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

Syaiful Khoiri, Tri Asmira Damayanti and Giyanto*  
Department of Plant Protection, Faculty of Agriculture, Bogor Agricultural University  
Jl. Kamper Kampus IPB Dramaga, Bogor, Indonesia, 16680  
*Corresponding author E-mail: giyanto2@yahoo.com

Received: August 12, 2015 / Accepted: March 24, 2016

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 *aiiA* 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 *aiiA* 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; *aiiA* gene; *Bacillus*; quorum sensing; soft rot disease

INTRODUCTION

*Dickeya dadantii* (synonym *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. dadantii* 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-C6-HSL or C6-HSL 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 *lacZ* 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 atrosepticum* 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

Cite this as: Khoi, S., Damayanti, T. A., & 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. http://doi.org/10.17503/agrivita.v39i1.633

Accredited: SK No. 81/DIKTI/Kep/2011

Permalink/DOI: http://dx.doi.org/10.17503/agrivita.v39i1.633
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 aiiA which is found in *Bacillus* spp. (Dong, Xu, Li, & Zhang, 2000). *B. weihenstephanensis* strain P65 produces lactonase that is encoded by aiiA 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 C₆HSL to run the QS system and produces violacein which is violet in colour (McCLean et al., 1997).

**Screening Method of AHL-lactonase-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 °C) 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)](image-url)
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<sub>600</sub>=0.05 (approximately 10<sup>3</sup>-10<sup>4</sup> cfu ml<sup>-1</sup>) as starting culture and 50 µl supernatant was added into tube containing *D. dadantii*. Supernatant that used in this assay was to avoid bias data with growth activity of bacteria were assayed. LB containing antibiotic kanamycin of 25 µg ml<sup>-1</sup> was used as positive control and sterile LB for negative control. The tube was incubated for 12 h at 30 °C. 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<sub>600</sub>=0.5, approximately 10<sup>8</sup> cfu ml<sup>-1</sup>). 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 °C 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 manufacturer. The amplification of lactonase gene was conducted using *aiiA* (5′-ATG ACA GTA AAR AAR CTT TAT TTC-3′) and *aiiA* (5′-TCA CTA TAT AYT CCG 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 °C for 5 min, followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1.5 min, extension at 72 °C for 2 min, and final extension at 72 °C 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 GGT AGC 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 °C for 3 min, followed by 30 cycles of denaturation 94 °C for 30 s, annealing at 57 °C for 30 sec, extension at 72 °C for 1.5 min and final extension at 72 °C 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 1<sup>st</sup> Base Asia, Malaysia. The nucleotide sequences of *aiiA* 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.
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\textsubscript{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 \(a\)ii\(A\) 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 \(a\)ii\(A\) gene of 19 isolates which were having anti-QS activity with no anti-microbial activity against \(D.\) \(d\)adantii showed that there were only four isolates (B37, GG3, GG6, and BT2) amplified \(a\)ii\(A\) gene encoding AHL-lactonase, respectively (Figure 3). Meanwhile 15 other isolates were not detected \(a\)ii\(A\) 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 \(a\)ii\(A\) 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 \(a\)ii\(A\) gene of 19 bacteria isolates showed only four isolates positively had the gene with size similar (753 bp) to \(a\)ii\(A\) gene from \(B.\) \(s\)ubtilis 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 \(B.\) \(t\)huringiensis. In addition, \(a\)ii\(A\) genes of those four isolates had three large conserved domain in AiiA proteins, such as: GloB, metallo-beta-lactamase superfamily, and metal dependent hydrolases. It indicates that those four \(a\)ii\(A\) gene had the character of AHL-lactonase that belongs to metallo-beta-lactamase group (Dong, Xu, Li, & Zhang, 2000).
Figure 3. Amplification of DNA aiIA 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 Bacillus subtilis | AF350929.1    |
| GG3      | 99           | Bacillus cereus Bacillus thuringiensis | JF501512.1    |
| GG6      | 99           | Bacillus cereus Bacillus thuringiensis | JF501512.1    |
| BT2      | 99           | Bacillus thuringiensis Bacillus subtilis | AF350929.1    |

Remarks: 1) Databases under accession number(s) LC055758, LC055760, LC055761, and LC055759

Table 3. Identity of the deduced amino acid sequences of aiIA 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 Bacillus subtilis | AAY51612.1     |
| GG3      | 99           | Bacillus cereus Bacillus thuringiensis | AEA48310.1     |
| GG6      | 99           | Bacillus cereus Bacillus thuringiensis | AEA48310.1     |
| BT2      | 99           | Bacillus thuringiensis Bacillus cereus Bacillus subtilis | AAY51612.1     |

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 aiIA (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).
Identification of AHL-Lactonase-Producing Bacteria

The four isolates are Gram positive bacteria. Based on the homology of 16S rRNA sequences, four isolates having aiiA 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 aiiA gene had not reported yet previously. Thus, it is newly reported of aiiA gene on *B. brevis* and their ability to inhibit virulence factor of *D. dadantii* and suppress violacein production on *C. violaceum*. The sequence of aiiA 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 *rpsL* 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 LAPH3-2 | KT216600.1 |
| GG3      | 95           | 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 *Pectobacterium atrosepticum* is regulated through QS system with 3-oxo-C6-HSL as signal recognized by ExpI/ExpR regulator protein (Nasser, Bouillant, Salmon, & 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 C6-HSL and *Agrobacterium tumefaciens* 3-oxo-C6-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-Uzcanga, Hayward-Jones, & Barboza-Corona, 2004).

These results extended the information on AHL-lactonase-producing bacteria as the source of AHL-lactonase (aiA) 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 engineered 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 aiA 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 aiiA gene on orchids or several plant.

**ACKNOWLEDGEMENTS**

This work partially supported by Superior Postgraduate Degree Scholarships for S.K. with contract no. 1094/E4.4/2013 granted by Ministry of Education and Cultural, Directorate General of Higher Education, Indonesia.

**REFERENCES**

Bainton, N. J., Stead, P., Chhabra, S. R., Bycroft, B. W., Salmond, G. P., Stewart, G. S., & Williams, P. (1992). N-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochemical Journal*, 288, 997–1004. http://doi.org/10.1042/bj2880997

Burr, T., Barnard, A. M. L., Corbett, M. J., Pemberton, C. L., Simpson, N. J. L., & Salmond, G. P. C. (2006). Identification of the central quorum sensing regulator of virulence in the enteric phytopathogen, *Erwinia carotovora*: The VirR repressor. *Molecular Microbiology*, 59(1), 113–125. http://doi.org/10.1111/j.1365-2958.2005.04939.x

Chandel, S., Allan, E. J., & Woodward, S. (2010). Biological control of *Fusarium oxysporum* f.sp. *lycopersici* on tomato by *Brevibacillus brevis*. *Journal of Phytopathology*, 158(7–8), 470–478. http://doi.org/10.1111/j.1439-0434.2009.01635.x

Chhabra, S. R., Stead, P., Bainton, N. J., Salmond, G. P., Stewart, G. S., Williams, P., & Bycroft, B. W. (1993). Autoregulation of carbapenem biosynthesis in *Erwinia carotovora* by analogues of N-(3-oxohexanoyl)-L-homoserine lactone. *The Journal of Antibiotics*, 46(3), 441–454. Retrieved from https://www.jstage.jst.go.jp/article/antibiotics1968/46/3/46_3_441/pdf

Chong, T. M., Koh, C. L., Sam, C. K., Choo, Y. M., Yin, W. F., & Chan, K. G. (2012). Characterization of quorum sensing and quorum quenching soil bacteria isolated from Malaysian tropical montane forest. *Sensors*, 12(4), 4846–4859. http://doi.org/10.3390/s120404846

Christiano, B., & Yogiara, Y. (2011). Screening of quorum quenching activity of bacteria isolated from ant lion. *Microbiology Indonesia*, 5(1), 46–49. http://doi.org/10.5454/mi.5.1.8

Conway, B. A., & Greenberg, E. P. (2002). Quorum-sensing signals and quorum-sensing genes in *Burkholderia vietnamiensis*. *Journal of Bacteriology*, 184(4), 1187–1191. http://doi.org/10.1128/jb.184.4.1187-1191.2002

de Kievit, T. R., & Iglewski, B. H. (2000). Bacterial quorum sensing in pathogenic relationships. *Infection and Immunity*, 68(9), 4839–4849. http://doi.org/10.1128/IAI.68.9.4839-4849.2000

Dong, Y. H., Gusti, A. R., Zhang, Q., Xu, J. L., & Zhang, L. H. (2002). Identification of quorum-quenching N-acyl homoserine lactonases from *Bacillus species*. *Applied and Environmental Microbiology*, 68(4), 1754–1759. http://doi.org/10.1128/AEM.68.4.1754-1759.2002

Dong, Y. H., Wang, L. H., Xu, J. L., Zhang, H. B., Zhang, X. F., & Zhang, L. H. (2001). Quenching quorum-sensing-dependent bacterial infection by an N-acyl homoserine lactonase. *Nature*, 411, 813–817. http://doi.org/10.1038/35081101

Dong, Y. H., Xu, J. L., Li, X. Z., & Zhang, L. H. (2000). AiiA, an enzyme that inactivates the acylhomoserine lactone quorum-sensing signal and attenuates the virulence of *Erwinia carotovora*. *Proceedings of the National Academy of Sciences of the United States of America*, 97(7), 3526–3531. http://doi.org/10.1073/pnas.97.7.3526

Dong, Y. H., Zhang, X. F., Xu, J. L., & Zhang, L. H. (2004). *Insecticidal Bacillus thuringiensis* silences *Enwinia carotovora* virulence by a new form of microbial antagonism, signal interference. *Applied and Environmental Microbiology*, 70(2), 954–960. http://doi.org/10.1128/AEM.70.2.954-960.2004

Escobar, A. M. F., Gonzalez, A., Pedroza, C. J., Correa, E., Rueda, N. J., & Orduz, S. (2014). Identification, cloning and lactonase activity of recombinant protein of N-acyl homoserine lactonase (AiiA) from *Bacillus thuringiensis* 147-115-16
strain. *Revista Colombiana de Biotecnología*, 16(1), 153–162. http://doi.org/10.15446/rev.colomb.biotecnologia.v16n1.40495

Flavier, A. B., Ganova-Raeva, L. M., Schell, M. A., & Denny, T. P. (1997). Hierarchical autoinduction in *Ralstonia solanacearum*: Control of acyl-homoserine lactone production by a novel autoregulatory system responsive to 3-hydroxypalmitic acid methyl ester. *Journal of Bacteriology*, 179(22), 7089–7097. Retrieved from http://jb.asm.org/content/179/22/7089.long

Handelsman, J., Raffel, S., Mester, E. H., Wunderlich, L., & Grau, C. R. (1990). Biological control of damping-off of alfalfa seedlings with *Bacillus cereus UW85*. *Applied and Environmental Microbiology*, 56(3), 713–718. Retrieved from http://www.ncbi.nlm.nih.gov/pmc/articles/PMC183411/pdf/aem00068-0139.pdf

Hanudin, & Rahardjo, I. P. (2012). Penyakit Busuk Lunak (pbl) pada anggrek: penyebab dan upaya pengendaliannya [Soft rot bacterial disease on orchids: Causal agent and its control]. *Prosiding Seminar Nasional Anggrek* 2012, 275-281. Retrieve from http://balithi.litbang.pertanian.go.id/jurnal-pf2012-275-281.pdf

Jiang, H., Dong, H., Zhang, G., Yu, B., Chapman, L. R., & Fields, M. W. (2006). Microbial diversity in water and sediment of Lake Chaka, an Athalassohaline Lake in Northwestern China. *Applied and Environmental Microbiology*, 72(6), 3832–3845. http://doi.org/10.1128/AEM.02869-05

Jones, A. L., & Schnabel, E. L. (2000). The development of streptomycin-resistant strains of *Erwinia amylovora*. In J. L. Vanneste (Ed.), *Fire blight: the disease and its causative agent, Erwinia amylovora* (pp. 235-251). Wallingford, UK: CAB International. http://doi.org/10.1079/9780851992945.0235

Lin, Y. H., Xu, J. L., Hu, J., Wang, L. H., Leong, O. S., Renton, L. J., & Zhang, L. H. (2003). Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Molecular Microbiology*, 47(3), 849–860. http://doi.org/10.1046/j.1365-2958.2003.03351.x

Liu, D., Momb, J., Thomas, P. W., Moulin, A., Petsko, G. A., Fast, W., & Ringe, D. (2008). Mechanism of the quorum-quenching lactonase (AiiA) from *Bacillus thuringiensis*. 1. Product-bound structures. *Biochemistry*, 47(29), 7706–7714. http://doi.org/10.1021/bi800368y

Lu, X., Yuan, X., Xue, X. L., Zhang, G. P., & Zhou, S. N. (2006). Identification of the critical role of Tyr-194 in the catalytic activity of a novel N-Acyl-Homoserine lactonase from marine *Bacillus cereus* strain Y2. *Current Microbiology*, 53(4), 346-350. http://doi.org/10.1007/s00284-006-0224-1

Ma, B., Hibbing, M. E., Kim, H., Reedy, R. M., Yedidia, I., Breuer, J., … Charkowski, A. O. (2007). Host range and molecular phylogenies of the soft rot enterobacterial genera *Pectobacterium* and *Dickeya*. *Phytopathology*, 97(9), 1150–1163. http://doi.org/10.1094/PHYTO-97-9-1150

MacIntosh, S. C., Stone, T. B., Sims, S. R., Hunst, P. L., Greenplate, J. T., Marrone, P. G., … Fuchs, R. L. (1990). Specificity and efficacy of purified *Bacillus thuringiensis* proteins against agronomically important insects. *Journal of Invertebrate Pathology*, 56(2), 258–266. http://doi.org/10.1016/0022-1201(90)90109-J

McCLean, K. H., Winson, M. K., Fish, L., Taylor, A., Chhabra, S. R., Camara, M., … Williams, P. (1997). Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acylhomoserine lactones. *Microbiology*, 143, 3703–3711. http://doi.org/10.1099/00221287-143-12-3703

Nasser, W., Bouillant, M. L., Salmon, G., & Reverchon, S. (1998). Characterization of the *Erwinia chrysanthemi* expl-expR locus directing the synthesis of two N-acyl-homoserine lactone signal molecules. *Molecular Microbiology*, 29(6), 1391–1405. http://doi.org/10.1046/j.1365-2958.1998.01022.x

Pan, J., Huang, T., Yao, F., Huang, Z., Powell, C. A., Qiu, S., & Guan, X. (2008). Expression
and characterization of aiiA gene from *Bacillus subtilis* BS-1. *Microbiological Research, 163*(6), 711–716. http://doi.org/10.1016/j.micres.2007.12.002

Perombelon, M. C. M., & Kelman, A. (1980). Ecology of the soft rot Erwinias. *Annual Review of Phytopathology, 18*, 361-387. http://doi.org/10.1146/annurev.py.18.090180.002045

Piper, K. R., von Bodman, S. B., & Farrand, S. K. (1993). Conjugation factor of *Agrobacterium tumefaciens* regulates Ti plasmid transfer by autoinduction. *Nature, 362*, 448–450. http://doi.org/10.1038/362448a0

Reyes-Ramírez, A., Escudero-Abarca, B. I., Aguilar-Uscanga, G., Hayward-Jones, P. M., & Barboza-Corona, J. E. (2004). Antifungal activity of *Bacillus thuringiensis* Chitinase and its potential for the biocontrol of phytopathogenic fungi in soybean seeds. *Journal of Food Science, 69*(5), M131–M134. http://doi.org/10.1111/j.1365-2621.2004.tb10721.x

Sakr, M. M., Aboshanab, K. M. A., Aboulwafa, M. M., & Hassouna, N. A. H. (2013). Characterization and complete sequence of lactonase enzyme from *Bacillus weihenstephanensis* isolate P65 with potential activity against acyl homoserine lactone signal molecules. *BioMed Research International, 2013*, 1–10. http://doi.org/10.1155/2013/192589

Song, C., Ma, H., Zhao, Q., Song, S., & Jia, Z. (2012). Inhibition of quorum sensing activity by ethanol extract of *Scutellaria baicalensis* Georgi. *Journal of Plant Pathology & Microbiology, S7*, 001-004. http://doi.org/10.4172/2157-7471.S7-001

Stockwell, V. O., & Duffy, B. (2012). Use of antibiotics in plant agriculture. *Revue Scientifique et Technique (International Office of Epizootics), 31*(1), 199–210. Retrieved from http://ir.library.oregon state.edu/xmlui/bitstream/handle/1957/39357/StockwellVirginiaBotanyPlantPathologyUseAntibioticsPlant.pdf?sequence=1

Thomas, P. W., Stone, E. M., Costello, A. L., Tierney, D. L., & Fast, W. (2005). The quorum-quenching lactonase from *Bacillus thuringiensis* is a metalloprotein. *Biochemistry, 44*(20), 7559–7569. http://doi.org/10.1021/bi050050m

Thompson, J. D., Higgins, D. G., & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research, 22*(22), 4673–4680. http://doi.org/10.1093/nar/22.22.4673

von Bodman, S. B., & Farrand, S. K. (1995). Capsular polysaccharide biosynthesis and pathogenicity in *Erwinia stewartii* require induction by an N-acylhomoserine lactone autoinducer. *Journal of Bacteriology, 177*(17), 5000–5008. Retrieved from http://jb.asm.org/content/177/17/5000.long

White, C. E., & Finan, T. M. (2009). Quorum quenching in *Agrobacterium tumefaciens*: Chance or necessity? *Journal of Bacteriology, 191*(4), 1123–1125. http://doi.org/10.1128/JB.01681-08

Yin, X. T., Xu, L., Fan, S. S., Xu, L. N., Li, D. C., & Liu, Z. Y. (2010). Isolation and characterization of an AHL lactonase gene from *Bacillus amyloliquefaciens*. *World Journal of Microbiology and Biotechnology, 26*(8), 1361–1367. http://doi.org/10.1007/s11274-010-0308-8