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Identification of Quorum Quenching Bacteria and Its Biocontrol Potential Against Soft Rot Disease Bacteria, *Dickeya dadantii*

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ABSTRACT

Dickeya dadantii is one of newly found bacteria causing soft rot on orchids in Indonesia. Infected plants showed severe rot rapidly only in few days. An effort to control the bacteria was conducted by utilizing selected quorum quenching (QQ) inducer bacteria which produce AHL-lactonase by *aii*A gene. The aims of this research were to screen and identify of quorum quenching bacteria, and also assayed their biocontrol potential ability against *D. dadantii* in laboratory. The screening of QQ bacteria was achieved using the anti-QS test, anti-microbial activity, and detection of *aii*A gene using specific primer. The determination of the ability against *D. dadantii* was done using the soft rot assay on potato and orchid. Among thirty one bacteria isolates screened, four isolates (in succession namely B37, BT2, GG3, and GG6) were selected to control *D. dadantii*. All of these bacteria showed QQ ability to suppress the virulence of *D. dadantii* infection on orchids, significantly. Based on nucleotide sequences of 16S ribosomal RNA, those of bacteria isolates had the highest identity with *Brevibacillus brevis*, *Bacillus cereus* ATCC14579, *Bacillus cereus* ATCC14579 and *Bacillus thuringiensis* ATCC 10792. *Brevibacillus brevis* was reported for the first time as QQ bacteria in this study.

Keywords: AHL-lactonase; *aii*A gene; *Bacillus*; quorum sensing; soft rot disease

INTRODUCTION

Dickeya dadantii (synonim *Erwinia chrysanthemi*) is a member of the pectinolytic erwiniae; it is the causal of soft rot disease. It has a wide range of host plant species such as potato, orchid, carrot, onion, sweet potato, etc. (Perombelon & Kelman, 1980; Ma et al., 2007).

In Indonesia, the distribution area of *D. dadanti* is still limited, and considered as one A2-group of quarantine pest. The bacteria infects orchids in West Java with severe symptoms and disease incidence up to 100 % (Hanudin & Rahardjo, 2012). *D. dadantii* is known to express pectate lyase as virulence factor used in quorum sensing (QS) system with 3-oxo-C_6HSL or C_6HSL as the signal that interacts with ExpI/ExpR regulator protein (Nasser, Bouillant, Salmond, & Reverchon, 1998).

Quorum sensing (QS) is an intercellular communication mechanism in gram negative bacteria with *N*-acyl homoserine lactone (AHL) inducing molecule as a biosensor. AHL at relatively high concentration induce protein synthesis by activation of transcriptional activator or protein regulator which induces gene expression. The biosensor that is related to quorum sensing control promoter had been reported previously such as *lac*Z or *lux* operon (de Kievit & Iglewski, 2000). In other report previously, QS is involved in the regulation of important biological functions such as antibiotic production, biofilm formation, luminescence, motility, plasmid transfer, regulation of the expression of pathogen genes, and virulence. In relation to the virulence of phytopathogenic bacteria infecting their host, AHL plays a role in the activation of virulence factors i.e. enzymes and toxins. Some bacteria such as *Ralstonia solanacearum*, *Pseudomonas aeruginosa*, *Pectobacterium antrosepticum* and several *Erwinia* species activate their virulence factors expression by QS (Dong, Xu, Li, & Zhang, 2000; Conway & Greenberg, 2001; Burr et al., 2006).

Studies on the enzymes that degrade AHL was identified from several bacteria with potential as anti-QS (Yin et al., 2010). One of the enzymes that degrade AHL is acyl homoserine lactonase (AHL-lactonase). AHL-lactonase enzyme is produced by several bacterial species that have the target substrate and deactivates AHL (Dong, Xu, Li, & Zhang, 2000). AHL-lactonase is a metallo-beta-lactamase (metalloenzyme) that deactivates AHL through the hydrolysis of ester

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ring from homoserine lactone. The hydrolysis of lactone ring enables the lactonase to protect this signal molecule from being bound by the target transcriptional regulator. The process of AHL degradation as a QS signal molecule is known as quorum quenching (QQ) (Dong et al., 2001). This enzyme is encoded by certain genes such as *aii*A which is found in *Bacillus* spp. (Dong, Xu, Li, & Zhang, 2000). *B. weihenstephanensis* strain P65 produces lactonase that is encoded by *aii*A gene (Sakr, Aboushanab, Aboulwafa, & Hassouna, 2013).

Based on those reports, it is necessary to screen several AHL-lactonase producing bacteria and test their potential in suppressing virulence expression factors that are involved in QS system with AHL as the inducer. Therefore, the aim of the research was to select and identify of AHL-lactonase producing bacteria from several isolates collection of Laboratory of Plant Bacteriology, Bogor Agricultural University, and also to study the ability of selected bacteria to control the causal of orchid soft rot *D. dadantii*.

MATERIALS AND METHODS

The nucleotide sequences data reported are available in the DDJB/EMBL/GenBank databases under accession number(s): LC055677, LC055679, LC055680, LC055678, LC055758, LC055760, LC055761 and LC055759.

Bacteria Isolates

D. dadantii, Escherichia coli strain DH5α and 31 Gram positive bacteria strains used in this work were obtained from the collection of Plant Bacteriology Laboratory, Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University, Indonesia. The selection of bacteria that produce AHL-lactonase is conducted using *Chromobacterium violaceum* which utilizes C_6HSL to run the QS system and produces violacein which is violet in colour (McClean et al., 1997).

Screening Method of AHL-lactanose-Producing Bacteria

Anti-QS activity against *C. violaceum* test was conducted to examine the inhibition of AHL-induced violacein expression on *C. violaceum*. The method was carried out using disc diffusion assay with double layer culture plates with minor modification as described by Song, Ma, Zhao, Song, & Jia (2012). Double layer agar with different concentration was poured on petri dish sterile. First layer is LB medium (1.5 %) and the second layer is LB medium (0.5 %) containing *C. violaceum* 1 % (v/v). The 1.5 ml of supernatant of the tested bacteria was centrifuged at 12000 x g for 10 min and filtered using milipore 0.2 µm (Minisart, Sartorius Stediem Biotech, Germany). Filter paper with diameter 6 mm was soaked in the supernatant of tested bacteria for 20 seconds, then placed onto agar surface, then was incubated at room temperature (30 ºC) overnight. QS-inhibition was shown by the presence of clear zone around the filter paper. QS-inhibition category was differentiated by size of clear zone with illustration in Figure 1.

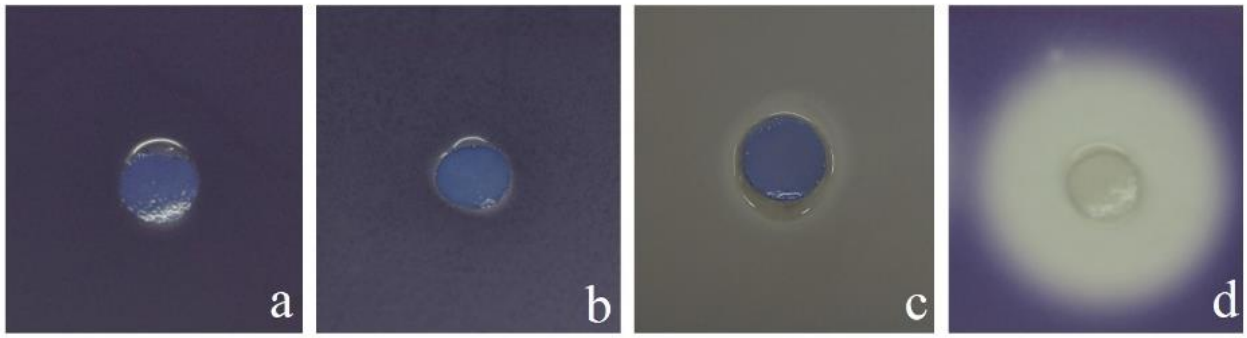

Figure 1. QS-inhibition category base on formation of *C. violaceum* clear zone a. not detected (clear zone 0 mm), b. low (clear zona < 2 mm), c. medium (2 mm ≤ clear zone <4 mm), d. high (clear zone ≥ 4 mm)

Anti-bacterial activity test to examine whether the clear zone formed in anti-QS activity test against *C. violaceum* due to QQ or not, anti-microbial activity test was conducted as described by Song, Ma, Zhao, Song, & Jia (2012). A total of 6 ml LB was inoculated with *D. dadantii* until OD_{600}=0.05 (approximately 10^3-10^4 cfu ml^{-1}) as starting culture and 50 µl supernatant was added into tube containing *D. dadantii*. Supernatant that used in this assay was to avoide bias data with growth activity of bacteria were assayed. LB containing antibiotic kanamycin of 25 µg ml^{-1} was used as positive control and sterile LB for negative control. The tube was incubated for 12 h at 30 ºC. The absorbance value of all tested bacteria was measured to determine the bacterial concentration.

Bio-assay of the Potential AHL-Lactonase-Producing Bacteria to Control *D. dadantii*

Isolates producing AHL-lactonase were tested for their ability to inhibit the virulence of *D. dadantii* using method as described by Dong, Zhang, Xu, & Zhang (2004) with minor modification. Potato was sterilised using 70 % ethanol for 1 min and rinsed using sterile water before slicing and wounded 6 mm in depth on the centre part. The supernatant of tested bacteria was added by 0.1X the volume of *D. dadantii* (OD_{600}=0.5, approximately 10^8 cfu ml^{-1}). 20 µl of a total mixture were dropped to the potato slices. *E. coli* DH5α supernatant was used as negative control. All the potato slices were incubated at 28 ºC for 24 h. The diameter of soft rot was measured for the examination.

The suppression of *D. dadantii* virulence was examined on orchid (*Phalaenopsis* sp. Hybrid MP-152) by using same inoculation as described above. After 24 h post inoculation, the diameter of soft rot on tested leaf plants were measured.

DNA Amplification and Sequencing

The total DNA was extracted by using DNA Extraction kit (Geneaid) according to protocol provided by manufacture. The amplification of lactonase gene was conducted using *aii*A1 (5'-ATG ACA GTA AAR AAR CTT TAT TTC-3') and *aii*A2 (5'-TCA CTA TAT ATA YTC MGG GAA CTC-3) with PCR product size approximately 753 bp (Pan et al., 2008). PCR reaction consisted of 12.5 µl PCR ready mix DreamTaq 2X (Thermo Scientific), 1 µl 20 pmol of each primer, 1 µl template DNA and water up to total volume 25 µl. The PCR was conducted as follows: pre-denaturation at 94 ºC for 5 min, followed by 30 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1.5 min, extension at 72 ºC for 2 min, and final extension at 72 ºC for 5 min.

The amplification of 16S rRNA was conducted using universal primer 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′) and 1492R (5'-GGT TAC CTT GTT ACG ACT T-3') with PCR product size approximately 1.5 Kb (Jiang *et al*., 2006). The PCR reaction consisted reagent as described previously. The PCR was conducted as follows: pre-denaturation at 94 ºC for 3 min, followed by 30 cycles of denaturation 94 ºC for 30 s, annealing at 57 ºC for 30 sec, extension at 72 ºC for 1.5 min and final extension at 72 ºC for 10 min. The PCR products were electrophorated on 1 % gel agarose at 75 V for 45 min. The DNA was then visualised using UV trans-illuminator and documented digitally.

The PCR products were directly subjected to nucleotide sequencing at 1st Base Asia, Malaysia. The nucleotide sequences of *aii*A genes and its deduced amino acid sequence were compared to corresponding genes in GenBank database by the BLAST search program. The 16S ribosomal RNA were aligned with those other corresponding sequences deposited in GenBank using the program ClustalW (Thompson, Higgins, & Gibson, 1994), while sequences identities were calculated using "sequence identity matrix" option in the program BioEdit version 7.05.

Experimental Design and Statistical Analysis

Experimental design used to examine the inhibition of soft rot symptom on potato and orchid was completely randomized design (CRD) under laboratory condition. Each treatment was repeated 6 times. The data obtained was analysed using variance (ANOVA) with Microsoft Office Excel 2010 and SPSS Statistics software version 17.0. Significantly different treatments were then further tested using Duncan's Multiple Range Test (DMRT) at P=0.05.

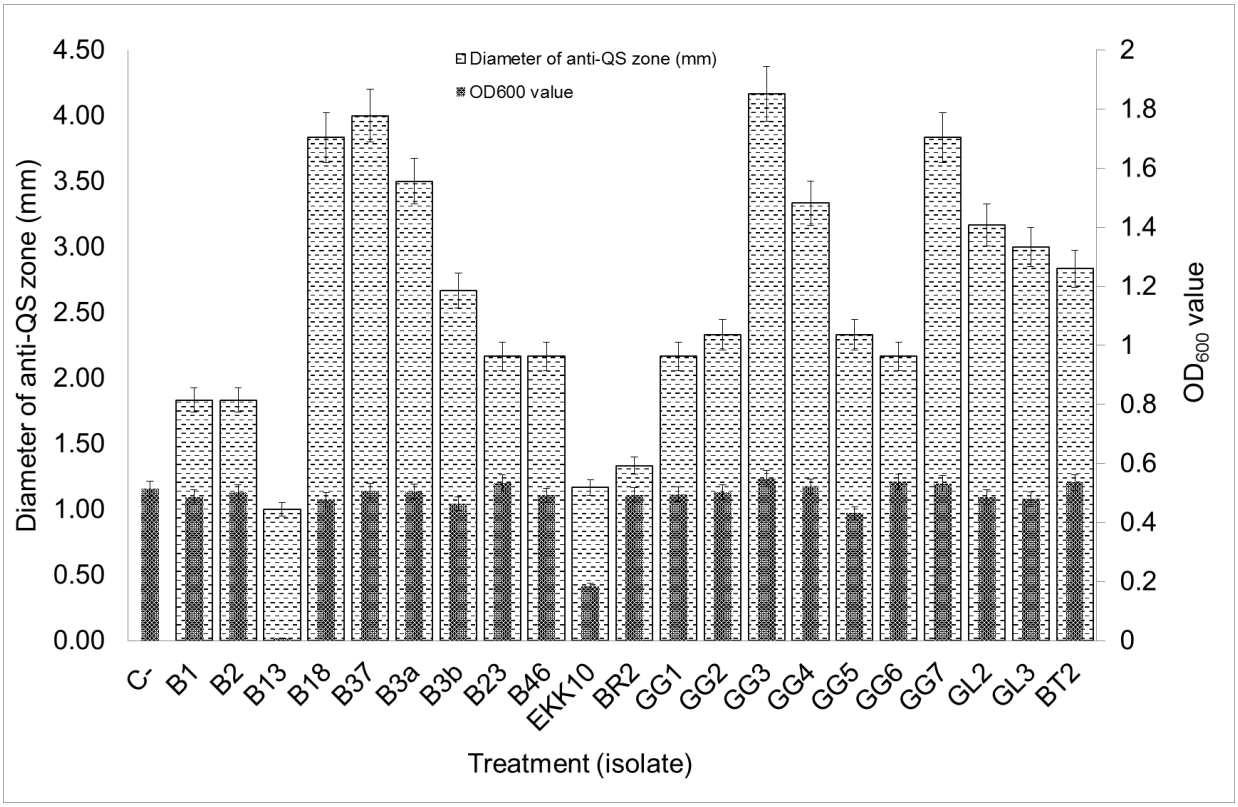

Figure 2. The screening result of anti-QS and antimicrobial activity of isolates on *C. violaceum.* C-=negative control, B1-BT2=isolates were assayed

RESULTS AND DISCUSSION

Screening of Quorum Quenching Bacteria

Screening of anti-QS activity against *C. violaceum* showed that 21 among 31 bacterial isolates namely B1, B2, B13, B18, B37, B3a, B3b, B23, B46, EKK10, BR2, GG1, GG2, GG3, GG4, GG5, GG6, GG7, GL2, GL3, BT2 exhibited anti-QS activity by forming clear zone around filter paper soaked in supernatant. The size of clear zone varied, indicating the difference ability of tested bacteria isolates to inhibit violacein production. Isolate GG3 and B37 produced total diameter clear zones 10.17 mm and 10 mm in diameter. This result showed the largest diameter of clear zones than other isolates, suggesting a high anti-QS activity (Figure 2; Table 1). *C. violaceum* is used as model for the screening of AHL-lactonase-producing bacteria was reported previously on the screening of AHL-lactonase-producing bacteria from ant lion (Myrmeleontidae) (Christianto & Yogiara, 2011) and bacteria from soil by Chong et al., (2012).

Anti-microbial activity of that of isolates showed that isolates B13 and EKK10 produced clear zones on anti-QS activity test against *C. violaceum*, however those isolates had anti-microbial activity based on OD_{600} measurement after 12 h incubation, while the remain isolates were not (Figure 2). It indicated that clear zone produced by both isolates might due to anti-microbial activity rather than anti-QS. Anti-QS only inhibited QS mechanism of *C. violaceum* for producing violacein, so *C. violaceum* still growth but not expression violet colour. The loss of violet pigment around *C. violaceum* cultured was indication of QS inhibition by supernatant bacteria were assayed (McClean *et al.,* 1997; Song, Ma, Zhao, Song, & Jia 2012).

Table 1. Bacteria isolates used in this study and screening result of isolates as quorum quenching bacteria

Isolate	Origin	anti-QS activity	anti-microbial activity	Detection of *aiiA* gene
B1	Tembilahan, Riau	Low	No	No
B2	Tembilahan, Riau	Low	-	-
B13	Tembilahan, Riau	Low	Yes	-
B18	Bogor, West Java	Medium	No	No
B37	Bogor, West Java	High	No	Yes
B47	Bogor, West Java	ND	-	-
B48	Bogor, West Java	ND	-	-
Bs168	Bogor, West Java	ND	-	-
B16	Tembilahan, Riau	ND	-	-
B21	Bogor, West Java	ND	-	-
B3a	Tembilahan, Riau	Medium	No	No
B3b	Tembilahan, Riau	Medium	No	No
B23	Bogor, West Java	Medium	No	No
B46	Bogor, West Java	Medium	No	No
B19	Bogor, West Java	ND	-	-
B17	Tembilahan, Riau	ND	-	-
EKK10	Bogor, West Java	Low	Yes	-
BR2	Bogor, West Java	Low	No	No
GG1	Bogor, West Java	Medium	No	No
GG2	Bogor, West Java	Medium	No	No
GG3	Bogor, West Java	High	No	Yes
GG4	Bogor, West Java	Medium	No	No
GG5	Bogor, West Java	Medium	No	No
GG6	Bogor, West Java	Medium	No	Yes
GG7	Bogor, West Java	Medium	No	No
GL1	Bogor, West Java	ND	-	-
GL2	Bogor, West Java	Medium	No	No
GL3	Bogor, West Java	Medium	No	No
GL4	Bogor, West Java	ND	-	-
GL5	Bogor, West Java	ND	-	-
BT2	Bogor, West Java	Medium	No	Yes

Remarks: (ND) not detected, (-): not assayed

Amplification of *aii*A gene of 19 isolates which were having anti-QS activity with no anti-microbial activity against *D. dadantii* showed that there were only four isolates (B37, GG3, GG6, and BT2) amplified *aii*A gene encoding AHL-lactonase, respectively (Figure 3). Meanwhile 15 other isolates were not detected *aii*A genes can be caused by other mechanisms, such as: other enzyme i.e. AHL-acylase (Lin et al., 2003).

The nucleotide sequences analysis of the isolates showed that all of isolates had similar *aii*A gene with corresponding species in GenBank (Table 2 and 3). BLAST-X analysis showed that all four sequences had lactamase_B specific hits and superfamily, and also showed GloB as multi-domain. Detection of *aii*A gene of 19 bacteria isolates showed only four isolates positively had the gene with size similar (753 bp) to *aii*A gene from *B. subtilis* BS-1 reported previously (Pan et al., 2008). All four isolates had 94-99% amino acid sequences homology to that of N-acyl homoserine lactonase from *B. thuringiensis.* In addition, *aii*A genes of those four isolates had three large conserved domain in AiiA proteins, such as: GloB, metallo-beta-lactamase super-family, and metal dependent hydrolases. It indicates that those four *aii*A gene had the character of AHL-lactonase that belongs to metallo-beta-lactamase group (Dong, Xu, Li, & Zhang, 2000).

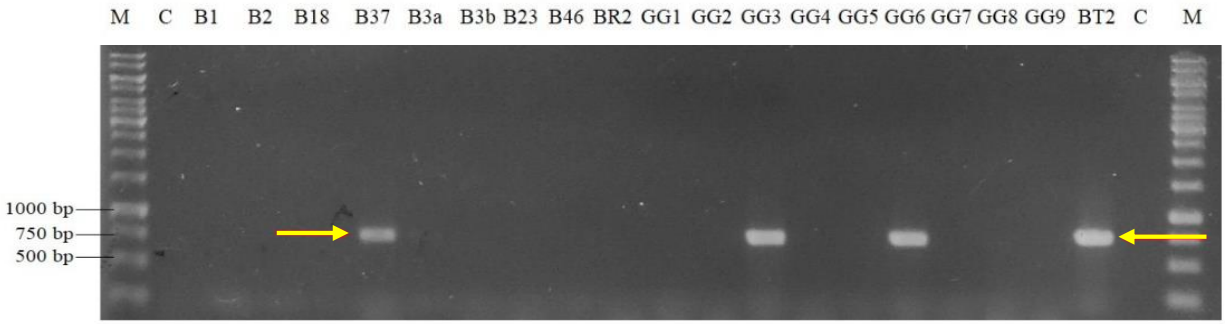

Figure 3. Amplification of DNA *aii*A gene from several isolates AHL lactonase producing bacteria. *Yellow Arrow* indicates size of amplified DNA. M=DNA ladder 1 Kb (Thermo Scientific), C=negative control

Table 2. Identity of *aiiA* gene of QQ bacteria in this study compared with other of *aiiA* gene deposited in GenBank

Isolates*)	Homology (%)	Species identity of *aiiA* gene	Accession no.
B37	97	*Bacillus thuringiensis*	AF350929.1
		Bacillus subtilis	DQ00640.1
GG3	99	*Bacillus cereus*	JF501512.1
		Bacillus thuringiensis	AY195570.1
GG6	99	*Bacillus cereus*	JF501512.1
		Bacillus thuringiensis	AY195570.1
BT2	99	*Bacillus thuringiensis*	AF350929.1
		Bacillus subtilis	DQ00640.1

Remarks: *) Databases under accession number(s) LC055758, LC055760, LC055761, and LC055759

Table 3. Identity of the deduced amino acid sequences of *aii*A in this study and that of the other AiiA amino acid sequences deposited in GenBank

Isolates	Homology (%)	Identity of amino acid sequences	Accession no.
B37	94	*Bacillus thuringiensis*	AAY51612.1
		Bacillus subtilis	AAY51610.1
GG3	99	*Bacillus cereus*	AEA48310.1
		Bacillus thuringiensis	WP000216593.1
GG6	99	*Bacillus cereus*	AEA48310.1
		Bacillus thuringiensis	WP000216593.1
BT2	99	*Bacillus thuringiensis*	AAY51612.1
		Bacillus cereus	WP016111450.1
		Bacillus subtilis	AAY51610.1

The Potential of AHL-Lactonase-Producing Bacteria as *D. dadantii* Biocontrol

Soft rot is caused by the activity of pectate lyase enzyme synthesized through QS system. Potato slices inoculated by *D. dadantii* showed severe softrot symptom. Whereas, potato slices treated with anti-QS bacteria inoculated by *D. dadantii* showed less rot. It indicates virulence inhibition of *D. dadantii* on treated potato slices (Figure 4). Based on the measurement of the diameter of the soft rot symptoms from each treatment result that the highest virulence inhibition of *D. dadantii* was indicated by BT2 treated potato slices and isolate GG6 on orchid, significantly. Escobar et al. (2014) reported that *B. thuringiensis* which has *aii*A (AHL-lactonase) was able to suppress virulence of *Pectobacterium carotovorum* cause softrot disease on potato.

This result also showed that AHL-lactonase release in LB medium. As a control, between *D. dadantii* alone treatment and *D. dadantii* with supernatant of *E. coli* DH5α showed no obvious difference (Table 4).

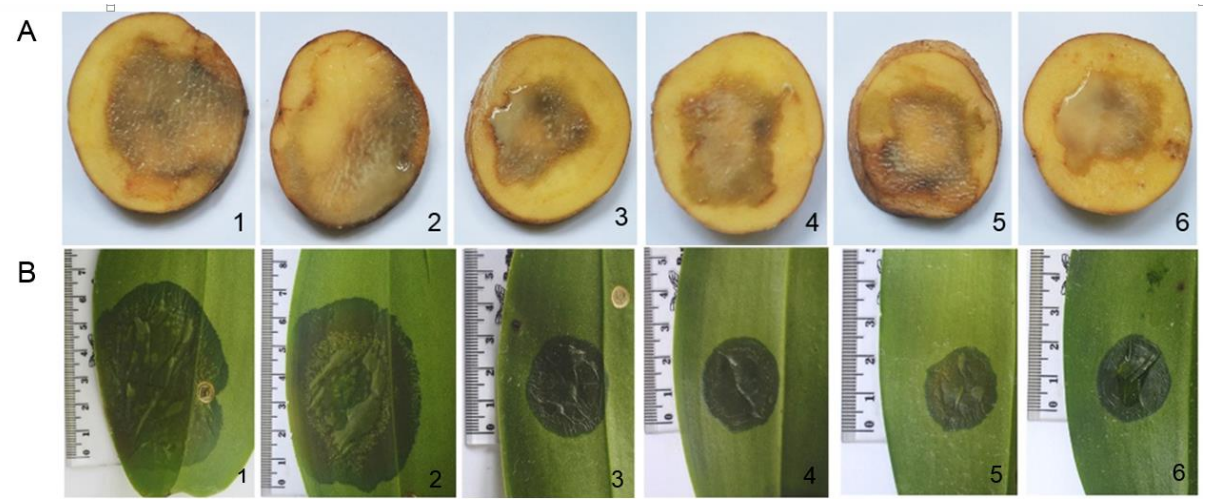

Figure 4. Inhibition activity of AHL-lactonase-producing bacteria against *D. dadantii* on potato (A1-6) and orchid (B1-6) at one day post-inoculation. **1** control *D. dadantii* only, **2** *D. dadantii* + DH5α, **3** *D. dadantii* + B37 , **4** *D. dadantii* + GG3, **5** *D. dadantii* + GG6, **6** *D. dadantii* + BT2

Table 4. Development of soft rot symptoms caused by *D. dadantii* in potato slices and orchid leaves[+]

Treatment	Diameter of soft rot (mm)		Relative inhibition of soft rot (%)[++]	
	Potato	Orchid	Potato	Orchid
D. dadantii (control)	55.00a	46.25a	00.00a	00.00a
D. dadantii +DH5α	48.83ab	42.50ab	11.04b	08.78a
D. dadantii +B37	42.00bc	34.00abc	23.01c	29.46b
D. dadantii +GG3	38.00bc	32.50abc	30.85cd	32.62b
D. dadantii +GG6	38.50bc	19.50c	30.99cd	55.22c
D. dadantii +BT2	34.50c	22.75bc	36.74d	49.03c

Remark: [+]Values within a column followed by the same letters are not significantly different at P=0.05, [++]Values obtained from the formula: PI=(D0-D1)/D0 x100% (PI=percentage inhibition, D0=diameter soft rot of control, D1=diameter soft rot of treatment)

Identification of AHL-Lactonase-Producing Bacteria

The four isolates are Gram positive bacteria. Based on the homology of 16S rRNA sequences, four isolates having *aii*A gene encoding AHL-lactonase of *Bacillus* group bacteria. The highest identity of those isolates with corresponding species deposited in GenBank were as follows: B37 was identified closely to as *Brevibacillus brevis*, GG3 and GG6 closely to *Bacillus cereus*, and BT2 closely to *Bacillus thuringiensis* (Table 5). In this study, QQ bacteria was identified as *Brevibacillus brevis*, *Bacillus cereus*, and *Bacillus thuringiensis. B. thuringiensis,* and *B. cereus* as lactonase producing bacteria have been reported (Thomas, Stone, Castello, Tierney, & Fast, 2005; Liu et al., 2008; Lu, Yuan, Xue, Zhang, & Zhou, 2006; Dong, Gusti, Zhang, Xu, & Zhang, 2002). However, *B. brevis* producing lactonase base on detection *aii*A gene had not reported yet previously. Thus, it is newly reported of *aii*A gene on *B. brevis* and their ability to inhibit virulence factor of *D. dadantii* and suppress violacein production on *C. violaceum*. The sequence of *aii*A gene and deduce amino acid from *B. brevis* had similarity with *B. thuringiensis* and *B. subtilis.*

The use of anti-QS compounds as quencher continued to be developed as inhibitors of bacterial virulence. In contrast, the anti-microbial compound that can cause resistance because of selection pressure. In the next generation of pathogens that are more resistant to anti-microbial compounds because of some adaptation mechanism (White & Finan, 2009). For example, *Erwinia amylovora* resists to streptomycin because its spontaneous mutation on *rps*L gene,. This mutation can prevent binding of streptomycine, and *E. amylovora* is resistant to this antibiotic (Jones & Schanabel, 2000). In other case, resistant bacteria to other antibiotics have been reported by Stockwell & Duffy (2012).

Table 5. Identities of QQ bacteria based on 16S ribosomal RNA sequences

Isolates*	Homology (%)	Species	Accession no.
B37	**99**	***Brevibacillus brevis* NBRC15304**	**NR_041524.1**
		Brevibacillus brevis DSM30	NR_112204.1
		Brevibacillus brevis GDXJ1	JN999872.1
		Brevibacillus brevis LAHP3-2	KT216600.1
GG3	**96**	***Bacillus cereus* ATCC14579**	**NR_074540.1**
		Bacillus cereus JCM2152	NR_113266.1
		Bacillus cereus CCM 2010	NR_115714.1
		Bacillus cereus NBRC15305	NR_112630.1
GG6	**97**	***Bacillus cereus* ATCC14579**	**NR_074540.1**
		Bacillus cereus JCM2152	NR_113266.1
		Bacillus cereus CCM 2010	NR_115714.1
		Bacillus cereus NBRC15305	NR_112630.1
BT2	**94**	***Bacillus thuringiensis* ATCC10792**	**NR_114581.1**
		Bacillus thuringiensis IAM12077	NR_043403.1
		Bacillus thuringiensis NBRC101235	NR_112780.1
		Bacillus thuringiensis BT407	NR_102506.1

Remark: *Databases under accession number(s) LC055677 for isolate B37, LC055678 for isolate BT2, LC055679 for isolate GG3, and LC055680 for isolate GG6

In controlling plant disease caused by bacteria, QQ mechanism is important to be studied in order to inhibit the regulation of plant pathogenic bacteria virulence factors involving QS system with AHL as inducer. The virulence factor of *D. dadantii*, *Erwinia carotovora* subsp. *carotovora, Erwinia stewartii,* and *Pectobac terium atrosepticum* is regulated through QS system with 3-oxo-C_6HSL as signal recognized by Expl/ExpR regulator protein (Nasser, Bouillant, Salmond, & Revechon, 1998; Bainton et al., 1992; Chhabra et al., 1993; von Bodman & Farrand, 1995; Burr et al., 2006). Whereas QS system of *Ralstonia solanacearum* is regulated by C_8-HSL and *Agrobacterium tumefaciens* 3-oxo-C_8-HSL (Flavier, Ganova-Raeva, Schell, & Denny, 1997; Piper, von Bodman, & Farrand, 1993).

AHL degradation using AHL-lactonase is potential as one of plant pathogenic bacteria controls. The present results also supported the previous reports that all four bacteria isolates produced lactonase which could inhibit the soft rot development caused by *D. dadantii* either on potato or orchids. Knowledge on AHL-producing bacteria and their potential to inhibit the virulence of *D. dadantii* can be used as alternative to control plant pathogenic bacteria that the virulence factor of which is expressed through QS system. In addition to biocontrol potential. Four isolates producing AHL-lactonase were reported previously, such us: *B. brevis* for controlling *Fusarium oxysporum* f. sp. *lycopersici* caused wilt in tomato (Chandel, Allan, & Woodward, 2010), *B. cereus* (strain UW85) for controlling *Phytophthora megasperma* f. sp. *medicaginis* causing damping off in alfalfa (Handelsman, Raffel, Mester, Wundurlich, & Grau, 1990), and *B. thuringiensis* for controlling some lepidopteran pest and fungi (MacIntosh et al., 1990; Reyes-Ramirez, Escudero-Abarea, Aguilar-Uscanga, Hayward-Jones, & Barboza-Corona, 2004).

These results extended the information on AHL-lactonase-producing bacteria as the source of AHL-lactonase (*aii*A) genes in Indonesia. In the future, it is necessary to test the effectiveness of these AHL-lactonase producing bacteria against other plant pathogenic bacteria. Alternatively, the lactonase gene might be utilized to enginered into plant to create resistant orchids.

CONCLUSION AND SUGGESTION

The four isolates namely B37, GG3, GG6, and BT2 were able to reduce the virulence of *D. dadantii* since they are having *aii*A gene encode AHL-lactonase. Among that of isolates, BT2 showed highest inhibition of *D. dadantii* virulence either growth or disease development. Based on pair wise comparison of 16S rRNA with corresponding nucleotide sequences in the GenBank showed that B37 had 99 % identity with *Brevibacillus brevis*, while GG3 and GG6 had 96 % and 97 % identity with *Bacillus cereus*, and BT2 had 94 % identity with *Bacillus thuringiensis*.

Brevibacillus brevis was reported for the first time as QQ bacteria in this study. Further research activities need to be done to characterize of AHL-lactonase and clone *aii*A gene on orchids or several plant.

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