 2013; Peterson 2019) and *Curvularia* sp. (Sunpapao et al. 2014; Agustika et al. 2019). *Ganoderma* sp. infection on the basal stems of palm tree (*Elaeis guineensis* Jacq.) is a serious problem that leads to stem rot and has significant negative impacts on oil palm production (Rees et al. 2009; Naher et al. 2013). *Curvularia* sp. attacks palm seedlings and cause leaf blight or leaf spots disease (Agustika et al. 2019), and it also infects other important crops, such as corn (Garcia-Aroca et al. 2018) and rice (Majeed et al. 2015). Therefore, the diseases caused by fungal infection should be controlled.

One sustainable control strategy is to use bacteria as biocontrol agents (Tiwari et al. 2019; Dame et al. 2021), as these can replace synthetic or chemical pesticides that can cause problems in human health and pollute the earth's ecosystems (Shafi et al. 2017). The use of chemicals can also threaten the survival of non-target organisms (Prapagdee et al. 2008); therefore, the use of bacteria as biocontrol agents is an environmentally friendly approach for overcoming fungal infections in agricultural plantations. One promising bacterial genus is *Bacillus*, which are major plant growth-promoting bacteria (PGPB) known to promote and protect plant growth. *Bacillus* spp. produce many enzymes and bioactive compounds, including antifungal compounds. The antifungal compounds belong to cyclic lipopeptides biosurfactant compounds, such as iturin, fengycin, and surfactin (Dame et al. 2021). The antifungal compounds contribute to their function as biocontrol agents (Shafi et al. 2017; Dame et al. 2021). *Bacillus* cells have thick peptidoglycan walls and produce endospores (Akinrinlola et al. 2018; Tiwari et al. 2019), allowing these bacteria to survive under unfavorable environmental conditions (Radhakrishnan et al. 2017; Tiwari et al. 2019). Consequently, *Bacillus* spp. have longer viability compared to Gram-negative or non-endospore bacteria.

In the present research, *B. siamensis* LDR isolated from coconut peat was evaluated for its antifungal compounds using an antibiosis assay (Santoso et al. 2021a). This *Bacillus* species has shown antagonistic activity against several fungal pathogens, such as *Aspergillus niger* (Santoso et al. 2021a), *Colletotrichum siamense* KA (Fadhilah et al. 2021), *Fusarium* sp., *Chaetomium globosum* InaCC F228, *Ganoderma* sp. TB 4 (Santoso et al. 2021b), *A. clavatus*, *A. flavus*, *A. tamarii* (Pertiwi et al. 2021), and *C. lunata* BM (Safitri et al. 2021). The aim of this research was to evaluate the possibility of using starch from hanjeli (*Coix lacryma-jobi* L.) as a local carbon source in the bacterial fermentation medium. The other aims were to extract the antifungal compounds produced by *B. siamensis* LDR and to characterize and identify them using thin layer chromatography (TLC) and ultraperformance liquid chromatography-mass spectrometry (UPLC-MS).

Materials and methods

Microorganisms

Bacillus siamensis LDR and fungal phytopathogen, *C. lunata* BM and *Ganoderma* sp. TB4 were provided from Laboratory of Microbiology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. The microorganisms were purified using a quadrant streak method on potato dextrose agar (PDA) medium. The pure culture of microorganisms were maintained in PDA medium.

Growth of *Bacillus siamensis* LDR

The *Bacillus siamensis* LDR culture was grown in modified Czapex-Dox broth (CDB) medium containing 3 g $NaNO_3$, 1 g K_2HPO_4, 0.5 g $MgSO_4$, 0.5 g KCl, 0.01 g $FeSO_4$, 15 g agar (Kusanggraeni et al. 2021) and supplemented with 2 g hanjeli as a carbon source in 1,000 mL distilled water. The modified CDB medium was inoculated with 10^5 CFU/mL *B. siamensis* LDR, and bacterial growth was measured at 7 and 14 days using total plate counts (TPC).

Fermentation of antifungal compounds

The fermentation of antifungal compounds by *B. siamensis* LDR was performed in the modified CDB medium containing hanjeli starch. A 1% (v/v) cell suspension of *B. siamensis* LDR was inoculated into 200 mL modified CDB medium and incubated for 12, 14, and 16 days. After incubation, the medium was centrifuged at 2,000 g for 20 min to obtain a cell-free filtrate, which was assumed to contain antifungal compounds produced by *B. siamensis* LDR. The cell-free filtrate was used to dissolve PDA powder to make a PDA-filtrate medium for the antibiosis assay.

Antibiosis agar assay

The antibiosis agar assay was conducted to determine the antifungal activity of compounds produced by *B. siamensis* LDR. The antibiosis agar assay was performed using the paper disk method for *C. lunata* BM and the agar plug method for *Ganoderma* sp. TB4. Spores of *C. lunata* BM in PDA medium were suspended in sterile distilled water, 10 µL of the spore suspension was inoculated to a paper disk (Ø 6 mm), and the paper disk was placed in the center of a plate containing the PDA-filtrate medium. An agar plug (Ø 5 mm) of *Ganoderma* sp. TB4 was placed in the center of a plate containing the PDA-filtrate medium. As a control, a paper disk containing spores of *C. lunata* BM and an agar plug of *Ganoderma* sp. TB4 were placed on normal PDA medium.

The antibiosis agar plates were incubated for 5 days, and then the antifungal activity of *B. siamensis* LDR was tested by determining the inhibition of fungal growth. The diameter of the fungal colony was measured using caliper and the inhibition was calculated according to Hussain

and Khan (2020) as the growth inhibition rate (GIR), where D1 is the diameter of the controlled fungal colony and D2 is the diameter of the threatened fungal colony.

$$GIR(\%) = \frac{D1 - D2}{D1} \times 100\%$$

Production and extraction of antifungal compound

A fourteen-day-old *B. siamensis* LDR in modified CDB medium was used for the extraction of antifungal compounds. The medium was centrifuged at 10,000 g for 10 min at 30 °C to obtain a cell-free filtrate medium. The pH of the cell-free filtrate was adjusted to pH 2.0 with 10 N HCl and stored overnight at 4 °C (Hussain and Khan 2020). The resulting precipitate was collected by centrifugation at 10,000 g for 10 min at 4 °C and then mixed and shaken vigorously with chloroform:methanol (2:1, v/v) at room temperature (Hussain and Khan 2020). The solution mixture was left until the solvent and precipitate were separated. The solvent was separated and evaporated at 40 °C to obtain the crude extract. The crude extract was dissolved in methanol to obtain a 4,500 ppm extract solution. The extract solution was sterilized using a syringe filter (0.45 µm) before use in antifungal extract assay.

Antifungal assay of the crude extract

The antifungal activity of the crude extract from *B. siamensis* LDR was assayed on PDA using the paper disk method. Spores of *C. lunata* BM were suspended in sterile distilled water, and 10 µL spore suspension was inoculated onto sterile paper disks, which were then placed in the center of each PDA plate. An agar plug of *Ganoderma* sp. TB4 was placed in the center of another set of PDA plates. The antifungal extract (100 µL) was inoculated onto paper disks and placed on each plate 3 cm away from the edge of the *C. lunata* BM and *Ganoderma* sp. TB4 isolates. Methanol (100 µL) was used as a control. The inoculated plates were incubated for 5 days for *C. lunata* BM and 10 days for *Ganoderma* sp. TB4. The antifungal activity was determined by the inhibition of growth of the fungal phytopathogen and was represented as the GIR.

Antifungal compound characterization and identification

The crude extract from *B. siamensis* LDR was characterized by TLC to detect the lipopeptide compounds suspected to act as antifungal compounds. Silica gel 60 F254 (Merck) was used as the stationary phase, and isopropanol: acetic acid: water (5:1:1) (v/v) was used as the mobile phase (Habib et al. 2020). The extract was spotted on the TLC plate, run for 30 min in the solvent and dried. The TLC plate was sprayed with water to observe the lipid moiety contained in the lipopeptide (Meena et al. 2018) and to detect protein moiety in the lipopeptide, the TLC plate was sprayed with 0.2% ninhydrin reagent and placed in the oven at 110 °C for 20 min. The pink spot on TLC plate was indicated protein moiety, meanwhile white spot was indicated lipid moiety in the lipopeptide compounds (Meena et al. 2018; Hussain and Khan 2020).

The crude extract was also characterized by UPLC-MS by separation on a C18 (1.8 µm 2.1 × 100 mm) HSS column (ACQUITY UPLC HSS, Waters, USA) using a mobile phase consisting of water and 5 mM ammonium formate (solvent A) and acetonitrile and 0.05% formic acid (solvent B) at a flow rate of 0.2 mL/min for 23 min. The separated compounds were then analyzed by MS (Xevo G2-S Q-TOF, Waters, USA) using electrospray ionization in positive mode and a mass analysis range of 50–1200 *m/z*. The operating parameters were collision 4 V and a ramp collision of 25–60 V.

Results and discussion

Growth of *Bacillus siamensis* LDR

Bacillus siamensis LDR grew well in the modified CDB medium with 0.2% hanjeli starch as the carbon source. The cell population increased to 3.5×10^7 CFU/mL after 7 days of incubation, and the cell population remained relatively constant (4×10^7 CFU/mL) at 14 days of incubation, indicating that the cells had achieved the stationary phase. *Bacillus siamensis* LDR has been reported to produce amylase, which can hydrolyze various types of starch (Kusanggraeni et al. 2021), including hanjeli starch (Safitri et al. 2021). This ability allowed *B. siamensis* LDR to grow well in the modified CDB medium. According to Corke et al. (2016), hanjeli contains nutritious substances, such as starch, protein, and lipids, as well as other micronutrients, such as phosphate, magnesium, and zinc. Hanjeli also contains niacin, thiamine, and riboflavin (Mulyono et al. 2019). The starch, protein, and fat contained in hanjeli are up to 68%, 15.6%, and 9%, respectively (Tensiska et al. 2021). Therefore, the hanjeli starch can substitute for the potato starch used in commercial culture media.

Hanjeli starch is a complex carbohydrate consisting of both amylose and amylopectin components. Corke et al. (2016) reported that hanjeli starch contains about 15.9–25.8% amylose. Amylose is considered a difficult compound to degrade (Wang et al. 1999); therefore, the metabolism of hanjeli starch will be gradual and it should support the growth of *B. siamensis* LDR for quite a long time. Antifungal compounds can therefore be produced using modified CDB containing hanjeli starch. Safitri et al. (2021) also reported that *B. siamensis* LDR has antagonistic activity against *Ganoderma* sp. TB4 and *C. lunata* BM when grown on modified Czapex-Dox agar (CDA) medium.

Antibiosis agar assay

The results of the antibiosis assay of *B. siamensis* LDR are presented in Table 1. The diameters were smaller for the colonies of *C. lunata* BM and *Ganoderma* sp. TB4 grown on the PDA-filtrate medium than on the control PDA

medium. Both fungi seemed to be inhibited by antifungal compounds produced by *B. siamensis* LDR and contained in the modified CDB medium. The percentage inhibition ranged from 44.14% to 47.08% for *C. lunata* BM and from 51.98% to 85.99% for *Ganoderma* sp. TB4. The highest percentage inhibition on both fungi were observed on PDA-filtrate containing 14th day of filtrate fermentation.

Antifungal compounds are commonly produced during the stationary phase when nutrients are depleted (Horak et al. 2019). Kumar et al. (2012) reported that the maximum production of antifungal compounds was achieved at the end of the stationary phase. Nevertheless, a longer fermentation time can decrease the antifungal activity (Kumar et al. 2012) due to degradation, re-utilization, or conversion of the antifungal compounds (Horak et al. 2019) by *B. siamensis* LDR.

The results of *B. siamensis* LDR growth suggested that the antifungal compounds might already be produced by 7 days of fermentation. The antibiosis assay at 12 days showed inhibition of fungal growth and greater inhibition at14 days, probably due to an accumulation effect. Beyond 14 days, however, the levels of antifungal compounds tended to decrease, as indicated by the assay conducted at 16 days. The growth inhibition of *C. lunata* BM is presented in Fig. 1, and that of *Ganoderma* sp. TB4 is presented in Fig. 2.

Antifungal assay of the crude extract

The antifungal activity of the crude extract was assayed based on the results of the antibiosis assay, which indicated that 14 days of incubation gave the highest percentage inhibition against the two fungal pathogens tested. The results of the antifungal assay using the crude extract of *B. siamensis* LDR are presented in Fig. 3. The crude extract inhibited the growth of both *C. lunata* BM and *Ganoderma* sp. TB4, indicating that an antifungal compound had been extracted successfully from the modified CDB medium. The percentage inhibition was up to 60.70% for *C. lunata* BM and up to 65.25% for *Ganoderma* TB4 (Table 2).

The percentage inhibition obtained by antifungal assay using the crude extract (Table 2) was higher than the percentage inhibition achieved with the antibiosis assay on the PDA-filtrate medium (Table 1). This difference probably reflects the increased purity of the extracted antifungal compound, which meant that the compound was more concentrated in the antifungal assay than in the antibiosis assay. Abdallah et al. (2015) also found that the percentage inhibition of fungal growth was higher in antifungal assay performed with the extracted compound than with a cell-free filtrate of *Bacillus* spp.

Antifungal compound characterization and identification

The antifungal compounds present in the crude extract of *B. siamensis* LDR were detected using TLC. Spraying the TLC plate with water revealed a white spot (Fig. 4a), indicating a lipid moiety in the compound (Meena et al. 2018); meanwhile spraying the TLC plate with 0.2% ninhydrin revealed

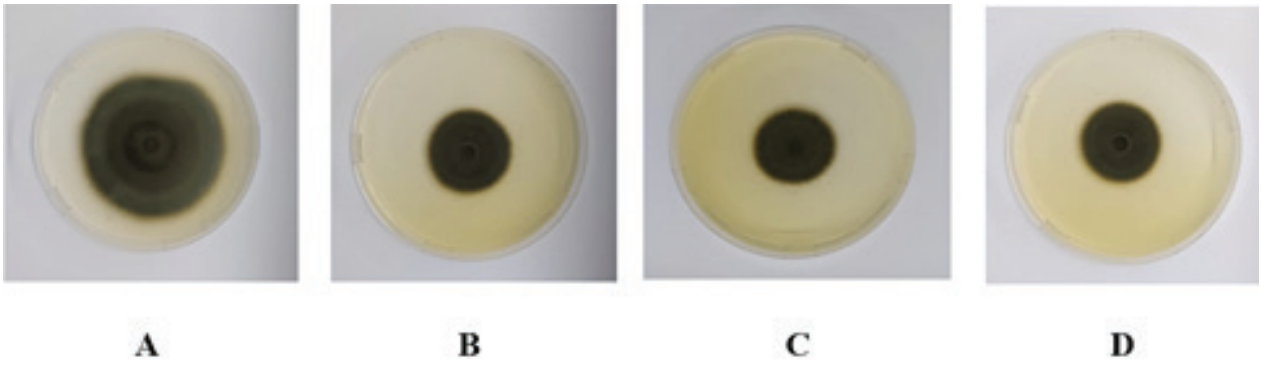

Figure 1. Growth inhibition of *Curvularia lunata* BM on potato dextrose agar (PDA)-filtrate medium: **A.** control, **B.** 12 days, **C.** 14 days, **D.** 16 days.

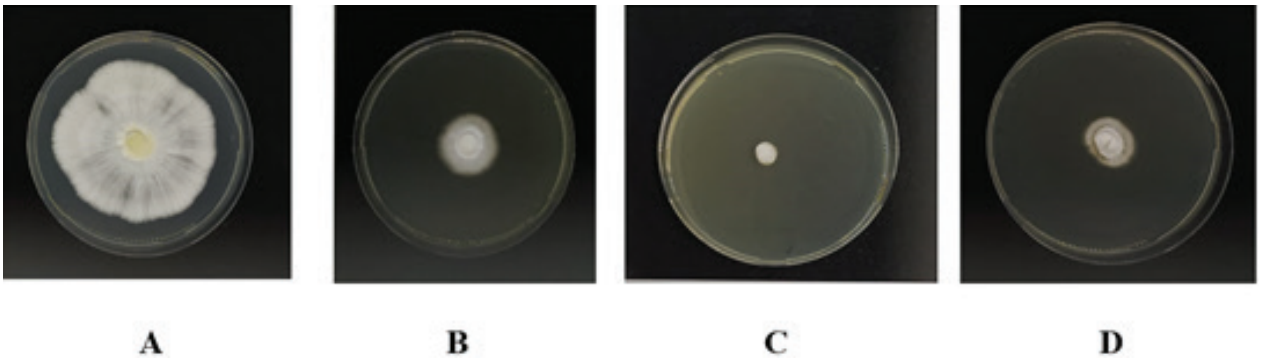

Figure 2. Growth inhibition of *Ganoderma* sp. TB4 on potato dextrose agar (PDA)-filtrate medium: **A.** control, **B.** 12 days, **C.** 14 days, **D.** 16 days.

Table 1. Antibiosis agar assay of *Bacillus siamensis* LDR against fungal pathogens.

Fungal Pathogen	Repetition	Control (mm)	12 days		14 days		16 days	
			Treatment (mm)	GIR (%)	Treatment (mm)	GIR (%)	Treatment (mm)	GIR (%)
Curvularia lunata BM	1	69.90	37.68	46.10	37.22	46.75	38.80	44.49
	2		41.33	40.87	36.91	47.20	37.42	46.47
	3		38.57	44.83	37.32	46.61	37.10	46.93
	4		39.20	43.92	36.75	47.43	36.89	47.23
	5		38.46	44.98	36.76	47.41	37.55	46.29
	average		**39.05±1.39**	**44.14±1.98**	**36.99±0.26**	**47.08±0.38**	**37.55±0.75**	**46.28±1.07**
Ganoderma sp. TB4	1	61.19	28.83	52.89	8.81	85.60	23.18	62.12
	2		30.83	49.61	8.26	86.50	25.26	58.72
	3		29.90	51.13	9.43	84.59	20.83	65.96
	4		28.28	53.78	7.89	87.11	23.59	61.44
	5		29.07	52.49	8.48	86.14	22.53	63.19
	average		**29.38±1.00**	**51.98±1.63**	**8.57 ± 0.58**	**85.99±0.95**	**23.08±1.61**	**62.28±2.64**

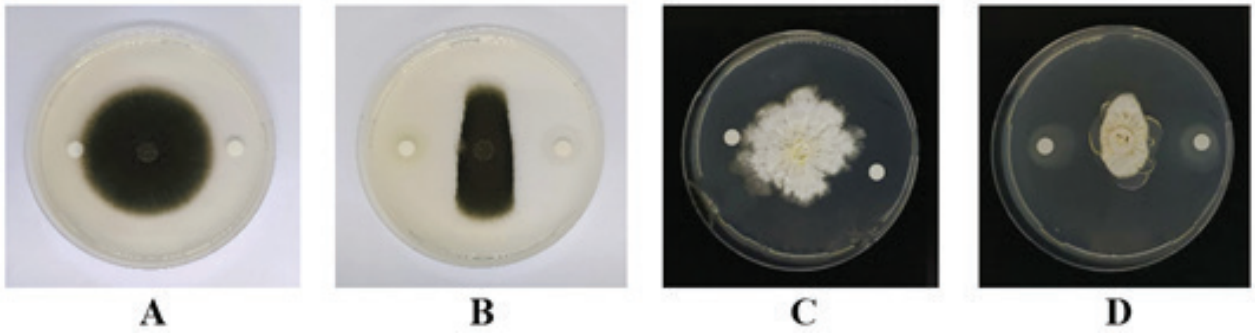

Figure 3. Growth inhibition of *Curvularia lunata* BM: **A.** control, **B.** treatment; *Ganoderma* sp. TB4 : **C.** control; **D.** treatment caused by the crude extract of *Bacillus siamensis* LDR.

Table 2. Antifungal assay of the crude extract from *Bacillus siamensis* LDR.

Fungal Pathogen	Repetition	Radius (mm)		GIR (%)
		Control	Treatment	
Curvularia lunata BM	1	35.53	12.64	56.03
	2		10.01	65.18
	3		11.43	60.24
	4		11.11	61.36
	Average		**11.30±1.08**	**60.70±3.76**
Ganoderma sp. TB4	1	32.58	9.81	60.24
	2		7.60	69.21
	3		8.23	66.64
	4		8.66	64.90
	Average		**8.58±0.93**	**65.25±3.78**

Table 3. Retention factor of protein moiety in antifungal compounds detected using TLC.

Spot	Rf value
A	0.89
B	0.69
C	0.64
D	0.40
E	0.37

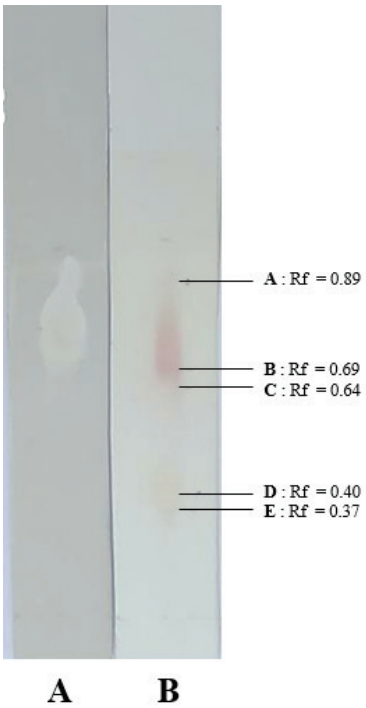

Figure 4. Thin layer chromatography of crude extract *Bacillus siamensis* LDR : **A.** lipid moiety; **B.** protein moiety.

five spots with pink and yellow color (Fig. 4b), labeled A to E, with Rf values ranging from 0.37 to 0.89 (Table 3) indicating a protein moiety of the compound. The spot B (Rf = 0.69) was the most dense pink color; meanwhile the spot E (Rf = 0.37) was quite distinguish with a yellowish color (Fig. 4b).

According to Habib et al. (2020), the appearance of red-to-pink spots indicates the presence of lipopeptides in the crude extract. The pink color on the TLC plate indicated that amino acids were contained in the extract (Hussain and Khan 2020). Therefore, it indicated the antifungal activity of the crude extract of *B. siamensis* LDR appeared to be due to a group of lipopeptide compounds.

The chromatogram profile of the crude extract of *B. siamensis* LDR are shown in Fig. 5. The extract contained

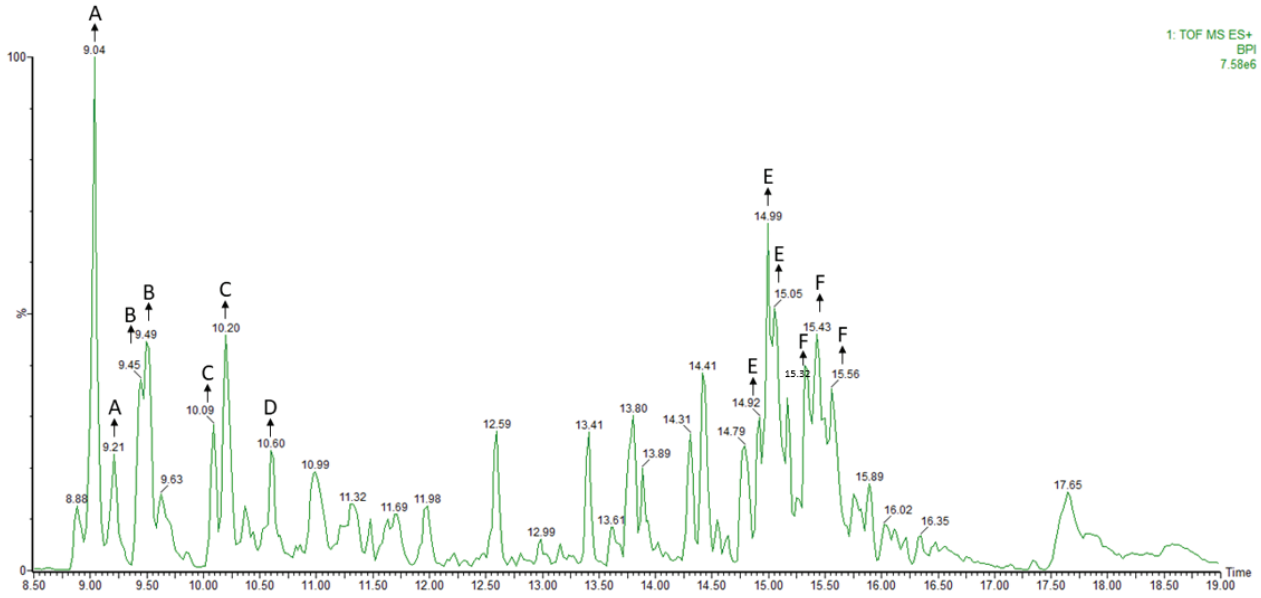

Figure 5. The chromatogram profile of the crude extract of *Bacillus siamensis* LDR : **A.** C_{14} iturin A; **B.** C_{14} bacillomycin F; **C.** C_{15} bacillomycin F; **D.** C_{16} bacillomycin F; **E.** C_{12} surfactin; **F.** C_{13} surfactin.

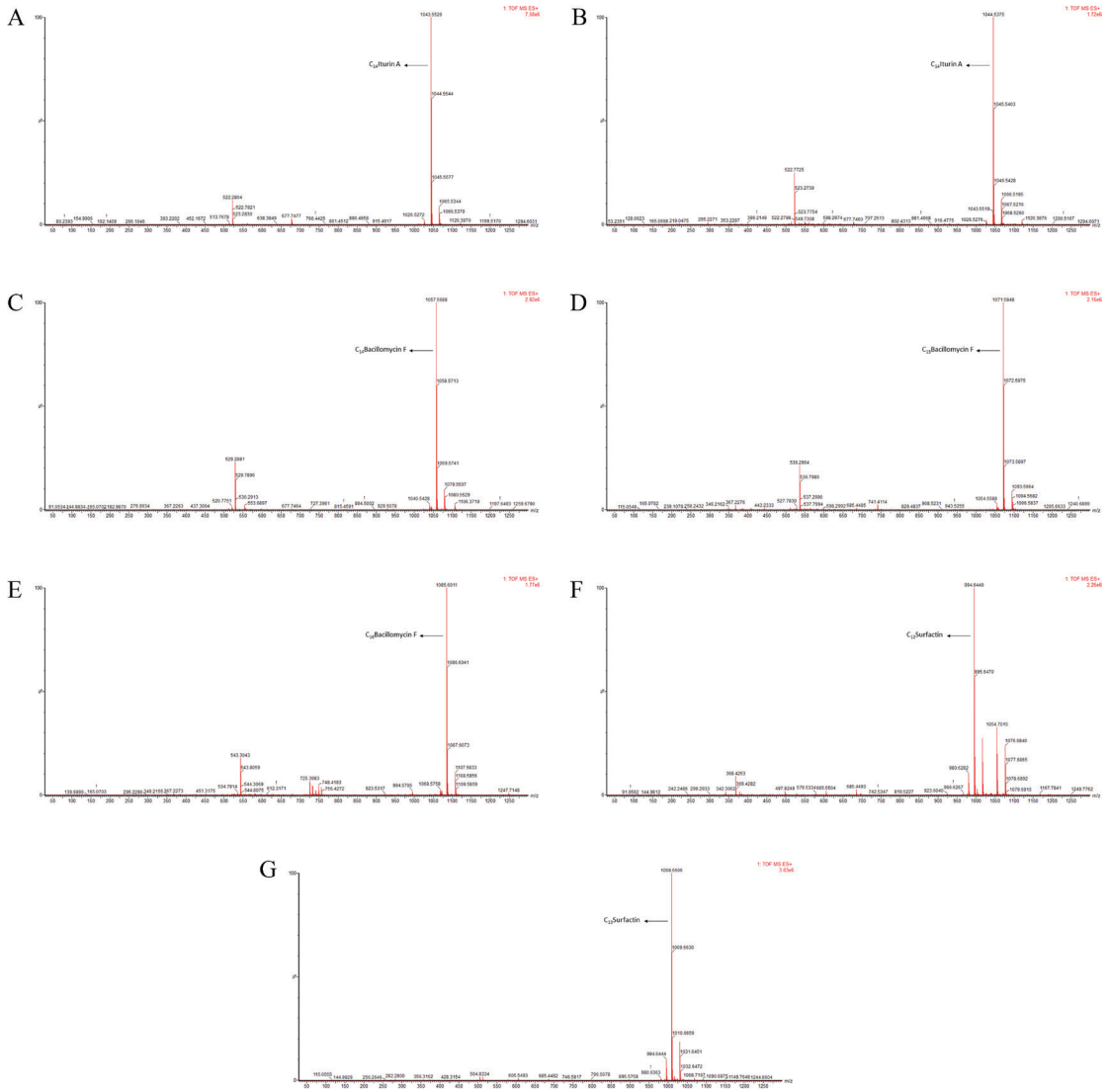

Figure 6. The results of mass spectrometry analysis: **A.** C_{14} iturin A (*m/z* 1043.5526); **B.** C_{14} iturin A (*m/z* 1044.5375); **C.** C_{14} bacillomycin F (*m/z* 1057.5688); **D.** C_{15} bacillomycin F (*m/z* 1071.5848); **E.** C_{16} bacillomycin F (*m/z* 1085.6011); **F.** C_{12} surfactin (*m/z* 994.6440); and **G.** C_{13} surfactin (*m/z* 1008.6606)

Table 4. Mass spectrometry results for the crude extract.

Peak No	RT	*m/z* $(M+H)^+$	Compound	Peak No	RT	*m/z* $(M+H)^+$	Compound
1	9.04	1043.5526	C_{14} Iturin A	8	14.92	994.6440	C_{12} Surfactin
2	9.21	1044.5375	C_{14} Iturin A	9	14.99	994.6434	C_{12} Surfactin
3	9.45	1057.5688	C_{14} Bacillomycin F	10	15.05	994.6451	C_{12} Surfactin
4	9.49	1057.5687	C_{14} Bacillomycin F	11	15.32	1008.6606	C_{13} Surfactin
5	10.09	1071.5848	C_{15} Bacillomycin F	12	15.43	1008.6603	C_{13} Surfactin
6	10.20	1071.5859	C_{15} Bacillomycin F	13	15.56	1008.6616	C_{13} Surfactin
7	10.60	1085.6011	C_{16} Bacillomycin F				

32 different compounds eluting at 8.88 to 18.57 min. Based on the mass spectrometry analysis of the molecular ions $[M+H]^+$ data and the comparison of the findings from previous studies by Xu et al. (2018) and Gorai et al. (2021), it was showed that 13 peaks were tentatively identified as C_{14} iturin A (Rt 9.04 and 9.21), C_{14} bacillomycin F (Rt 9.45 and 9.49), C_{15} bacillomycin F (Rt 10.09 and 10.20), C_{16} bacillomycin F (Rt 10.60), C_{12} surfactin (Rt 14.92, 14.99, and 15.05), and C_{13} surfactin (Rt 15.32, 15.43, and 15.56) (Table 4). The MS profiles of iturin A, bacillomycin F, and surfactin are shown in Fig. 6. Those annotation compounds were mostly detected as the major peaks of the present extract (Fig. 5). Indeed, they constitute the common antifungal compounds produced by *Bacillus* species and are cyclic lipopeptides (Kumar and Johri 2012; Dame et al. 2021). Another research by Ali et al. (2020) reported that iturin and surfactin was also detected in *B. siamensis* S3 extract at *m/z* 1023.6 to 1122.7 using MALDI-TOF mass spectrometry. Sharma et al. (2021) also reported surfactin and bacillomycin (*m/z* 994.8 to 1096.86) that has been isolated from *B. siamensis* NKIT9 extract. All the given studies showed that the *B. siamensis* commonly produced lipopeptide biosurfactant, such as iturin and surfactin.

Conclusion

Bacillus siamensis LDR can produce antifungal compounds when grown in a modified CDB medium with hanjeli starch as the carbon source. The antifungal compounds can inhibit the growth of both *C. lunata* BM and *Ganoderma* TB4. The percentage inhibition by a crude extract containing the antifungal compounds was up to 60.70% for *C. lunata* BM and 65.25% for *Ganoderma* sp. TB4. The crude extract apparently contained several antifungal compounds, including iturin A, bacillomycin F, and surfactin.

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References

Abdallah RAB, Jabnoun-Khiareddine H, Mokni-Tlili S, Medimagh-Saidana S, Daami-Remadi M (2015) Endophytic *Bacillus* spp. from wild solanaceae and their antifungal potential against *Fusarium oxysporum* f. sp. *lycopersici* elucidated using whole cells, filtrate cultures and organic extracts. Journal of Plant Pathology & Microbiology 6: 1–7. http://dx.doi.org/10.4172/2157-7471.1000324

Agustika, D, Prihartna C, Suwanto A (2019) Rapid inoculation technique and biological control of leaf spot disease in oil palm. International Journal of Oil Palm 2: 1–11. https://doi.org/10.35876/ijop.v2i1.24

Akinrinlola RJ, Yuen RY, Drijber RA, Adesemoye AO (2018) Evaluation of *Bacillus* strains for plant growth promotion and predictability of efficacy by in vitro physiological traits. International Journal of Microbiology 2018: 1–11. https://doi.org/10.1155/2018/5686874

Ali MA, Ren H, Ahmed T, Luo J, An Q, Qi X, Li B (2020) Antifungal effects of rhizospheric *Bacillus* species against bayberry twig blight pathogen *Pestalotiopsis versicolor*. Agronomy 10: 1–16. https://doi.org/10.3390/agronomy10111811

Almeida F, Rodrigues ML, Coelho C (2019) The still underestimated problem of fungal iseases worldwide. Frontiers in Microbiology 10: 1–5. https://doi.org/10.3389/fmicb.2019.00214

Corke H, Huang Y, Li JS (2016) Coix: Overview. In: Wrigley C, Corke H, Seetharaman K, Faubion J (Eds) Encyclopedia of Food Grains 2nd edn.: The World of Food Grains. Oxford, Academic Press, 184–189. https://doi.org/10.1016/B978-0-12-394437-5.00008-5

Dame ZT, Rahman M, Islam T (2021) Bacilli as sources of agrobiotechnology: recent advances and future directions. Green Chemistry Letters and Reviews 14: 246–271. https://doi.org/10.1080/17518253.2021.1905080

Fadhilah QG, Santoso I, Maryanto AE, Yasman (2021) Antifungal potential from *Bacillus* sp. Against phytopathogenic fungus *Colletotrichum* sp. AIP Conference Proceedings 2374: 1–8. https://doi.org/10.1063/5.0058769

FAO [Food and Agriculture Organization of the United Nations] (2017) Country fact sheet on food and agriculture policy trends: Indonesia: Socio-economic context and role agriculture. https://www.fao.org/3/i7696e/i7696e.pdf [Accessed on: 29.12.2021]

Garcia-Aroca T, Doyle V, Singh R (2018) First report of *Curvularia* leaf spot on corn, caused by *Curvularia lunata*, in the United States. Plant Health Progress 19: 140–142. https://doi.org/10.1094/PHP-02-18-0008-BR

Gorai PS, Ghosh R, Mandal S, Ghosh S, Chatterjee S, Gond SK, Mandal NC (2021) *Bacillus siamensis* CNE6- a multifaceted plant growth promoting endophyte of *Cicer arietinum* L. having broad spectrum antifungal activities and host colonizing potential. Microbiological Research 252: 1–12. https://doi.org/10.1016/j.micres.2021.126859

Habib S, Ahmad SA, Johari WLW, Shukor MYA, Alias SA, Smykla J, Saruni NH, Razak NSA, Yasid NA (2020) Production of Lipopeptide biosurfactant by a hydrocarbon-degrading Antarctic *Rhodococcus*.

International Journal of Molecular Sciences 21: 1–21. https://doi.org/10.3390/ijms21176138

Horak I, Engelbrecht G, Jansen van Rensburg PJ, Claassens (2019) Microbial metabolomics: essential definitions and the importance of cultivation conditions for utilizing *Bacillus* species as bionematicides. Journal of Applied Microbiology 127: 326–343. https://doi.org/10.1111/jam.14218

Hushiarian R, Yusof NA, Dutse SW (2013) Detection and control of *Ganoderma boninense*: strategies and perspectives. Springerplus 2: 1–12. https://dx.doi.org/10.1186%2F2193-1801-2-555

Hussain T, Khan AA (2020) Determining the antifungal activity and characterization of *Bacillus siamensis* AMU03 against *Macrophomina phaseolina* (Tassi) Goid. Indian Phytopathology 73: 507–516. https://doi.org/10.1007/s42360-020-00239-6

Kumar A, Saini S, Wray V, Nimtz M, Prakash A, Johri BN (2012) Characterization of an antifungal compound produced by *Bacillus* sp. strain A5F that inhibits *Sclerotinia sclerotiorum*. Journal of Basic Microbiology 52: 1–9. https://doi.org/10.1002/jobm.201100463

Kumar A, Johri BN (2012) Antimicrobial lipopeptide of *Bacillus*: natural weapon for biocontrol of plant pathogens. In: Satyanarayana T, Johri BN, Prakash A (Eds) Microorganisms in Sustainable Agriculture and Biotechnology. Springer, Netherlands, 91–111. https://doi.org/10.1007/978-94-007-2214-9

Kusanggraeni I, Santoso I, Yasman (2021) Antifungal production of *Bacillus siamensis* LDR against *Aspergillus niger* ABP and ART using rice starch as carbon source. Ecology, Environment and Conservation 27: S101–S107. http://www.envirobiotechjournals.com/article_abstract.php?aid=11129&iid=324&jid=3

Majeed RA, Shahid AA, Ashfaq M, Saleem MZ, Haider MS (2015) First report of *Curvularia lunata* causing brown leaf spots of rice in Punjab, Pakistan. Disease Note. https://doi.org/10.1094/PDIS-05-15-0581-PDN

Meena KR, Tandon T, Sharma A, Kanwar SS (2018) Lipopeptide antibiotic production by *Bacillus velezensis* KLP2016. Journal of Applied Pharmaceutical Science 8: 091–098. http://dx.doi.org/10.7324/JAPS.2018.8313

Mulyono E, Kusuma A, Dewandari KT, Darniadi S (2019) Preliminary study of hanjeli (*Coix lacryma-jobi L*) flour for food uses. IOP Conference Series: Earth and Environmental Science 309: 1–6. https://doi.org/10.1088/1755-1315/309/1/012057

Naher L, Yusuf UK, Tan SG, Ismail A (2013) Ecological status of *Ganoderma* and basal stem rot disease of oil palms (*Elaeis guineensis* Jacq.) Australian Journal of Crop Science 7: 1723–1727. http://www.cropj.com/naher_7_11_2013_1723_1727.pdf

Pertiwi M, Santoso I, Fadhilah QG (2021) Antagonistic activity screening of *Bacillus siamensis* LDR against fungal associated with crop contamination. IOP Conference Series: Earth and Environmental Science 846: 1–7. https://doi.org/10.1088/1755-1315/846/1/012002

Peterson RRM (2019) *Ganoderma boninense* disease of oil palm to significantly reduce production after 2050 in Sumatra if projected climate change occurs. Microorganisms 7: 1–8. https://doi.org/10.3390/microorganisms7010024

Prapagdee B, Kuekulvog C, Mongkulsuk S (2008) Antifungi potential of extracellular metabolites produced by *Streptomyces hygrospicus* against phytophatogenic fungi. International Journal of Biological Science 4: 330–337. https://doi.org/10.7150/ijbs.4.330

Radhakrishnan R, Hashem A, Abd_Allah EF (2017) *Bacillus*: a biological tool for crop improvement through bio-molecular changes in adverse environments. Frontiers in Physiology 8: 1–14. https://doi.org/10.3389/fphys.2017.00667

Rees RW, Flood J, Hasan Y, Potter U, Cooper RM (2009) Basal stemrot of oil palm (*Elaeis guineensis*); mode of root infection and lower stem invasion by *Ganoderma boninense*. Plant Pathology 58: 982–989. https://doi.org/10.1111/j.1365-3059.2009.02100.x

Safitri SD, Santoso I, Fadhilah QG, Maryanto AE, Yasman (2021) Antagonistic activity of *Bacillus siamensis* LDR against *Curvularia lunata* BM. AIP Conference Proceedings, in press.

Santoso I, Fadhilah QG, Maryanto AE, Yasman (2021a) Antagonist effect of *Bacillus* spp. against *Aspergillus niger* CP isolated from cocopeat powder. IOP Conference Series: Earth and Environmental Science 846: 1–10. https://doi.org/10.1088/1755-1315/846/1/012001

Santoso I, Fadhilah QG, Maryanto AE, Yasman (2021b) The potency of *Bacillus siamensis* LDR as biocontrol agent against fungal phytopathogen. Advances in Biological Sciences Research 14: 463–468. https://dx.doi.org/10.2991/absr.k.210621.079

Shafi J, Tian H, Ji M (2017) *Bacillus* species as versatile weapons for plant pathogens: a review. Biotechnology & Biotechnological Equipment 31: 446–459. https://doi.org/10.1080/13102818.2017.1286950

Sharma A, Kaushik N, Sharma A, Bajaj A, Rasane M, Shouche YS, Marzouk T, Djèbali N (2021) Screening of tomato seed bacterial endophytes for antifungal activity reveals lipopeptide producing *Bacillus siamensis* strain NKIT9 as a potential bio-control agent. Frontiers in Microbiology 12: 1–17. https://doi.org/10.3389/fmicb.2021.609482

Sunpapao A, Kittimorakul J, Pornsuriya C (2014) Disease Note: Identification of *Curvularia oryzae* as cause of leaf spot disease on oil palm seedlings in nurseries of Thailand. Phytoparasitica 2: 1–6. https://doi.org/10.1007/s12600-014-0390-9

Tensiska, Cahyana Y, Nurhadi B, Desani, WD, Subroto E (2021) In vitro digestibility and the characteristics of puffed products from different degrees of milling and moisture content of adlay grain (*Coix lacryma-jobi L.*). Food Research 5: 317–378. http://dx.doi.org/10.26656/fr.2017.5(2).548

Tiwari S, Prasad V, Lata C (2019) *Bacillus*: plant growth promoting bacteria for sustainable agriculture and environment. In: Singh JS, Singh DP (Eds) New and Future Developments in Microbial Biotechnology and Bioengineering: Microbial Biotechnology in Agro-Environmental Sustainability. Elsevier, The Netherlands, 43–45. https://doi.org/10.1016/B978-0-444-64191-5.00003-1

Wang X, Conway PL, Brown IL, Evans AJ (1999) In vitro utilization of amylopectin and high-amylose maize (amylomaize) starch granules by human colonic bacteria. Applied and Environmental Microbiology. 65: 4848–4854. https://doi.org/10.1128/aem.65.11.4848-4854.1999

Xu B, Lu Y, Ye Z, Zheng Q, Wei T, Lin J, Guo L (2018) Genomics-guided discovery and structure identification of cyclic lipopeptides from the *Bacillus siamensis* JFL15. PLoS ONE. 13: 1–18. https://doi.org/10.1371/journal.pone.0202893