AJAB

Asian J Agric & Biol. 2019;7(3):404-411.

Original Article

Endophytic bacteria from *Theobroma cacao* L. with antifungal activities against *Phytophthora palmivora*

Ishak Zubir[1,2], Ernie Eileen Rizlan Ross[3], Ainon Hamzah[1], Wan Syaidatul Aqma[1*]

[1]School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia 43600 Bangi, Selangor Malaysia

[2]Malaysia Cocoa Board, Cocoa Innovative and Technology Centre, Lot 12621, Kawasan Perindustrian Nilai 71800 Nilai, Negeri Sembilan, Malaysia

[3]Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam Selangor, Malaysia

Received:
July 13, 2018

Accepted:
July 17, 2019

Published:
September 30, 2019

**Corresponding author email:*
ishak_z@koko.gov.my

Abstract

Black pod disease caused by the pathogenic fungus *Phytophthora palmivora* is a serious threat to the cocoa industry causing the destruction of cocoa plants in many plantations across Malaysia. The objectives of this study are to determine the antifungal activities of the endophytic bacteria isolates and to identify the isolates. Four selected endophytic bacteria previously isolated from tissues of healthy *Theobroma cacao* L. designated as isolate LKM-UL, LKM-PA, LKM-PD, and LKM-BL were assessed for their abilities to inhibit the growth of *P. palmivora in vitro*. Preliminary tests using dual culture method showed that the isolate LKM-BL had the strongest inhibition towards the growth of *P. palmivora* after 24 h of incubation compared to the other isolates. The growth of LKM-UL, LKM-PA, LKM-PD, and LKM-BL revealed that the antifungal activities against cocoa pathogen increases as the number of endophytic cells increases. The cell-free supernatant from isolate LKM-BL produced the highest antifungal activity against cocoa pathogen with an inhibition zone of 19.5 ± 0.50 mm during the highest cell growth at 24 h of incubation. The endophytic bacteria were characterized morphologically and based on biochemical tests. Based on the analysis of the 16S ribosomal DNA sequences, isolate LKM-UL, LKM-PA, LKM-PD, and LKM-BL were identified as *Bacillus amyloliquefaciens*, *Pantoea agglomerans*, *Bacillus pumilus* and *Bacillus subtilis* respectively. The findings indicate that among the four endophytic bacterial isolates studied, *B. subtilis* LKM-BL showed the highest antifungal activity and has the potential to be used as a biological control agent towards the cocoa pathogen *P. palmivora*.

Keywords: Endophytic bacteria, Antifungal, *Phytophthora palmivora*, Black pod, Cocoa

How to cite this:

Zubir I, Ross EER, Hamzah A and Aqma WS, 2019. Endophytic bacteria from *Theobroma cacao* L. with antifungal activities against *Phytophthora palmivora*. Asian J. Agric. Biol. 7(3):404-411.

Introduction

Theobroma cacao L. is an economically important crop in the chocolate industry. Unfortunately, the crop is suffering from the damaging effects of the cocoa pathogenic fungi (Vanegtern et al., 2015). *Phytophthora palmivora* infection is one of the most threatening diseases that have been reported to significantly drop the annual pod yield production in Malaysia. This pathogen is known to cause the black pod disease in cocoa plants and it is highly destructive towards cocoa pods (Acebo-Guerrero et al., 2012).

Initial infection appears as small brownish spots which may cover the entire pod in a few days causing loss of valuable cocoa pods. The pathogen then invades deeper into the pod where it infects and destroys the beans (Vanegtern et al., 2015). Controlling the infection by spraying chemical fungicide has long been practiced but can be costly. In addition, the extensive use of fungicide has been shown to contribute to chemical contamination in the environment. Furthermore, spraying of fungicide as an attempt to control fungal diseases of cocoa is not completely effective especially in high rainfall areas because the agent will be easily washed off (Agbeniyi et al., 2014). The public awareness of agriculture and food contamination due to chemical usage targeted on gaining productivity by shielding plants from pathogen, has highlighted the need for an innovative approach for disease management (Paul et al., 2013). Endophytic bacteria are potential candidate to be utilized as biological control agent because of their capability to reside inside plants and their ability to exert antifungal activity to control fungal pathogens (Li et al., 2015).

Endophytic bacteria, as previously reported, have the capability to produce antifungal substances that can be used in controlling pathogens of tomato (Yi et al., 2015), rapeseed (Chen et al., 2014), poplar (Ren et al., 2013), cucumber (Cao et al., 2012), corn (Petatan-Sagahon et al., 2011), banana (Fu et al., 2010), and many other plants. The advantage of utilizing endophytic bacteria as biocontrol agents against the cocoa pathogen is that they have the ability to effectively colonize the cocoa plants, therefore, they could be effective in the suppression of pathogen (Lin et al., 2013) without causing environmental contamination (Paul et al., 2013). Applying endophytic bacteria as biological agents may establish a symbiotic relationship between the plant and the endophyte; thus conferring longer protection of the plant compared to using fungicide, as reported by Adejumo (2005).

This study is targeted on endophytic bacteria for their antifungal activities against cocoa fungal pathogen, specifically *P. palmivora*. The endophytes were then characterized morphologically and biochemically prior to identification via 16S rDNA sequence analysis.

Material and Methods

Selected endophytic bacteria and pathogen culture

The cultures of endophytic bacteria previously isolated from tissues of healthy *T. cacao* L. designated as isolate LKM-UL, LKM-PA, LKM-PD, LKM-BL and the cocoa pathogen *Phytophthora palmivora* were obtained from Malaysia Cocoa Board Culture Collection. Stock cultures of the endophytic isolates were stored at−80 °C in nutrient broth (NB) with 15% glycerol (Shin et al., 2007). The working cultures were established by a streaking technique from the stock cultures onto freshly prepared nutrient agar (NA) in Petri dishes and incubated for 24 h at 28 °C. Meanwhile, the cocoa pathogen, *P. palmivora* was grown by transferring from stock agar onto freshly prepared potato dextrose agar (PDA) in Petri dishes and incubated for 7 days in the dark at 30 °C.

Antifungal activities of selected endophytic bacteria against cocoa pathogen

Dual culture method (Shin et al., 2007) was used to determine the antagonistic capabilities of the isolated endophytes against *P. palmivora*. The selected endophyte was streaked on one side of a freshly prepared PDA, while a 6 x 6 mm agar block extracted from 7 days-old agar cultures containing the mycelia of *P. palmivora* were subsequently placed next to the bacterial streak. As control, mycelia of *P. palmivora* were inoculated at the middle of PDA without any endophytes isolate. The Petri dishes were incubated at 30 °C for one week. The inhibition zones (mm) were recorded by measuring the clear distance between the edges of the fungal mycelium and the bacterial streak.

Growth pattern of endophytic bacteria and their antifungal activities

The selected endophytic bacteria isolates were separately inoculated in 100 mL NB in a 250 mL conical flask and incubated overnight at 28 °C with agitation at 120 rpm. The cultures were centrifuged at 4000 rpm for 30 min and the cell pellets were suspended in normal saline (0.85% NaCl) for

preparation of standard inoculums (Ainon et al., 2017). Subsequently, 10% (v/v) of standard inoculums of each bacterium was transferred into 100 mL NB and incubated overnight at 28 °C with agitation at 120 rpm respectively. The bacterial growth was monitored by measuring the absorbance at 550 nm (A_{550}) using a UV-spectrophotometer (Dynamica, Australia) after 4, 8, 12, 16, 24, 28, 32, 36, 40, 44 and 48 hours of incubation.

For preparation of cell-free supernatant, 1 mL of each culture at different growth time interval was then centrifuged at 4000 rpm for 30 min at 4 °C. Sterile filter paper disc (BD 6 mm, USA) was impregnated with 100 µL of cell-free supernatants (after filtered through 0.2 µm filter). About 100 µL of mycelia suspension culture of *P. palmivora* was spread on PDA plate and paper disc with cell-free supernatants was placed at the centre of the plate and incubated for 7 days at 30 °C. The antifungal activity was determined by measuring the inhibition zone of mycelial growth of the pathogen around the filter paper disc.

Characterization and identification of endophytic bacteria

Endophytic bacteria isolates LKM-UL, LKM-PA, LKM-PD and LKM-BL were characterized based on morphological, biochemical and physiological characteristics. Colony color was recorded after 24 h of incubation on NA plates at 28 °C. The Gram staining of the individual bacterial isolates was observed by light microscopy (Olympus BX41, Tokyo). Cell shape and size of each endophyte isolate was observed by scanning electron microscopy (SEM) (Hitachi S3400N, Japan). Tests for motility, catalase and methyl red-voges proskauer (MR-VP) were also conducted according to standard microbiological procedures (Gerhardt et al., 1994). Turbidity around the line of inoculation of the endophytic bacteria in the sulfide indole motility medium showed the positive indicator of motility (Islam et al., 2016). Formation of oxygen bubbles by adding few drops of 3% hydrogen peroxide solution onto the microscopic slides containing the isolates indicated positive reaction of catalase activity (Gupta et al., 2015). Positive observation of VP tests showed the ability of all isolates to produce neutral by-product to form acetoin from glucose fermentation (Labrador et al., 2014). Endophytic bacteria isolates LKM-UL, LKM-PA, LKM-PD, and LKM-BL were also identified using 16S rDNA gene sequences approach. DNeasy Blood and Tissue kit (QIAGEN) was used to extract bacterial genomic DNA according to procedures recommended by the manufacturer. The primers used to amplify the 16S rDNA region in this study were universal primers 27F and 1492R. Amplified PCR products were purified using QIAquick PCR Purification Kit (QIAGEN) before they were sent to 1st Base Laboratories Sdn. Bhd., Malaysia for sequencing. The sequences obtained were subjected to the Basic Local Alignment Search Tool (BLAST) analysis and DNA sequences similarities were determined by comparing them with sequences available in the National Centre for Biotechnology Information (NCBI) databases. Sequences were selected from Blast-Webpage and added directly into MEGA7 software. Furthermore, all the analyzed sequences were deposited in GenBank and the accession numbers were obtained.

Statistical analysis

All experiments were conducted in triplicate and the data were expressed as mean ± standard deviation after analyzed via one-way ANOVA (SPSS Ver. 13.0). The statistical significance was set at a confidence level of $p < 0.05$.

Results and Discussion

Antifungal activity against cocoa pathogen

The antifungal activity of the selected endophytic bacteria designated LKM-UL, LKM-PA, LKM-PD and LKM-BL against *P. palmivora* were carried out using the dual cultures method. In this study, all four isolates demonstrated promising antifungal activity against cocoa pathogen *P. palmivora*. Among the tested isolates, isolate LKM-BL significantly showed the biggest inhibition zone against *P. palmivora*, while isolate LKM-PA showed the smallest inhibition zone (Table 1). The findings, in fact, are in accordance with previous reports, in which the endophytic bacteria produced antifungal activity to protect the plant from its pathogen. Various results have revealed the potential of endophytic bacteria to inhibit the growth of plant pathogenic fungi. Kefi et al., (2015) demonstrated that the endophytic bacteria *Bacillus* strains BL1, BT5, BR8, and BR11 isolated from tomato plants showed inhibitory activities against *Botrytis cinerea* using dual culture methods. Chen et al., (2014) reported that endophytic bacterium *Bacillus subtilis* strain EDR4 showed inhibitory effect on a stem rot caused by *Sclerotinia sclerotiorum*. Meanwhile, *Bacillus amyloliquefaciens* isolated from

tomato produced antifungal activity to protect the plant from early blight of tomato disease caused by *Alternaria solani* (Yi et al., 2015).

Table 1. Antifungal activity of endophytic bacteria against cocoa pathogen *Phytophthora palmivora* using dual cultures method

Selected endophytic bacteria	Inhibition zones of *P. palmivora* (diameter, mm)*
LKM-UL	32.2 ± 0.52[c**]
LKM-PA	20.6 ± 0.95[a]
LKM-PD	29.0 ± 1.51[b]
LKM-BL	41.2 ± 1.44[d]

* Antifungal activities in triplicate with the mean and standard deviations. **The number followed by different letters in the column showed significantly different results based on Duncan test at $p < 0.05$.

Growth pattern and antifungal activity

In general, exponential phase of the four endophytes isolate started at the 4 hours incubation and ended before 24 hours incubation period. At this phase, bacterial cells begin to actively reproduce by binary fission (Llorens et al., 2010). After 24 h incubation period, all the four endophytes were found to have entered their stationary phase of growth where the bacterial cell growth and death rates have reached an equilibrium. Eventually, the death rate exceeds the growth rate at 28 h after the incubation period. This might have due to limited nutrients in the culture medium, production of secondary metabolites as a defense against changes of culture conditions (Muhsinin et al., 2016), and accumulation of waste products.

The effect of cultivation period of the four endophytes isolates on their abilities to inhibit the cocoa pathogen *P. palmivora* is illustrated in Figure 1. All four endophytes isolate LKM-UL, LKM-PA, LKM-PD, and LKM-BL supernatants showed that the antagonistic capability against *P. palmivora* was relatively increased as the number of bacterial cells increased. The maximal antagonistic capability for all isolates was recorded at 24 h of cultivation, and a further incubation period (28 and 48 h) showed a gradually decreased in the antagonistic capability. This pattern of antifungal capability was observed among all the four endophytes (LKM-UL, LKM-PA, LKM-PD, and LKM-BL). As reported by Li et al. (2011), the production of bioactive compounds from endophytic bacteria *Bacillus subtilis* ZZ120 was positively correlated with cell growth and was at the highest level during stationary phase 24 h after inoculation. Interestingly, a study by Yi et al., (2015) revealed that *Bacillus amyloliquefaciens* isolated from tomatoes required longer cultivation periods of 48 h to inhibit *Alternaria solani*, a pathogenic fungus that caused early blight in tomato. In this study, results suggested that the bioactive compound production and antifungal activity of the four endophyte isolates increased as the concentration of the cells increased throughout the exponential phase, and were highest at the end of exponential phase at 24 h of cultivation period.

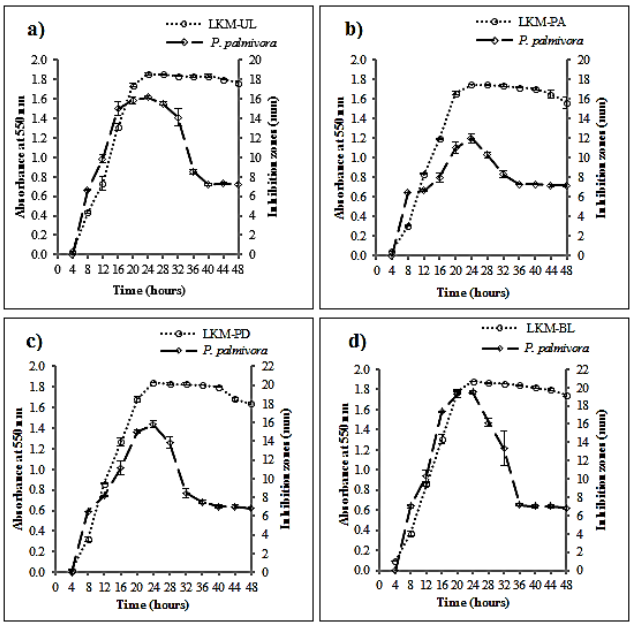

Figure 1. The growth of endophytic bacteria; a) LKM-UL; b) LKM-PA; c) LKM-PD and d) LKM-BL and inhibition zones of cell-free supernatant against cocoa pathogen *P. palmivora*

Surprisingly, the inhibition zones produced by endophytic cells (dual cultures method) were larger than the inhibition zones produced by cell-free supernatant. For example, results from the dual cultures method from LKM-BL cells gave an average of 41.2 ± 1.44 mm inhibition zone compared to the cell-free supernatant of 19.5 ± 0.5 mm. A similar finding was reported by Wang and Liang (2014), where the endophytic bacteria *Bacillus amyloliquefaciens* BZ6-1 cells produced slightly higher antimicrobial substances compared to the cell-free supernatant.

Table 2. Morphology and biochemical characteristics of selected endophytic bacteria isolates

Morphology and biochemical test	Endophytic bacteria			
	LKM-UL	LKM-PA	LKM-PD	LKM-BL
Color	White	Yellowish	Yellowish	Yellowish
Shape	Rod	Rod	Rod	Rod
Length	2.1-2.6 µm	2.0-2.5 µm	2.3-2.5 µm	2.5-2.6 µm
Gram staining	+	-	+	+
Motility	+	+	+	+
Catalase	+	+	+	+
MR	-	+	-	-
VP	+	+	+	+

Note: +, positive; -, negative

It was reported that the rapid growth of endophytic bacteria, nonetheless, offered an advantage in the race for space and nutrients against pathogenic fungi, before any antifungal activity was deployed (Zivkovic et al., 2010). In short, the continuous production of secondary metabolites by the actively growing bacterial cells on solid culture media would result in greater antagonistic activity in comparison to the cell-free supernatant. This finding also indicates the potential of using endophyte isolate LKM-BL as a coating agent for cocoa pods that are growing in the field.

Characterization and identification of endophytic bacteria

The morphological and biochemical characterization of all four endophytes isolate LKM-UL, LKM-PA, LKM-PD, and LKM-BL was summarized in Table 2. In addition, the SEM micrographs of each isolate were shown in Figure 2. There were 3 Gram-positive (isolate LKM-UL, LKM-PD, and LKM-BL) and 1 Gram-negative (LKM-PA) endophytic bacteria screened in this study. Generally, all isolates revealed positive results for motility, catalase, and VP tests. Meanwhile, only endophytic bacteria LKM-PA displayed a positive result in MR test indicating the ability to produce acidic end products. On the basis of morphology and biochemical test, it is obvious that isolates LKM-UL and LKM-PA were a different isolate due to their different characteristics in colony color, Gram staining reaction, and MR test. However, as the tests performed are not sufficient to identify these isolates, a 16S rDNA sequence analysis was subsequently performed.

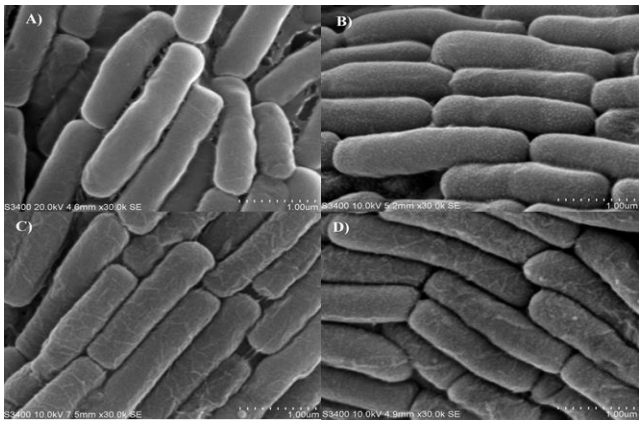

Figure 2. SEM micrographs of four selected endophytic bacteria: A) LKM-UL; B) LKM-PA; C) LKM-PD and D) LKM-BL (30 000 × magnification)

The 16S rDNA sequences of the isolates LKM-UL, LKM-PA, LKM-PD and LKM-BL were submitted to the GenBank with the accession numbers of KR560041, KR560042, KR560043, and KR560044 respectively. Analysis of the 16S rDNA sequence via BLASTn search from the National Center of Biotechnology Information (NCBI) revealed that all endophytes isolate possessed 99% sequences homology to those found in the database. A phylogenetic tree of each endophytic isolates constructed by using a software MEGA7 are shown in Figure 3. Endophytic bacterium LKM-UL was identified as *Bacillus amyloliquefaciens* and designated as *B. amyloliquefaciens* LKM-UL. Isolate LKM-PA was identified as *Pantoea agglomerans* and was designated as *P. agglomerans* LKM-PA. The sequences from endophytic bacterium LKM-PD was identified as *Bacillus pumilus* and was designated as *B. pumilus* LKM-PD, while sequences from 2endophytic bacterium LKM-BL was identical to that of *Bacillus subtilis* and was designated as *B. subtilis* LKM-BL.

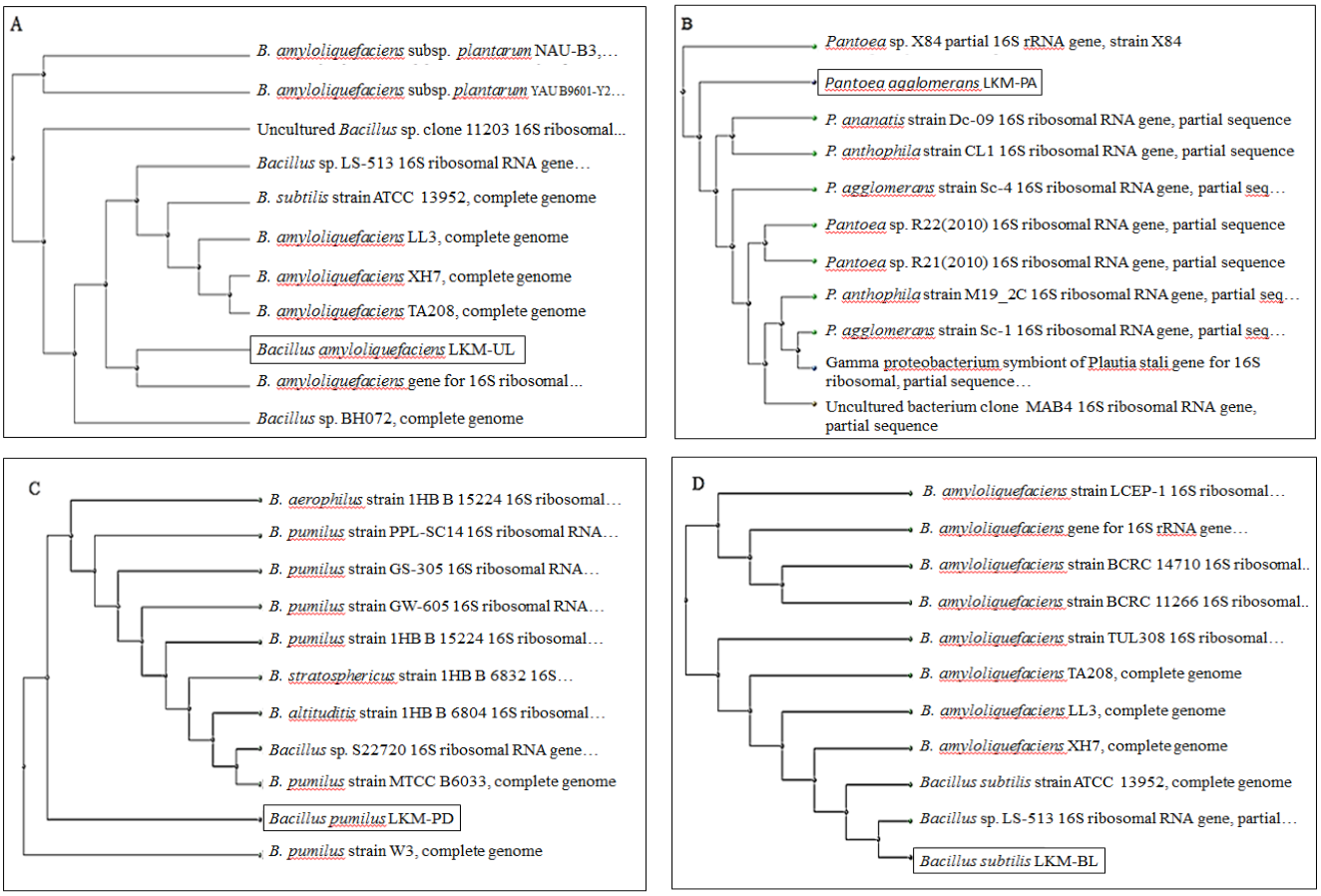

Figure 3. Phylogenetic tree obtained from 16S rDNA sequences showing the position of endophytic bacteria; A) *B. amyloliquefaciens* LKM-UL; B) *P. agglomerans* LKM-PA; C) *B. pumilus* LKM-PD and D) *B. subtilis* LKM-BL

The *Bacillus* species have presented an interesting study on the production of bioactive compounds with a wide spectrum of antifungal activity. For instance, endophytic *B. pumilus* JK-SX001, as depicted by Ren et al., (2013), had the potential to be functioned as a biocontrol agent to protect poplar canker disease caused by three fungal pathogens (*Cytospora chrysosperma*, *Phomopsis macrospora*, and *Fusicoccum aesculi*), while *B. subtilis* EDR4 was reported to inhibit the growth of *Sclerotinia sclerotiorum* fungal (Chen et al., 2014). Other than that, *P. agglomerans* ENA1, which had been isolated from soybean nodule, possessed the potential as to be used as a biocontrol agent against *Macrophomia phaseolina*, the causal agent of charcoal rot in soybean (Vasebi et al., 2015). In addition, *P. agglomerans* was also used to protect pear and apple trees from fire blight caused by the bacterium *Erwinia amylovora* (Stockwell et al., 2002), to control *Pseudomonas syringae* pv. *syringae* the causal agent of basal kernel blight barley (Braun-Kiewnick et al., 2000) and causal agents of corn ear rot by *Stenocarpella maydis* and *S. macrospora* fungi (Petatan-Sagahon et al., 2011).

Conclusion

In conclusion, results obtained from this study has reveal that the endophytic bacteria previously isolated from healthy tissue of cocoa plant have the potential to be used as biological control agents inhibiting the growth of *P. palmivora* the causal agent of cocoa black pod disease. Particularly the endophyte *B. subtilis* LKM-BL possess the greatest antifungal activity against *P. palmivora* during the end of exponential phase at 24 hours of cultivation period in a culture medium. Further investigation to identify bioactive compounds that produce by *B. subtilis* LKM-BL.

Acknowledgment

This work was supported by Intensive Research in Priority Area (IRPA) of Ministry of Sciences, Technology and Environment of Malaysia.

Contribution of Authors

Zubir I: Conceived idea, designed research methodology, literature search, data collection, data interpretation, statistical analysis and manuscript writing.
Ross EER: Conceived idea, literature review and manuscript final reading and approval.
Hamzah A: Conceived idea, literature review and data interpretation.
Aqma WS: Conceived idea, data interpretation and manuscript final reading and approval.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: This work was funded by Intensive Research in Priority Area (IRPA) of Ministry of Sciences, Technology and Environment of Malaysia.

References

Acebo-Guerrero Y, Hernandez-Rodriguez A, Heydrich-Perez M, El Jaziri M and Hernandez-Lauzardo AN, 2012. Management of black pod rot in cocoa (*Theobroma cocoa* L.): a review. Fruits. 67: 41-48.

Adejumo TO, 2005. Crop protection strategies for major disease of cocoa, coffee and cashew in Nigeria. Afr. J. Biotechnol. 4: 143-150.

Agbeniyi SO, Adedeji AR and Adeniyi DO, 2014. On-farm evaluation of *Trichoderma asperellum* on the suppression of *Phytophthora megakarya* causing pod rot of *Theobromae cacao* in Nigeria. Br. J. Appl. Sci. Technol. 4(22): 3153-3159.

Ainon H, Ishak Z, Ernie ERR and Wan SA, 2017. Antagonistic effect and plant growth hormone produced by endophyte *Bacillus amyloliquefaciens* LKM-UL isolated from cocoa plant. Int. J. Biosci. Biochem. Bioinformat. doi: 10.17706/ijbbb.2017.7.3.169-176.

Braun-Kiewnick A, Jacobsen BJ and Sands DC, 2000. Biological control of *Pseudomonas syringae* pv. *syringae*, the causal agent of basal kernel blight of barley, by antagonistic *Pantoea agglomerans*. Phytopathol. 90: 368-375.

Cao Y, Xu Z, Ling N, Yuan Y, Yang X, Chen L, Shen B and Shen Q, 2012. Isolation and identification of lipopeptides produced by *B. subtilis* SQR 9 for suppressing *Fusarium* wilt of cucumber. Scientia Horticulturae. 135: 32-39.

Chen Y, Gao X, Chen Y, Qin H, Huang L and Han Q, 2014. Inhibitory efficacy of endophytic *Bacillus subtilis* EDR4 against *Sclerotinia sclerotiorum* on rapeseed. Biol. Control. 78: 67-76.

Fu G, Huang S, Ye Y, Wu Y, Cen Z and Lin S, 2010. Characterization of a bacterial biocontrol strain B106 and its efficacy in controlling banana leaf spot and post-harvest anthracnose diseases. Biol. Control. 55: 1-10.

Gerhardt P, Murray R, Wood W and Krieg N, 1994. Methods for general and molecular bacteriology. Washington D.C. American Society for Microbiology.

Gupta RM, Kale PS, Rathi ML and Jadhav NN, 2015. Isolation, characterization and identification of endophytic bacteria by 16S rRNA partial sequencing technique from roots and leaves of *Prosopis cineraria* plant. Asian J. Plant Sci. Res. 5(6): 36-43.

Islam S, Akanda AM, Prova A, Islam MT and Hossain MM, 2016. Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. Front Microbiol.http://dx.doi.org/10.3389/fmicb.2015. 01360.

Kefi A. Slimene IB, Karkouch I, Rihouey C, Azaeiz S, Bejaoui M, Belaid R, Cosette P, Jouenne T and Limam F, 2015. Characterization of endophytic *Bacillus* strains from tomato plants (*Lycopersicon esculentum*) displaying antifungal activity against *Botrytis cinerea* Pers. World J. Microbiol. Biotechnol. 31: 1967-1976.

Labrador KL, Lustica ELT and Novero AU, 2014. Isolation and characterization of bacterial endophytes associated with sago palm (*Metroxylon sagu* ROTTB.) in tissue culture. Asian Jr. Microbiol. Biotechnol. Environ. Sci. 16(4): 877-885.

Li H, Soares MA, Torres MS, Bergen M and White Jr JF, 2015. Endophytic bacterium, *Bacillus amyloliquefaciens*, enhances ornamental hosta

resistance to diseases and insect pests. J. Plant Interact. 10(1): 224-229.

Li H, Wang X, Han M, Zhao Z, Wang M, Tang Q, Liu C, Kemp B, Gu Y, Shuang J and Xue Y, 2011. Endophytic *Bacillus subtilis* ZZ120 and its potential application in control of replant diseases. Afr. J. Biotechnol. 11(1): 231-242.

Lin T, Zhao L, Yang Y, Guan Q and Gong M, 2013. Potential of endophytic bacteria from *Sophora alopecuroides* nodule in biological control against *Verticillium* wilt disease. Austr. J. Crop Sci. 7: 139-146.

Llorens JMN, Tormo and Garcia EM, 2010. Stationary phase in Gram-negative Bacteria. FEMS Microbiol. Rev. 34: 476-495.

Muhsinin S, Budiarto RM and Mulyani LN, 2016. Isolation of endophytic bacteria from plant basil (*Ocimum sanctum* L.) as antibacterials against *Staphylococcus aureus*. J. Innov. Pharmaceut. Biol. Sci. 3(4): 92-96.

Paul NC, Ji SH, Deng JX and Yu SH, 2013. Assemblages of endophytic bacteria in chili pepper (*Capsicum annuum* L.) antifungal activity against phytopathogens *in vitro*. Plant Omics. 6: 441-448.

Petatan-Sagahon I, Anducho-Reyes MA, Silva-Rojas HV, Arana-Cuenca A, Tellez-Jurado A, Cardenas-Alvarez IO and Mercado-Flores Y, 2011. Isolation of bacteria with antifungal activity against the phytopathogenic fungi *Stenocarpella maydis* and *Stenocarpella macrospora*. Int. J. Mol. Sci. 12(9): 5522-5537.

Ren JH, Li H, Wang YF, Ye JR, Yan AQ and Wu XQ, 2013. Biocontrol potential of an endophytic *Bacillus pumilus* JK-SX001 against poplar canker. Biol. Control. 67: 421-430.

Shin DS, Park MS, Jung S, Lee MS, Lee KH, Bae KS and Kim SB, 2007. Plant growth-promoting potential of endophytic bacterial isolated from roots of coastal sand dune plants. J. Microbiol. Biotechnol. 17: 1361-1368.

Stockwell VO, Johnson KB, Sugar D and Loper JE, 2002. Antibiosis contributes to biological control of blight by *Pantoea agglomerans* strain Eh252 in orchards. Phytopathol. 92(11): 1202-1209.

Vanegtern B, Rogers M and Nelson S, 2015. Black pod rot of cacao caused by *Phytophthora palmivora*. Plant Dis. 108: 1-5.

Vasebi Y, Alizadeh A and Safaie N, 2015. *Pantoea agglomerans* ENA1 as a biocontrol agent of *Macrophomia phaseolina* and growth enhancer of soybean. J. Crop Prot. 4(1): 43-57.

Wang X and Liang G, 2014. Control efficacy of an endophytic *Bacillus amyloliquefaciens* strain BZ6-1 against peanut bacterial wilt, *Ralstonia solanacearum*. BioMed. Res. Int. 2014: 1-11.

Yi YJ, Li YS, Xia B, Li WP, Pang L and Tong ZD, 2015. Optimization of medium composition and culture conditions for antifungal activity of a tomato endophytic bacterium. Biol. Control. 82: 69-75.

Zivkovic S, Stojanovic S, Ivanovic Z, Gavrilovic V, Popovic T and Balaz J, 2010. Screening of antagonistic activity of microorganisms against *Colletotrichum acutatum* and *Colletrichum gloeosporioides*. Arch.Biol. Sci. Belgrade. 62(3): 611-623.