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ACYL HOMOSERINE LACTONE LACTONASE BACTERIA POTENTIAL AS BIOCONTROL AGENT OF SOFT ROT INFECTION

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ABSTRACT

The virulence gene expression of phytopathogenic bacteria, such as *Dickeya dadantii* is regulated by quorum sensing (QS) mechanism. The QS mechanism is induced by acyl homoserine lactone (AHL) that is universal autoinducer of Gram-negative bacteria. Degradation of AHL compound can be used to control the virulence of phytopathogenic bacteria. The enzyme of AHL lactonase is encoded by the *aiiA* gene and can degrade the lactone ring of AHL. This study was aimed to characterize *aiiA* gene encoding AHL lactonase from 20 isolates of shrimp pond and soil forest, Indonesia and examine the isolates ability to reduce violacein pigment of *Chromobacterium violaceum* and soft rot disease symptoms caused by *D. dadantii*. The results showed nine bacterial isolates (B4, B10, B11, B13, B14, B16, B17, B18 and B19) containing the *aiiA* gene encoding AHL lactonase. Based on 16S rRNA gene identification, 9 isolates had the highest similarity with *Bacillus toyonensis*, *B. cereus, B. thuringiensis, B. mycoides* and *B. subtilis*. These isolates had the capability to reduce the formation of violacein pigment and the symptoms of soft rot disease in potatoes. These results show that the AHL lactonase produced by these isolates can be used as Quorum Quenching (QQ) agent to inhibit the QS mechanism

Key words: Acyl-Homoserine lactone, lactonase, *C. violaceum, D. dadantii,* Quorum quencing

INTRODUCTION

Phytopathogenic bacteria can reduce agricultural and horticultural production of plants. Infection and colonization of the plants results in abnormal symptoms, such as spot, soft rot, cancer, or hormone imbalance, which lead to plant overgrowth, stunting, root branching, or death. These condition results in the lower quality and quantity of plants (Mansfield *et al.,* 2012). *Pseudomonas viridiflava, Pectobacterium carotovorum*, and *Dickeya* spp. are responsible of soft rot disease in wide range of crops and ornamental plants (Reverchon & Nasser, 2013). These bacteria can produce extracellular enzymes, such as protease, pectinase, and cellulose, as a virulence factor causing maceration of plant tissue. *Dickeya* spp. are a broad range spectrum phytopathogens, responsible in causing soft rot and wilt disease in carrot, banana, corn, tomato, sweet potato, cabbage, chrysanthemum, *Begonia, Dahlia, Freesia, Hyacinthus, Iris, Kalanchoe* dan *Zantedeschia* (Lee *et al.,* 2002; Samson *et al.,* 2005).

Virulence factor of some phytopathogenic bacteria are regulated through quorum sensing mechanisms (Defoirdt *et al.,* 2004). Quorum sensing (QS) is a signaling system that occurs in pathogenic bacteria to sense its own population density and synchronize the expression of the virulence genes via secretion of small, diffusible signal molecules or autoinducers (AIs) (Fuqua & Greenberg, 2002). QS can regulate the expression of genes associated with pathogenicity including biofilm formation and virulence of bacterial pathogens such as *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Erwinia* spp. (Crepin *et al.,* 2012). This signaling process or quorum sensing plays an important role in coordinating many physiological phenomena such as regulation of the expression of pathogenicity genes, formation of

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biofilm, bioluminescence, sporulation, antibiotic production, and formation of pigment such as violacein (Fuqua & Greenberg, 2002). The QS mechanism depends on the density of bacterial cells that produced and accumulated signal molecules or autoinducers (AIs).

Based on the importance of the QS process, the anti-QS mechanism can be used as biocontrol for phytopathogenic bacteria. One of the anti-QS mechanisms is quorum quenching (QQ) through the degradation of N-acyl homoserine lactones (AHLs) compounds using AHL lactonase enzyme. AHLs are universal autoinducer compounds of Gram-negative pathogenic bacteria such as *Erwinia carotovora* and *Agrobacterium tumefaciens* (Fuqua & Greenberg, 2002). The disruption of the QS mechanism occurs so that virulence genes of pathogenic bacteria are not expressed (Czajkowski & Jafra, 2009). The QQ mechanism is the environmental friendly mechanism because it suppress bacterial virulence without killing the pathogen (Zhang *et al.,* 2019).

The successive quorum quenching mechanism against the infection of phytopathogenic bacteria was firstly reported by Dong *et al.* (2000). *aiiA* gene from *Bacillus* 24B01 had been expressed in *E. carotovora* SCG1 that producing AHL lactonase which decreased the AHL compound and extracellular pectinolytic enzyme production. This QQ process in *E. carotovora* (*Pectobacterium caratovorum*) SCG1 brought positive impact on the reduction of soft rot disease symptoms in potato, eggplant, cabbage, carrot, celery, cauliflower, and tobacco (Dong *et al.,* 2000). Molina *et al.* (2003) reported that expressing of AHL lactonase from *Bacillus* spp. in *Pseudomonas fluroscene* P3 significantly reduced potato soft rot caused by *Erwinia carotovora* and crown gall of tomato caused by *Agrobacterium tumefaciens.* Several rhizospheric and phyllospheric bacteria that produce AHL lactonase has been known to control the pathogenic *D. dadantii* onset in potato and orchid (Khoiri *et al.,* 2016; Sari *et al.,* 2017; Satwika *et al*., 2017). Recent study found that QQ activity of *Pseudomonas segetis* P6 isolated from *Salicornia europaea* alleviated the soft rot disease symptoms caused by *Dickeya solani*, *Pectobacterium atrosepticum*, and *P. carotovorum* in potato and carrot (Rodriguez *et al.,* 2020). Exploration and characterization of potential quorum quencher bacterial isolates should be developed to control the phytopathogenic bacteria. This study aimed to characterize AHL lactonase producing bacteria of 20 bacterial isolates originated from shrimp pond and forest soil, Indonesia and observe their capability to reduce violacein pigment formation of *C. violaceum* and soft rots symptoms of *D. dadantii.*

MATERIAL AND METHODS

Materials

Bacteria used in this study are 20 bacterial isolates that are isolated from shrimp pond sediment and forest soil, Indonesia. We used 20 bacteria without prior isolation, obtained from Dr. Iman Rusmana and Dr. Alina Akhdiya collection, co-author of this journal, which was used in the previous research. *C. violaceum* and *D. dadantii* A3 were obtained from ICABIOGRAD Microbiology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, IPB University, Indonesia collection. All bacteria were cultured in LB liquid medium at 28°C.

Bioassay of AHL-degrading activity

The bioassay was determined using a disc diffusion assay based on Fitriyah *et al.* (2015). All bacteria were cultured in Luria Bertani Broth (LB). Then, bacterial culture was centrifuged at 16,000 g for 10 min and supernatant was filtered using 0.2 um membrane filter. As much as 20 µL supernatant was dropped on a 6 mm paper disc. The disc was placed on a LB agar plate containing 1% of *C. violaceum* (OD_{600} 0.8). Plate was incubated at room temperature for 24 h and 20 µl of sterile dH_2O was used as control. AHL degradation activity was indicated and determined by measuring non-purple zone diameter around paper disc. AHL-degradation index was calculated by the following formula:

$$\text{AHL degradation index} = \frac{\text{non-purple zone diameter} - \text{paper disc diameter}}{\text{paper disk diameter}} \times 100\%$$

Detection of *aiiA* gene encoding AHL lactonase

Genomic DNA extraction was performed by Presto™ Mini gDNA procedure Bacteria Kit (Geneaid). The concentration and the purity of DNA were calculated using Nanodrop 2000 (Thermo Scientific Wilminton DE, USA). Amplification of *aiiA* gene from selected bacterial isolates was performed using an *aiiA*F primer (5'-GGA AGA TCT ATG ACA GTA AAG AAG CTT TAT TTC G-3') and *aiiA*R primer (5'-CATG CCA TCA TCA ACA AGA TAC TA TGA TGA TGT- 3') (Dong *et al.,* 2000). The amplification was done with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of amplification at denaturation 95°C for 30 sec, annealing 55°C for 45 sec, elongation 72°C for 30 sec and then followed by final elongation 72°C for 10 min. The PCR products were sent to First Base, Malaysia for sequencing. The sequences were analyzed using Seqtrace and compared to *aiiA*

gene database using the BLASTN and amino acid database using the BLASTX from the NCBI (www.ncbi.nlm.nih.gov). Phylogenetic analysis was performed using MEGA 5.05 with Neighbour Joining (NJ) method with bootstrap 1000 replication.

Identification of bacterial isolates

Amplification of 16S rRNA gene was performed by using primers of 63f (5'-CAG GCC CAC ATG TAA CAA GTC-3') and 1387r (5'-GGG CGG GTA WGT CAA GGC-3') (Marchesi *et al.,* 1998). The amplification was done with an initial denaturation step at 95°C for 5 min, followed by 30 cycles of amplification at 95°C for 1 min denaturation, annealing at 55°C for 1 min, and extension at 72°C for 1.5 min with a final extension step at 72°C for 10 min then followed by final elongation 72°C for 10 minutes. The sequence results were analyzed as described earlier.

Bioassay of violacein production

A total of 30 mL LB broth was inoculated with 1% *C. violaceum* OD_{660} = 0.05. About 300 µL supernatant was added to LB broth containing *C. violaceum* followed by incubation for 24 and 48 hours at 27°C. The production of violacein was measured by extracting the violacein pigment from the culture of *C. violaceum* (Choo *et al.,* 2006). The positive control was *C. violaceum* culture without inoculation of the test bacterial supernatant. A total of 1.5 mL each *C. violaceum* culture was centrifuged at 16,000 g for 15 min. The supernatant was removed and pellet was resuspended in 1.5 mL DMSO. Pellet was centrifuged at 16.000 g for 15 minutes. The violacein extracted in the supernatant was estimated by measuring OD at 585 nm. Violacein unit was calculated as the ratio (OD585/OD660), which gives an indication of violacein production per unit of growth (Chaudhari *et al.,* 2004).

Bioassay of anti-QS activity against *Dickeya dadantii* A3

Bacterial isolates producing AHL-lactonase were tested for their ability to inhibit virulence of *D. dadantii* A3 using the method described by Dong *et al.* (2000). Potato tuber was surface sterilized by immersing in 70% ethanol for 1 min and sterile water for 3 min. The potato tuber was then wounded 0.6 cm in depth on the centre part and soaked in bacterial cultures then, the wound was inoculated with 1 µL of *D. dadantii* A3 (OD_{660} = 0.8). Symptoms of soft rot disease in potato tuber were observed after 24 hours of incubation at room temperature.

RESULTS AND DISCUSSION

AHL degradation activity

C. violaceum is the QS indicator bacteria due to its capablility to produce violacein pigment through QS mechanism. The violacein pigment formation of *C. violaceum* is determined by the concentration of N-hexanoyl homoserine lactone as an autoinducer compound (Morohoshi *et al.,* 2008). Among 20 bacterial isolates, 9 isolates exhibited AHL degradation activity against *C. violaceum* (Table 1). The non-purple zone indicated the inhibition of violacein pigment production by *C. violaceum* (Figure 1).

The results suggested that the nine bacterial isolates produce AHL degrading enzymes. The nine bacterial isolates showed AHL degradation index in the range of 0.33–4.17 (Table 1). Highest value of inhibition zone was B16 with AHL degradation index as 4.17 (Figure 1; Table 1). B13 and B18 showed the lowest AHL degradation index of 0.33 (Figure 1; Table 1). Fitriyah *et al.* (2015) reported that 6 bacteria isolated from the agricultural land had an AHL degradation index in the range of 0.53–2.61. AHL degrading bacteria from various

Table 1. The N-hexanoyl homoserine lactone degradation index of bacterial supernatant indicated

Isolate Code	Source	Diameter of non-purple zone formation (mm)	AHL Degradation Index
B4	Lampung Shrimp Pond	16	1.67
B10	Cangkuang Forest Sukabumi	18	2.00
B11	Cangkuang Forest Sukabumi	14	1.33
B13	Cangkuang Forest Sukabumi	8	0.33
B14	Cangkuang Forest Sukabumi	23	2.83
B16	Cangkuang Forest Sukabumi	32	4.17
B17	Cangkuang Forest Sukabumi	9	0.5
B18	Cangkuang Forest Sukabumi	8	0.33
B19	Cangkuang Forest Sukabumi	13	1.17

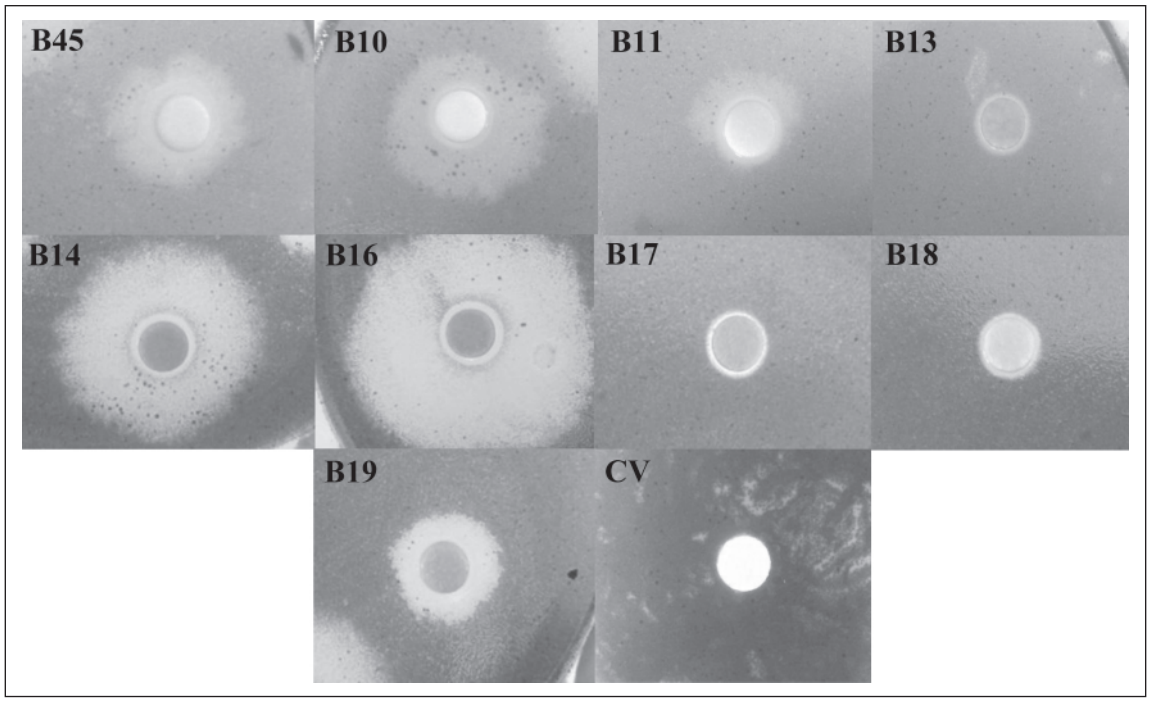

Fig. 1. Formation of non-purple zone around the paper disc soaked by supernatant of selected bacterial isolates. The symbols CV indicate negative control.

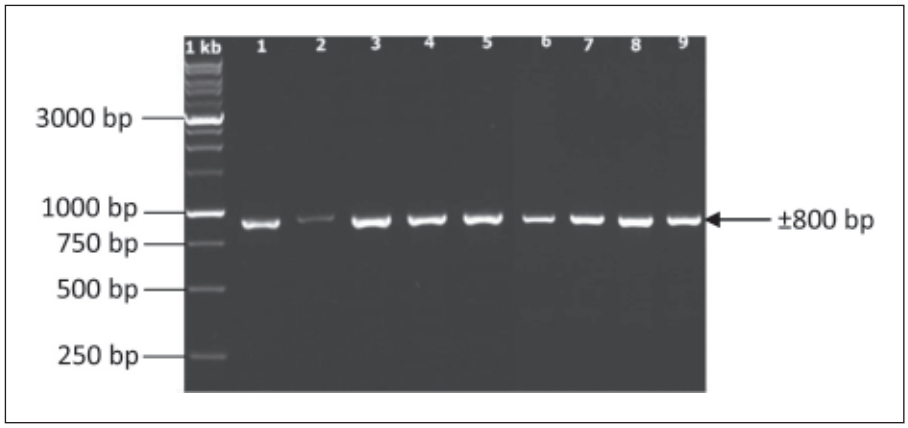

Fig. 2. PCR products (800 bp) of *aiiA* genes visualized on 0.8% agarose gel electrophoresis. M: 1 kb DNA marker; Lane 1, 2, 3, 4, 5, 6, 7, 8 and 9: indicating PCR products of of B4, B10, B11, B13, B14, B16, B17, B18, and B19 respectively.

rhizosphere and phylosphere samples in Indonesia were reported to have an AHL degradation index in the range of 0.077–3.08 (Satwika *et al.,* 2017).

***aiiA* gene of AHL degrading bacteria analysis**

The result showed that *aiiA* gene encoding AHL lactonase had amplified from all 9 bacterial isolates. These isolates had obtained AHL degrading activity based on bioassay using *C. violaceum* (Figure 2). Further amino acid analysis based on the *aiiA* sequences indicated high similarity to the amino acids of AHL lactonase from the *Bacillus* group such as *B. thuringiensis*, *B. cereus*, *B. mycoides*, and *B. wiedmannii* with homology 96–99% (Table 2). Phylogenetic tree based on the amino acid sequences of AHL lactonase of all the 9 AHL degrading bacteria showed that they had a close relationship with AHL lactonase of *Bacillus* group, however they had different clusters with the AHL lactonase produced by the *Rhodococcus facians* (Figure 3).

The AHL lactonase enzyme is a metallo-betalactamase (metalloenzyme) that is able to hydrolyze the ester bond on the homoserine lactone ring of AHL and the acyl homoserine compound is not recognized as a signal molecule. It cannot bind

Table 2. The amino acid sequence homology of AHL-degrading bacterial isolates

Bacterial Isolate	Reference bacterial strain (*Gene Bank Database*)	Identity (%)	*E-value*	Acc. Number
B4	MULTISPECIES: N-acyl homoserine lactone hydrolase [*Bacillus*]	99	0.0	WP_000216574.1
B10	N-acyl homoserine lactonase [*B. cereus]*	99	0.0	WP_059303559.1
B11	N-acyl homoserine lactonase [*Bacillus thuringiensis*]	97	8e-177	WP_071731229.1
B13	MULTISPECIES: N-acyl homoserine lactonase [*Bacillus cereus* group]	96	1e-176	WP_000216580.1
B14	MULTISPECIES: N-acyl homoserine lactonase *aiiA* [*Bacillus*]	96	6e-174	WP_016111450.1
B16	N-acyl homoserine lactonase [*Bacillus mycoides*]	93	2e-167	WP_044438838.1
B17	N-acyl homoserine lactonase [*Bacillus weihenstephanensis*]	90	3e-165	WP_070128835.1
B18	N-acyl homoserine lactonase [*Bacillus cereus*]	96	1e-174	WP_017656305.1
B19	N-acyl homoserine lactonase [*Bacillus wiedmannii*]	97	2e-176	WP_061677651.1

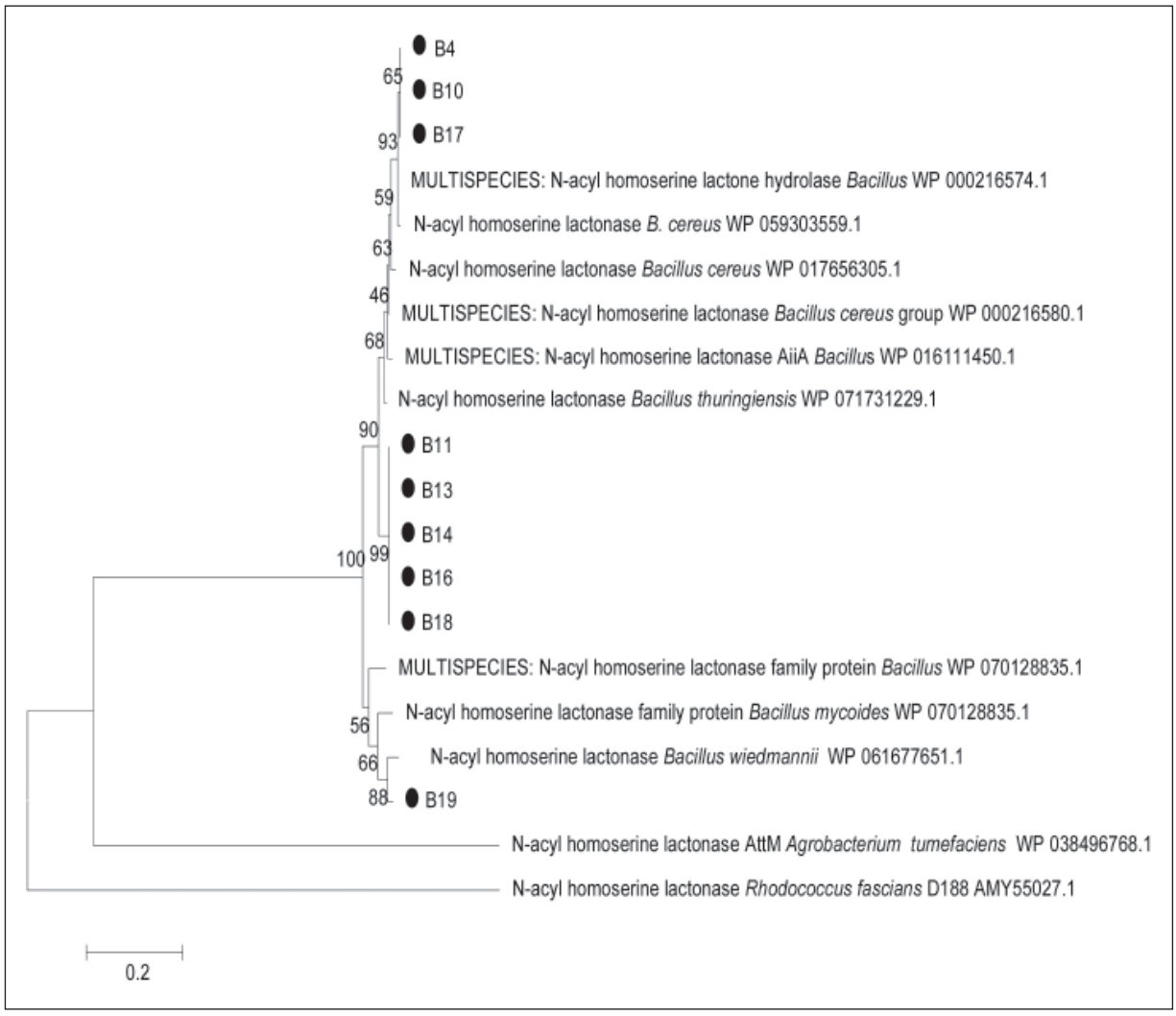

Fig. 3. Phylogenetic tree based on *aiiA* gene nucleotide sequences using maximum likelihood method (bootstrap 1000 replication).

Table 3. Homology of 16S rRNA gene sequence of AHL degrading bacteria isolate

Isolate code	Reference species(*Database Gene Bank*)	Identity (%)	*E-value*	Accession number
B4	*Bacillus cereus* strain EM20	100	99	KJ612546.1
B10	*Bacillus cereus* strain MER140	100	99	KT719718.1
B11	*Bacillus cereus* strain p20	100	98	KX783605.1
B13	*Bacillus thuringiensis* strain SSA550	100	98	KC534226.1
B14	*Bacillus cereus* strain EM6	100	99	KJ612533.1
B16	*Bacillus cereus* strain EM5	100	99	KJ612532.1
B17	*Bacillus toyonensis* strain BCT-7112	100	99	NR121761.1
B18	*Bacillus subtilis* strain PJS	100	99	KU672511.1
B19	*Bacillus mycoides* strain WJB140	100	99	KU877669.1

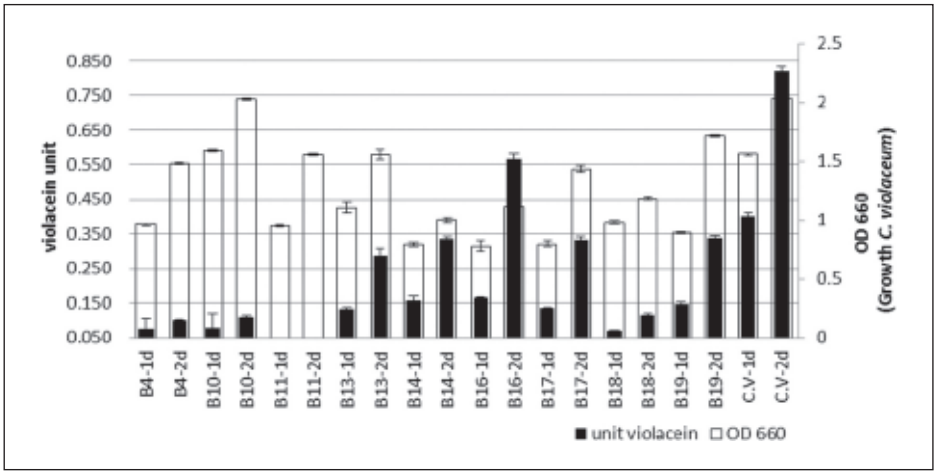

Fig. 4. Violacein production and growth of *C. violaceum* cultures treated with bacterial supernatant. CV = Control Treatment; B4, B10, B11, B13, B14, B16, B17, B18, B19 = treatment of supernatant bacterial code; 1d = 24 hours incubation; 2d = 48 hours incubation.

to the transcriptional regulatory protein, such as ExpR so that the degradation of AHL by the enzyme inhibits the QS mechanism. All genes regulated by the QS mechanism are not expressed such as the genes involve in virulence factors of bacterial phytophatogens (Defoirdt *et al.,* 2004).

AHL lactonase from *Bacillus* spp. reported to have high activity to degrade AHL with various side-chain groups and acyl chain lengths such as C4-HSL (N-butyryl-dl-homoserine lactone), C6-HSL (N-hexanoyl-dl-homoserine lactone), C8-HSL (N-octanoyl-dl-homoserine lactone), C10-HSL (N-decanoyl-dl-homoserine lactone) and C12-HSL (N-dodecanoyl-dl-homoserine lactone) (Wang *et al.,* 2004; Torres *et al.,* 2013).

Identification of AHL-lactonase producing bacteria

Isolates displaying positive results for *aiiA* gene were further identified through amplification of 16S rRNA gene. Blast-N analysis of 16S rRNA gene sequences showed that these isolates were closely related to genus *Bacillus* i.e. *B. thuringiensis*, *B. cereus, B. mycoides*, *B. subtilis*, and *B. toyonensis* (Table 3). Dong *et al.* (2002) reported that the *aiiA* gene was found in *Bacillus* species including *B. subtilis, B. cereus, B. mycoidies*, and some strains of *B. thuringiensis*. Huma *et al*. (2011) reported that the *aiiA* gene encoding the AHL lactonase enzyme is widespread among *Bacillus* sp. strains including *Bacillus cereus*, *B. thuringiensis*, *B. anthracis* and *B. mycoides.*

Inhibition of violacein formation produced by *C. violaceum*

The violacein pigment formation in *C. violaceum* is regulated by QS mechanisms (Hoshino, 2011). This ability can be calculated based on the violacein units, which can be measured at 585/660 nm (Chaudhari *et al.,* 2004). The former OD presented violacein pigment, while the latter represented bacterial growth. The result showed that each bacterial supernatant tested had different ability to inhibit the violacein pigment formation. In general, bacterial supernatant was able to reduce formation of the pigment after 24 hours and 48 hours incubation (Figure 4). The addition of B4, B11, B13, B14, B16, B17, B18 and B19 supernatant

also showed inhibition violacein formation and growth suppression of *C. violaceum*. The growth inhibition of *C. violaceum* was observed from OD 660 nm that was lower than the one without bacterial supernatant addition (control) (Figure 4). This result indicated that 8 AHL degradation isolates were also capable in producing antimicrobial compounds. The result showed that *B. cereus* B10 supernatant addition did not suppress *C. violaceum* growth. In consequence, *C. violaceum* growth with the B10 supernatant addition was same as control (Figure 4). The result showed that the capability of *B. cereus* B10 in violacein formation inhibition occurs through quorum quenching mechanism. Khori *et al.* (2017) reported that inoculated bacterial supernatants of EKK10 and B13 could inhibit violacein pigment and growth inhibition of *C. violaceum* based on 660 nm value.

Anti-quorum sensing activity against *Dickeya dadantii* A3

Dickeya dadantii (ex: *Erwinia chrysanthemi*) is a plant pathogenic bacterium generating soft rot diseases on various plants, including chicory, arabidopsis, orchids, saintpaulia, cabbages, and carrots (Lamas *et al.,* 2009). The anti-QS activity test was performed on potato tubers to determine the ability of the isolates in suppression of soft rot disease caused by *D. dadantii* A3. Expression of the genes associated with virulence factors on *D. dadantii* is regulated by quorum sensing mechanism, using 3-oxo-C6-HSL as an autoinducer (Czajkowski & Jafra, 2009).

The results showed that 9 AHL lactonase producing bacterial isolates were successful in suppressing the soft rot disease caused by *D. dadantii* A3 compared with control (Figure 5 and Figure 6). The results showed that soft rot symptom was not detected in potato after *B. cereus* B11, *B. cereus* B13, *B. thuringiensis* B14 inoculation. These bacterial isolates have the highest suppression of *D. dadantii* infection. This inhibition was not only impacted by the AHL lactonase, which was produced by these bacteria. The three bacteria also have antibiosis activity based on the *C. violaceum* growth analysis (Figure 4). Satwika *et al*. (2017) reported that AHL degrading bacteria also had antibiosis activity. In contrast to the other bacterial isolates, *B. cereus* B10 had no antibiotic activity based on the *C. violaceum* growth analysis. This result indicated the soft rot symptom reduction on potato occurs through the QQ mechanism. The AHL lactonase produced by nine bacteria could interfere QS *D. dadantii* in consequence of pectinolytic enzyme gene unsuccessfully expressed. *Bacillus* spp. known as biocontrol agent with various modes of action, including: antibiosis through antimicrobial compounds production such as lipopeptides, antibiotics, and bacteriocins; quorum sensing discruption through inactivation of acyl-homoserine lactones (Raaijmakers *et al.,* 2010; Gerayeli *et al.,* 2018). Krzyzanowska *et al.* (2012) reported the

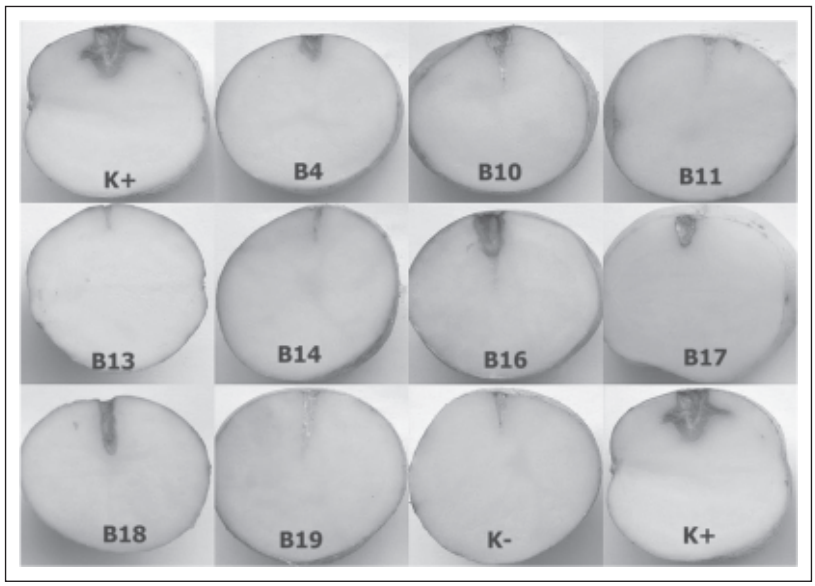

Fig. 5. Inhibition of soft rot symptoms caused by *D. dadantii A3* on potato tubers due to degradation of AHL signal by the bacterial isolates.
K+ = positive control (inoculated with *D. dadantii* A3 only); K- = negative control (inoculated with sterilewater); and B4, B10, B11, B13, B14, B16, B17, B18, B19 = treatment of potato tuber with bacterial supernatant before inoculation with *D. dadantii* A3.

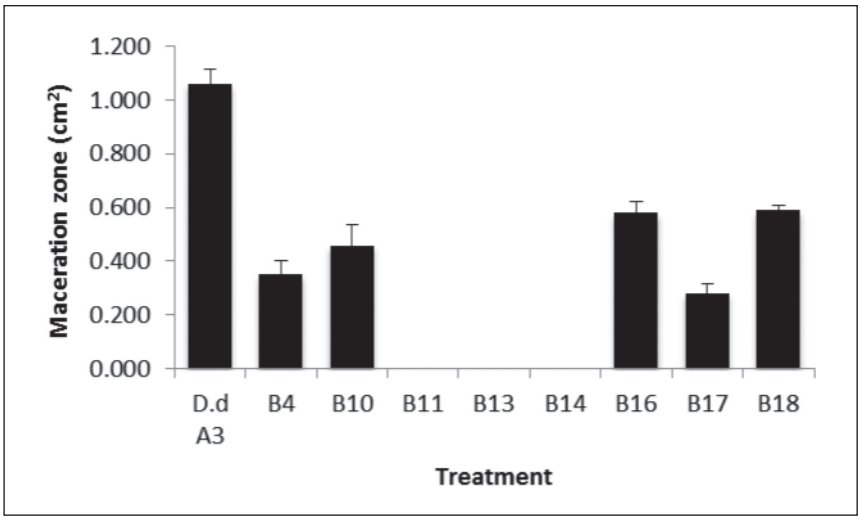

Fig. 6. The tuber maceration zone of soft rot symptoms caused by *D. dadantii A3* on potato tubers due to degradation of AHL signal by the bacterial isolates.
D.d A3 = positive control (inoculated by *D. dadantii* A3 only); and B4, B10, B11, B13, B14, B16, B17, B18, B19 = treatment of potato tuber by soaking in the bacterial isolate cultures (indicate by the symbol, respectively) before they were inoculated with *D. dadantii* A3.

role of qourum quenching mechanism is more effective than the antimicrobial mechanism against *Pectobacterium* spp. and *Dickeya* spp. infection in potato and chicory.

Recent study reported that transgenic tobacco, *Amorphophallus konjac*, and cabbage expressing AHL lactonase were able to reduce soft rot disease caused by *Erwinia carotovora* SCG1 (Dong *et al.,* 2001; Dong *et al.,* 2002; Ban *et al.,* 2009). The AHL lactonase of *Bacillus subtilis* BSI was used to control the soft rot symptoms caused by *Erwinia carotovora* var. *carotovora* on potato (Pan *et al.,* 2008). Our results also supported the previous research that reported AHL lactonase producing bacteria had potential to inhibit the virulence of *D. dadantii.* Many pathogenic bacteria perform quorum sensing to regulate the expression of virulence genes. Therefore, in order to control plant phatogenic bacteria it was suggested to interfere their bacterial quorum sensing system by quorum quenching for treating or preventing the phytopatogenic bacterial infection.

CONCLUSION

The nine bacterial isolates namely B4, B10, B11, B13, B14, B16, B17, B18, and B19 were successfully characterized as AHL lactonase degrading bacteria. These isolates have capability to inhibit violacein pigment formation of *C. violaceum* and reduce soft rot disease on potato tuber of *D. dadantii* infection. The nine AHL lactonase degrading bacteria could be developed as biocontrol agents through quorum quenching mechanism approach.

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REFERENCES

Ban, H., Chai, X., Lin, Y., Zhou, Y., Peng, D., Zhou, Y., Zou, Y., Yu, Z. & Sun, M. 0000. Transgenic *Amorphophallus konjac* expressing synthesized acyl-homoserine lactonase (*aiiA*) gene exhibit enhanced resistance to soft rot disease. *Plant Cell Report*, **28**: 1847-1855.

Chaudhari, V., Gosai, H., Raval, S. & Kothari, V. 2004. Effect of certain natural products and organic solvents on quorum sensing in *Chromobacterium violaceum*. *Asian Pacific Journal of Tropical Medicine*, **7(1)**: S204-S211.

Choo, J.H., Rukayadi, Y. & Hwang, J.K. 2006. Inhibition of bacterial quorum sensing by vanilla extract. *Letter in Applied Micro-biology*, **42(6)**: 637-641.

Crepin, A., Cirou, A.M., Barbey, C., Farmer, C., Helias, V., Burini, J.F., Faure, D. & Latour, X. 2012. *N-acyl homoserin lactone*s in diverse *Pectobacterium* and *Dickeya* plant pathogen: diversity, abudance and involvement in virulence. *Sensor*, **12(3)**: 3494-3497.

Czajkowski, R. & Jafra, S. 2009. Quenching of acyl-homoserine lactone dependent quorum sensing by enzymatic disruption of signal molecules. *Acta Biochimica Polonica*, **56(1)**: 1-16.

Defoirdt, T., Boon, N., Bossier, P. & Verstraete, W. 2004. Disruption of bacterial quorum sensing: an unexplored strategy to fight infections in aquaculture. *Aquaculture*, **240(14)**: 69-88.

Dong, Y.H., Xu, J.L., Li, X.C. & Zang, L.H. 2000. *aiiA*, a novel enzyme inactivates acyl homoserine-lactone quorum sesing signal and attenuates the virulence of *Erwinia caratovora. Proceedings of the National Academy of Sciences*, **97(7)**: 3526-3531.

Dong, Y.H., Gusti, A.R., Zhang, Q., Xu, J.L. & Zhang, L.H. 2002. Identification of quorum quoenching *N-acyl homoserin lactone*s from *Bacillus* spesies. *Applied and Environmental Microbiology*, **68(4)**: 1754-1759.

Fuqua, C. & Greenberg, E.P. 2002. Listening in on bacteria acyl-homoserine lactone signaling. *Nature Rev*., **3**: 683-695.

Fitriyah, D., Rusmana, I., Wahyudi, A.T. 2015. Characterization of bacteria producing acyl homoserine lactone (AHL) lactonase for agricultural lands. *Advance in Enviromental Biology*, **9(8)**: 140-148.

Gerayeli, N., Ravari, S.B. & Tarigh S. 2018. Evaluation of the antagonistic potential of *Bacillus* strains against *Pectobacterium carotovorum* subsp. *carotovorum* and their role in the induction of resistance to potato soft rot infection. *European Journal of Plant Pathology*, **150(4)**: 1049-1063.

Hoshino, T. 2011. Violacein and related tryptophan metabolites produced by *Chromobacterium violaceum*: biosynthetic mechanism and pathway for construction of violacein core. *Applied Microbiolgy and Biotechnology*, **91**: 1463-1475.

Huma, N., Shankar, P., Kushwah, J., Bhushan, A., Joshi, J., Mukherjee, T., Raju, S., Purohit, H.J. & Kalia, V.C. 2011. Diversity and polymorphism in AHL-lactonase gene (*aiiA*) of *Bacillus*. *Journal Microbiology and Biotechnology*, **21**: 1001-1011.

Khoiri, S., Damayanti, T.S. & Giyanto. 2017. Identification of quorum quenching bacteria and its biocontrol potential against soft rot disease bacteria, *Dickeya dadantii. AGRIVITA Journal of Agricultural Science*, **39(1)**: 45-55.

Krzyzanowska, D.M., Potrykus, M., Golanowska, M., & Polonis, K. 2012. Rhizosphere bacteria as potential biocontrol agents against soft rot caused by various *Pectobacterium* and *Dickeya* spp. strains. *Journal of Plant Pathology*, **94(2)**: 367-378.

Lamas, M.A., Ordonez, E.C., Solanilla, E.M., Raposo, R. & Salazar, O.T., Moreno, A.R., Palenzuela, P.R. 2009. Role of motility and chemotaxis in the pathogenesis of *Dickeya dadantii* 3937 (ex *Erwinia chrysanthemi* 3937. *Microbiology*, **155**: 434-442.

Lee, Y.A., Chen, K.P. & Chang, Y.C. 2002. First report of bacterial soft rot of white flowered calla lily caused by *Erwinia chrysanthemi* in Taiwan. *Plant Disease*, **86(11)**: 1273-1273.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V. & Beer, S.V. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular Plant Pathology*, **13(6)**: 614-629.

Marchesi, J.R., Sato, T., Weightman, A.J., Martin, A.T., Fry, J.C., Hiom, S.J. & Wade, W.G. 1998. Design and evaluation of useful bacterium-specific primers that amplify genes coding for bacterial 16S rRNA. *Applied and Environmental Microbiology,* **64(2)**: 795-799.

Molina, L., Constantinescu, F., Michel, L., Reimmann, C., Duffy, B. & Défago, G. 2003. Degradation of pathogen quorum-sensing molecules by soil bacteria: a preventive and curative biological control mechanism. *FEMS Microbiology Ecology*, **45(1)**: 71-81.

Morohoshi, T., Kato, M., Fukamachi, K., Kato, N. & Ikeda, T. 2008. N-Acylhomoserine lactone regulates violacein production in *Chromobacterium violaceum* type strain ATCC12472. *FEMS Microbiology Letter*, **279**: 124-130.

Pan, J., Huanga, T., Yao, F., Huang, Z., Charles, A., Powell, Qiu, S. & Guana, X. 2008. Expression and characterization of *aiiA* gene from *Bacillus subtilis* BS-1. *Microbiological Research*, **163(6)**: 711-716.

Raaijmakers, J.M., De Bruijn, I., Nybroe, O. & Ongena, M. 2010. Natural functions of lipopeptides from *Bacillus* and *Pseudomonas*: more than surfactants and antibiotics. *FEMS Microbiology Review*, **34**: 1037-1062.

Reverchon, S. & Nasser, W. 2013. *Dickeya* ecology, environment sensing and regulation of virulence programme. *Environmental Microbiology Reports*, **5(5)**: 622-636.

Rodriguez, M., Torres, M., Blanco, L., Béjar, V., Sampedro, I. & Llamas, I. 2020. Plant growth-promoting activity and quorum quenching-mediated biocontrol of bacterial phytopathogens by *Pseudomonas segetis* strain P6. *Scienific Reports*, **10**: 4121.

Samson, R., Legendre, J.B., Christen, R., Fischer, L., Saux, M., Achouak, W. & Gardan, L. 2005. Transfer of *Pectobacterium chrysanthemi* (Burkholder *et al.,* 1953) Brenner I. 1973 and Brenneria paradisiaca to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov and

Dickeya paradisiaca comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. *International Journal of Systematic and Evolutionary Microbiology*, **55**: 1415-1427.

Sari, P.E., Rusmana, I. & Akhidya, A. 2016. AHL-lactonase characteristics of *Bacillus thuringiensis* SGT3g and its effectiveness in inhibiting pathogenicity of *Dickeya dadantii*. *Malaysian Journal of Microbiology*, **12(4)**: 315-321.

Satwika, T.D., Rusmana, I. & Akhidya, A. 2017. Potensi *quorum quenching* bakteri filosfer dan rizosfer terhadap *Dickeya dadantii*. *Jurnal AgroBiogen*, **13(2)**: 101-110.

Torres, M., Romeroc, M., Pradoc, S., Dubertc, J., Tahriouia, A, Oteroc, A. & Lamasa, I. 2013. N-acylhomoserine lactone-degrading bacteria isolated from hatchery bivalve larval cultures. *Microbiological Research*, **168(9)**: 547-554.

Wang, L.H., Weng, L.X., Dong, Y.H. & Zhang, L.H. 2004. Specificity and enzyme kinetics of the quorum-quenching n-acyl homoserine lactone lactonase (AHL-lactonase). *The Journal of Biological Chemistry*, **279(14)**: 13645-13651.

Zhang, J., Feng, T., Wang, J., Wang, Y. & Zhang, X.H. 2019. The mechanisms and applications of Quorum Sensing (QS) and Quorum Quenching (QQ). *Journal of Ocean University of China*, **18**: 1427-1442.