Scientific Research Publishing

Advances in Microbiology, 2023, 13, 526-538
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Degradation of *N*-Acyl Homoserine Lactone Quorum Sensing Signals by *Bacillus thuringiensis* AHL Lactonase

Waeel H. Alramadhan, Anthony Ejiofor, Terrance Johnson

Department of Biological Sciences, Tennessee State University, Nashville, Tennessee, USA
Email: waeel550@gmail.com

How to cite this paper: Alramadhan, W.H., Ejiofor, A. and Johnson, T. (2023) Degradation of *N*-Acyl Homoserine Lactone Quorum Sensing Signals by *Bacillus thuringiensis* AHL Lactonase. *Advances in Microbiology*, **13**, 526-538.
https://doi.org/10.4236/aim.2023.1311034

Received: October 12, 2023
Accepted: November 26, 2023
Published: November 29, 2023

Abstract

Bacterial cells rely on signaling molecules to communicate with others from the same species and induce certain genes in a process known as quorum sensing (QS). A common molecule is *N*-acyl homoserine lactone (AHL) which is responsible for the expression of virulence and other factors that allow the organisms to compete in a given environment. On the other hand, other bacteria produce certain enzymes such as AHL-lactonase that break down AHL molecules and prevent gene expression of these factors. The aim of this work was to examine the level of degradation of AHL molecules by AHL-lactonase in 62 *Bacillus thuringiensis* (Bt) strains isolated from Middle Tennessee, Mississippi, and Alabama. *N*-hexanoyl-homoserine lactone (C_6-HSL) and *N*-3-oxo-hexanoyl homoserine lactone (3-oxo-C_6-HSL), which cause *Chromobacterium violaceum* (CV026) to produce a purple pigment were tested at different concentrations to view the Bt lactonase activity. In addition, PCR was used to test for the presence of the lactonase gene. The results showed that among the 62 Bt strains, there were 58 that possessed the AHL-lactonase (*aiiA*) gene and 48 strains were able to degrade C_6-HSL. At high concentrations of AHL, only 13 strains were able to completely degrade C_6-HSL. In addition, degradation of 3-oxo-C_6-HSL was weak compared to C_6-HSL. The results also revealed that AHL lactonase was thermostable, and it was concluded that the level of degradation varies in Bt strains. Only 13 of the strains studied have potent inhibitory activity against C_6-HSL, which may be good to be used in field applications to control agricultural pest.

Keywords

Quorum Sensing, Quorum Sensing Inhibitor, *N*-Acyl Homoserine Lactone, AHL Lactonase, *Bacillus thuringiensis*, CV026

1. Introduction

Quorum sensing (QS) is a signaling mechanism that bacterial cells within a community use to interact [1] [2]. Gram-negative bacteria within the same species produce and secret special molecules such as *N*-acyl homoserine lactone (AHL) to activate certain genes including virulence factors sporulation, biofilm formation, antimicrobial peptide synthesis, and others [3]. *Bacillus thuringiensis* (Bt) strains isolated from soil possess AHL lactonase which breaks down AHL molecules and consequently can be used as QS inhibitors. The degree of degradation varies from one strain to another in the same species. In addition, working with many strains of bacteria makes it hard to isolate the enzymes of each strain and examine the level of activity. A combination of several tests can ensure the presence of the AHL lactonase and ensure the level of enzymatic activity.

There is a wide distribution of quorum sensing inhibitory (QSI) enzymes, but the most common enzymes are AHL lactonase and AHL acylase. AHL lactonase hydrolyzes the ester bond of the homoserine lactone ring (HSL) to produce the corresponding acyl-homoserines [4] [5] while AHL acylase cleaves the AHL amide bond generating the corresponding free fatty acid and homoserine lactone ring [6] [7]. AHL lactonase is a common enzyme discovered in various Bacillus species and the gene responsible for the activity was named *aiiA* (autoinduder inactivator) which belongs to the metallo β-lactamase superfamily [8].

Since the discovery of AHL lactonase enzymes in Bacillus species and other enzymes from other bacteria, scientists have devoted a lot of time and effort to explore the benefits of quorum sensing inhibitors. In the agricultural field, for example, a study showed how AHL lactonase helps in reducing the impact *of Erwinia carotovoa*, a pathogenic bacterium that causes a major problem in agriculture by invading crops of potatoes and other vegetables [4]. Another study, conducted by Wang *et al.* (2010), demonstrated that *Microbacterium testaceum*, which harbors the surface of potato leaves can protect the plant from soft rot disease by degrading AHL produced by the plant pathogen *Pectobacterium carotovorum* [9]. The application of the benefits of quorum sensing inhibitors has extended to the aquaculture field as well. It showed that when the purified AHL lactonase attenuated the virulence of *Aeromonas hydrophila* and decreased the mortality rate of fish by co-injection with AHL lactonase [10]. As a result, it is anticipated that application of quorum sensing inhibitors will minimize the use of antibiotics and reduce the emergence of antibiotic resistant bacteria.

This study examines the activities of AHL lactonase from 62 Bt strains isolated in middle Tennessee, Mississippi, and Alabama, and it shows how the level of degradation of the AHL molecules varies in Bt strains. In addition, the results demonstrate that although the majority of Bt strains possess the *aiiA* gene and degrade AHL molecules, only a few strains have a potent activity degrading AHL molecules at high AHL concentrations. The results also showed that the AHL lactonase viewed in this study was a thermostable enzyme and can tolerate a

temperature up to 65˚C without losing activity.

2. Material and Methods

2.1. Bacterial Isolates and Growth Conditions

There were 62 strains of Bt used in this study which were isolated from Middle Tennessee. The Bt strains were grown in Luria Bertani (LB) broth and incubated overnight at 30˚C in a shaking incubator set at a speed of 200 rpm. In addition, LB agar was also used in the AHL-lactonase assay.

CV026, a mutant form of *Chromobacterium violaceum*, which produces *N*-acyl homoserine lactone to activate the gene responsible for a purple pigment, was used as biosensor. In contrast *E. coli*, was used in the study as a negative control. CV026 and *E. coli* were both grown in LB broth and incubated overnight at 30˚C in a shaking incubator set at a speed of 180 rpm.

2.2. DNA Purification, PCR and Gel Electrophoresis

Genetic features of AHL-lactonase of the Bt strains were examined. The DNA was extracted using a DNA purification kit from Sigma and the provided protocol was followed. Due to the cell wall thickness in Bt, proteinase-k and lysozyme were used in the first steps of DNA extraction. A Nanodrop spectrophotometer was used to measure, quantify, and assess the purity of the DNA nucleic acids in each Bt strain.

PCR was performed to determine the presence of AHL lactonase gene *aiiA* using the forward primer 5'-ATGGGATCCATGACAGTAAAGAAGCTTTAT-3' and reverse primer 5'-GTCGAATTCCTCAACAAGATACTCCTAATG-3' obtained from Thermo Fisher Scientific, USA. The PCR reaction was set up at a volume of 50 µl each reaction containing 45 µl supermix solution (Thermo Fisher Scientific, USA), 1 µl forward primer, 1 µl reverse primer, 1 µl of DNA, and 2 µl of ddH_2O. The PCR reaction was set to run 30 cycles as follows: (1) Denaturation at 94˚C for 30 s; (2) Annealing at 55˚C for 30 s; and (3) Extension at 72˚C for 60 s per cycle with the final extension set at 72˚C for 5 min.

After the PCR reaction was completed, gel electrophoresis of the products was performed. Prior to loading the reaction into the gel, the PCR reaction mixture was mixed with loading dye. Then agarose gel (1%) was prepared in 100 mL of TAE buffer. Ethidium bromide was added to the agarose and mixed well before it was poured into the tray. Once gel was solidified, PCR products were loaded into the wells where electrophoresis was set at 90 V for 40 min. Bands were expected to be seen around 800 bp under UV light and the ladder used in this experiment was 100 bp.

2.3. AHL Lactonase Assay

N-acyl homoserine lactone (AHL) was used a source of the quorum sensing molecule. Two types of AHL were tested in this study: *N*-hexanoyl homoserine lactone (C_6-HSL) and *N*-3-oxo-hexanoyl homoserine lactone (3-oxo-C_6-HSL)

(Sigma-Aldrich, USA).

To determine the enzymatic activity of the AHL lactonase, CV026 was used to detect the presence of C_6-HSL which would produce the purple pigment in CV026. This was done using the LB agar plates containing wells in which 100 µL of a mixture of the AHL with or without Bt was placed.

To microcentrifuge tubes, overnight Bt culture, and a solution of C_6-HSL were added in a 1:1 volume to volume ratio and incubated at room temperature. The optical density of the Bt samples was measured at a wavelength of 600 nm (OD_{600}) and the absorbance was set at 1.0 for standardization and mixed with AHL as follows: 1) Bt culture (OD_{600} of 1.0) with 10 µM C_6-HSL and 2) Bt culture (OD_{600} of 1.0) with 5 µM C_6-HSL.

After a one-hour-incubation at room temperature, the mixture of Bt culture/AHL was centrifuged, and the supernatant was loaded in the LB agar wells after streaking with CV026. The plates were incubated at 30˚C overnight. *E. coli* was used instead of Bt as a negative control. AHL-lactonase activity was accessed by the lack of a purple pigment around the wells of the plates.

In this experiment the C_6-HSL was not directly incubated with the Bt culture. After overnight growth, the bacterial cells were centrifuged first, and then the supernatant which would contain AHL-lactonase was taken incubated with C_6-HSL at different temperatures; room temperature, 30˚C, 40˚C, 50˚C, and 60˚C. After an hour incubation of the supernatant and C_6-HSL, the mixture was loaded into the wells of plates streaked with CV026, the plates incubated at different temperature and the presence or absence of the purple pigment was observed.

2.4. AHL Reformation Assay

Degraded AHL molecules by AHL-lactonase can be reformed back to the active molecule in an acidic environment. After Bt cultures were incubated with C_6-HSL and then centrifuged, HCL was added to the supernatant and incubated for 1 h at room temperature. The mixture was loaded into the LB agar wells of plates that were streaked with CV026. The plates were incubated at 30˚C for 24 h, and the presence or absence of the purple pigment was observed.

2.5. *N*-3-Oxo-Hexanoyl Homoserine Lactone (3-Oxo-C_6-HSL)

In addition to the activity of AHL lactonase on C_6-HSL in Bt, another test was performed on modified C_6-HSL which was 3-oxo-C_6-HSL. This test was run to assess whether AHL-lactonase in Bt strains had the ability to inactivate different AHLs. C_6-HSL was replaced by 3-oxo-C_6-HSL and the protocol was performed as described above.

2.6. Clavulanate Potassium Assay

Although clavulanate potassium has been used to inhibit β-lactamase from degrading beta lactam antibiotics, this study tested its action on AHL lactonase

since both β-lactamase and AHL lactonase belong to the same superfamily. In addition to the mixture of Bt culture and AHL, clavulanate potassium was added to the mixture. This mixture was incubated for different periods of time at room temperature and then centrifuged. The supernatant was loaded into the agar wells streaked with CV026 and incubated at 30˚C for 24 h. The plates were then observed for the presence or absence of the purple pigment.

3. Results

3.1. AHL-Lactonase (*aiiA*) Gene

A total of 62 strains of *Bacillus thuringiensis* were screened for the *aiiA* gene using PCR. The strains were numbered 1 through 72; however, strains 31, 55, 59, 61, 64, 66, 68, 70, 71 and 72 did not grow. As a result, the study included 62 strains of Bt, among which 56 possessed the *aiiA* gene, and six strains did not possess the gene (Figure 1).

3.2. AHL-Lactonase Activity in *B. thuringiensis* Strains

Although the PCR results showed that 56 strains possess the AHL lactonase gene, not all strains with the *aiiA* gene degraded C_6-HSL. As described below, the bioassay activity was categorized into three different groups based on the concentration of the bacterial culture as well as the concentration of C_6-HSL.

Group 1 assay was done to screen for the activity of lactonase in the Bt strains. The strains were grown overnight, when the optical density (OD_{600}) was determined to be between 2.0 and 2.2. At the beginning of the experiment different concentrations of C_6-HSL were tested and the best concentration was determined to be 10 µM and 5 µM. Once the screening was performed the assay showed that 45 strains out of the 56 strains completely degraded C_6-HSL at 10 µM (Figure 2).

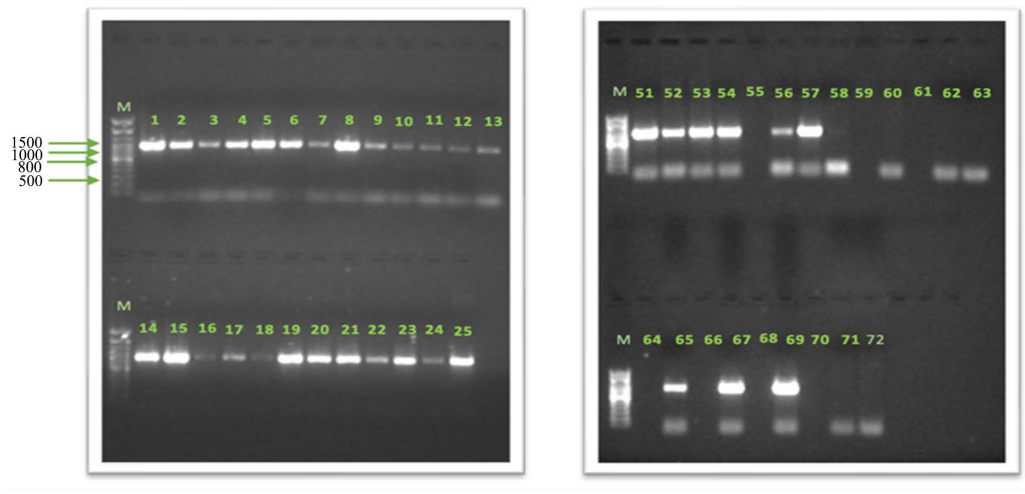

Figure 1. The results of PCR are shown. Gel electrophoresis of the amplified *aiiA* gene in the Bt strains (strain 1 to strain 25) and (strain 51 to strain 72) at 800 base pair are provided. The first lane (M) is the marker.

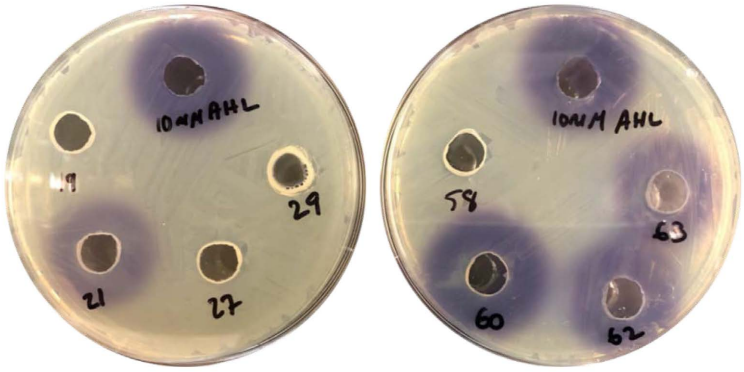

Figure 2. Rapid degradation of 10 uM of AHL in overnight grow of *B. thuringiensis* strains.

In group 2, the concentration of the Bt strains was set an OD_{600} of 1, and the concentration of C_6-HSL was 5 μM. The pigment was observed in this condition and the number of strains degrading C_6-HSL decreased to 20 strains inhibiting pigment formation.

The last group was performed by mixing 10 μM C_6-HSL with a low concentration of Bt cultures (OD_{600} of 1.0). This category revealed that only 11 strains were able to degrade 10 μM C_6-HSL and inhibit the production of the pigment.

3.3. Reformation of Degraded AHL

The degradation of C_6-HSL by AHL-lactonase can be repaired by lowering the solution's pH with hydrochloric acid (HCl). The acidic environment restores the structure of C_6-HSL by lactone-ring closing that was cleaved by AHL-lactonase activity. This resulted in exposing the degraded C_6-HSL to HCl which restores the function of C_6-HSL and thus, regenerated pigment production in CV026. The result showed that as the volume of the HCl increases which consequently lowers the pH, the intensity of the pigment increases as well.

3.4. Action of Clavulanate Potassium

There are different reasons that involve the hydrolysis of the lactone ring and inactivating quorum sensing. Inhibiting the lactonase activity produced by the Bt strains was assessed by clavulanate potassium which is a beta-lactamase inhibitor and prevents antibiotic inactivation by bacterial lactamases. It was used as an inhibitor of AHL-lactonase activity since both AHL-lactonase and β-lactamase are structurally similar. Bt strains were incubated with both clavulanate potassium and C_6-HSL at the same time. The results indicated that clavulanate potassium was a suitable inhibitor of the lactonase activity because the lactonase could not hydrolyze C_6-HSL when incubated with clavulanate potassium.

3.5. *N*-3-Oxo-Hexanoyl Homoserine Lactone (3-Oxo-C_6-HSL)

There are different molecules of *N*-acyl homoserine lactone produced by microbes. These molecules are specific to a species and a role. Due to this variation in

the AHL molecules, enzymatic activities of quorum sensing inhibitors are also specific to certain molecules. A slight variation could change the chemical characteristic of the molecules. For example, C_6-HSL is a non-polar molecule, while 3-oxo-C_6-HSL is a polar molecule. This experiment tested whether the lactonase could also degenerate 3-oxo-C_6-HSL as it degraded C_6-HSL. C_6-HSL was replaced with 3-oxo-C_6-HSL and tested for pigment production by CV026. It was found that CV026 turned purple, indicating that 3-oxo-C_6-HSL molecules were not degraded. These results show that the lactonase in the Bt strains have a specific activity on C_6-HSL but not on 3-oxo-C_6-HSL.

3.6. The Effect of Temperature and Time on AHL Lactonase Activity

In this experiment the C_6-HSL was not incubated directly with the Bt culture. After the Bt strains were grown overnight, the bacterial cells were first centrifuged, and then the supernatant which contained the lactonase was incubated with C_6-HSL at different temperatures; room temperature, 30˚C, 40˚C, 50˚C, and 60˚C. Following one-hour incubation of the supernatant with C_6-HSL, the mixture was loaded into the wells of plates streaked with CV026. The outcome of this experiment found that there was no degradation of C_6-HSL when the mixture was incubated at room temperature or at 30˚C as the intensity of the pigment at these temperatures was identical to the control. However, as the temperature increased, basically at 40˚C and 50˚C, the pigment around the wells was faint due to slight degradation of C_6-HSL. The pigment disappeared completely when the mixture was incubated at 60˚C. This indicates that the supernatant contained active AHL lactonase; however, the enzyme showed activity only at higher temperatures as CV026 did not produce the pigment at a temperature of 60˚C.

In addition, different incubation periods (0, 10 min, 20 min, 30 min, 40 min, 50 min, and 60 min) of supernatant/C_6-HSL mixture were set to determine at what time the lactonase completely degenerates C_6-HSL. It was found that a slight degradation occurred during the beginning of the incubation periods (20 min and 30 min). However, complete degradation was established at 40 min and a longer period of incubation.

4. Discussion

Many bacteria rely on quorum sensing to regulate gene expression of several virulence factors in human, animals, and plants resulting in health issues as well as economic issues [11] [12] [13] [14] [15]. Although there are several signal molecules produced by Gram-positive and Gram-negative bacteria, the most common type is AHL produced in Gram-negative bacteria [16] [17] [18]. These molecules are species specific and each molecule regulates certain genes. AHLs vary in the length of acyl chain, which could be four carbon atoms and up to 16 carbon atoms, as well as C-3, which could be a hydrocarbon, hydroxyl group, or

carbonyl group [19]. In this study, CV026 was used as a biosensor. It is a Gram-negative bacterium that requires C_6-HSL molecules to express violacein, a purple pigment protein [20].

Interestingly, gene expression regulated by AHL molecules is mitigated by quorum sensing inhibitor (QSI) enzymes. There are basically three types of quorum sensing inhibitor enzymes that can degrade AHL molecules; AHL-lactonase, AHL-acylase, and AHL-oxidoreductase. The difference between these three enzymes is the mechanism of degrading AHL molecules. AHL-lactonase, for example, break down AHLs by opening the lactone ring [4]. AHL-acylase, on the other hand, disrupts QS by hydrolyzing the acyl side chains from the homoserine lactone (HSL) [21] [22]. Finally, AHL-oxidoreductase modifies the acyl chain in AHL by either oxidizing or reducing the C-3 [9]. Such modifications by any of the three enzymes disrupt cell-cell communication in Gram-negative bacteria and attenuate gene expression involved in pathogenicity, biofilm production, and virulence factors. The study used Bt isolates to examine the activity of AHL-lactonase on C_6-HSL molecules by pigment production in CV026.

4.1. *aiiA* Gene and AHL-Lactonase

The AHL-lactonase, *aiiA*, gene is a common lactonase gene found in Gram-positive bacteria especially *Bacillus* species *including B. cereus*, *B. thuringiensis*, and *B. mycoides*. There are other AHL-lactonase genes found in different Gram-positive bacteria such as *aiiM* in *Microbacterium testaceum* [10] and *qsdA* in *Rhodococcus erythropolis* [23] as well as in Gram-negative bacteria such as *aiiB* in *Agrobacterium tumefaciens* [24] and *ahlK* in *Klebsiella pneumoniae* [25]. After performing PCR and gel electrophoresis, this study found that the majority, around 90%, of Bt isolates possess the *aiiA* gene. AHL-lactonase enzymes are not the same, that is, *aiiA* and AiiB enzymes belong to the metallo-β-lactamase family while AiiM and QsdA are related to α/β hydrolase family and phosphotriesterase family, respectively.

The AHL-lactonase from the different Bt isolates showed a variation in C_6-HSL degradation. Among the 56 strains with *aiiA* gene, 25 strains showed weak activity, 9 strains showed moderate activity, and 11 strains showed potent activity. The activity varies on the concentration of the bacterial cells based on the optical density as well as on the concentration of the C_6-HSL molecules (10 μM and 5 μM). A previous study also showed that some Bt strains showed intense degradation and others weak degradation [26]. Although the lactonase degraded C_6-HSL, it could not break down 3-oxo-C_6-HSL, indicating that the lactonase cannot hydrolyze an acyl group with a substitution in C-3, and it specifically hydrolyzes the C_6-HSL molecule.

4.2. Reformation of Hydrolyzed AHL

AHL molecules are affected by the pH of the medium. Increasing the pH leads to the hydrolysis of the homoserine lactone similar to the AHL lactonase. On the

other hand, lowering the pH to less than two leads to reformation of the hydrolyzed products to the original molecules [27]. In the findings of this work, the reformation of degraded AHL was demonstrated by purple pigment around CV026. When the hydrolysis product of lactonase was incubated with HCl and compared to the hydrolysis product of lactonase with no HCL the purple pigment was present. This reversible process by acidification is known as lactonolysis. Hydrolysis products of other enzymes such as AHL acylase or oxidoreductase cannot be restored by acidification. Thus, the hydrolysis product of lactonase is pH-dependent, and acidification can assure that the molecules were degraded by AHL lactonase [28].

It is known that the hydrolyzed product by lactonase restores the degraded AHL into active AHL when the pH is <2. In this study, pH was presented in the ratio between HCl and the mixture (Bt supernatant/AHL). It was found that at low pH, the lactonolysis starts to establish and thus gives light pigment. When pH was, the pigment got darker, indicating more reformation. Lowering the pH by adding more HCl, for example, 1:2 or 1:1 would increase the chance of getting more active AHL, but it would increase a clear zone around the wells as CV026 bacterium cannot grow in a highly acidic environment.

4.3. Inhibition of AHL-Lactonase

Studies have shown that AHL-lactonase belongs to the metallo-β-lactamase family [29]. Due to the high similarity between the two enzymes, clavulanate potassium, which has been used to block the activity of β-lactamase, was used to inhibit AHL-lactonase activity. When clavulanate potassium was added to the mixture (Bt cell/AHL), it inhibited the AHL lactonase from degrading C_6-HSL and thus, CV026 turned to purple. Hence, clavulanate potassium was found to be a suitable inhibitor and can inhibit AHL lactonase. This ensures that degradation occurred by AHL lactonase and not by other enzymes. It was a good inhibitor to be used to block lactonase activity and to test whether other AHL enzymes exist in a particular organism.

4.4. Effect of Temperature on AHL-Lactonase

This study showed that AHL lactonase was a temperature-dependent enzyme when the supernatant of the bacterial cells was used for the enzymatic activity. At a temperature of 20˚C and 30˚C, no enzymatic activity was observed as pigment appeared as dark as the control (AHL). When the mixture (Bt supernatant/AHL) was incubated at 40˚C, the pigment appeared faint, indicating slight hydrolysis of AHL. However, when the mixture was incubated at a high temperature (>60˚C), the pigment disappeared in each of Bt strains of this study. Therefore, this study showed that the activity of AHL lactonase depended upon temperature when the supernatant of the Bt culture was mixed with AHL molecules. In addition, the result showed that AHL-lactonase is a thermostable enzyme and can tolerate high temperature (>60˚C) without losing activity. Simi-

larly, a previous study showed that AidB, another lactonase belonging to the metallo β-lactamase superfamily, was thermostable in the range 30˚C to 80˚C and the optimum temperature of AidB was 60˚C [30].

5. Summary and Conclusions

This study reviews the quorum sensing inhibitor activity of soil bacteria isolated from middle Tennessee. Some Bt strains show strong degradation against AHL molecules and completely prevent pigment production in CV026 while other strains have mild degradation to no degradation of AHL molecules. The various test of these studies reveals that degradation of AHL molecules occurred by AHL lactonase. Furthermore, acidification tests were performed to confirm that quorum quenching activity occurred because of AHL lactonase. AHL lactonase was able to degrade C_6-HSL as well as 3-oxo-C_6-HSL indicating that the enzyme is not affected by the carbonyl group in 3-oxo-C_6-HSL. The last test performed in this study was blocking the AHL lactonase activity. Potassium clavulanate, a β-lactamase inhibitor, was tested and showed to be a great inhibitor of AHL lactonase. This was expected since lactonase and β-lactamase share some molecular similarities. By blocking AHL lactonase, further studies can be done on other quorum quenching enzymes such as acylase and oxidoreductase. It was concluded that only a few Bt strains showed a potent degradation and can compete with quorum sensing bacteria. Applying quorum quenching activity to biofilm formation and virulence factor producing bacteria would reduce the ability of these bacteria to become established pathogens.

In conclusion, quorum sensing inhibitory (QSI) enzymes have shown a great method to interfere with bacterial quorum sensing signals and thus inhibit virulence factors in pathogenic bacteria. Based on the various tests that were performed including the presence of *aiiA* gene, the reformation of hydrolyzed C_6-HSL, and the inhibition of the enzyme activity, it was demonstrated that the enzyme was AHL lactonase. The study also showed that some Bt strains possess AHL lactonase with strong activity against AHL molecules, C_6-HSL in specific. Studies have shown a significant effect of quorum sensing inhibitors on quorum sensing bacteria expressing virulence factors. The effects were in the agriculture and aquaculture fields. For example, purified lactonase *aiiA* from *Bacillus* co-injected with the fish pathogen *Aeromonas hydrophila* in carp decreased the mortality rate and delayed the mortality time of fish [11]. In addition, *Microbacterium testaceum*, which harbors the surface of potato leaves, can protect the plant from soft rot disease by degrading AHL produced by the plant pathogen *Pectobacterium carotovorum*. Once AHL lactonase has degraded AHL molecules, it reduces the expression of virulence genes [25]. QSI could be an alternative to antibiotics as it indirectly attenuates gene expression, thus reducing the pressure to identify new antibiotics that act on pathogenic bacteria.

Acknowledgements

Special thanks to Dr. Colin Dale and his lab mates at The University of Utah for

providing us with CV026 without which this study was not possible.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Miller, M.B. and Bassler, B.L. (2001) Quorum Sensing in Bacteria. *Annual Reviews in Microbiology*, **55**, 165-199. https://doi.org/10.1146/annurev.micro.55.1.165

[2] Turovskiy, Y., Kashtanov, D., Paskhover, B. and Chikindas, M.L. (2007) Quorum Sensing: Fact, Fiction, and Everything in between. *Advances in Applied Microbiology*, **62**, 191-234. https://doi.org/10.1016/S0065-2164(07)62007-3

[3] Czajkowski, R. and Jafra, S. (2009) Quenching of Acyl-Homoserine Lactone-Dependent Quorum Sensing by Enzymatic Disruption of Signal Molecules. *Acta Biochimica Polonica*, **56**, 1-16. https://doi.org/10.18388/abp.2009_2512

[4] Dong, Y.H., Xu, J.L., Li, X.Z. and 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*, **97**, 3526-3531. https://doi.org/10.1073/pnas.97.7.3526

[5] Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F. and Zhang, L.H. (2001) Quenching Quorum-Sensing-Dependent Bacterial Infection by an *N*-acyl Homoserine Lactonase. *Nature*, **411**, 813-817. https://doi.org/10.1038/35081101

[6] Leadbetter, J.R. and Greenberg, E.P. (2000) Metabolism of Acyl-Homoserine Lactone Quorum-Sensing Signals by *Variovorax paradoxus*. *Journal of Bacteriology*, **182**, 6921-6926. https://doi.org/10.1128/JB.182.24.6921-6926.2000

[7] Lin, Y.H., Xu, J.L., Hu, J., Wang, L.H., Ong, S.L., Leadbetter, J.R. and 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**, 849-860. https://doi.org/10.1046/j.1365-2958.2003.03351.x

[8] Wang, L.H., Weng, L.X., Dong, Y.H. and Zhang, L.H. (2004) Specificity and Enzyme Kinetics of the Quorum-Quenching *N*-acyl Homoserine Lactone Lactonase (AHL-Lactonase). *Journal of Biological Chemistry*, **279**, 13645-13651. https://doi.org/10.1074/jbc.M311194200

[9] Wang, W.Z., Morohoshi, T., Ikenoya, M., Someya, N. and Ikeda, T. (2010) AiiM, a Novel Class of *N*-Acylhomoserine Lactonase from the Leaf-Associated Bacterium *Microbacterium testaceum*. *Applied and Environmental Microbiology*, **76**, 2524-2530. https://doi.org/10.1128/AEM.02738-09

[10] Chen, R., Zhou, Z., Cao, Y., Bai, Y. and Yao, B. (2010) High Yield Expression of an AHL-Lactonase from Bacillus sp. B546 in *Pichia pastoris* and Its Application to Reduce *Aeromonas hydrophila* Mortality in Aquaculture. *Microbial Cell Factories*, **9**, Article No. 39. https://doi.org/10.1186/1475-2859-9-39

[11] Licciardello, G., Bertani, I., Steindler, L., Bella, P., Venturi, V. and Catara, V. (2009) The Transcriptional Activator rfiA Is Quorum-Sensing Regulated by Cotranscription with the luxI Homolog pcoI and Is Essential for Plant Virulence in *Pseudomonas corrugata*. *Molecular Plant-Microbe Interactions*, **22**, 1514-1522. https://doi.org/10.1094/MPMI-22-12-1514

[12] Licciardello, G., Strano, C.P., Bertani, I., Bella, P., Fiore, A., Fogliano, V. and Catara, V. (2012) *N*-acyl-homoserine-lactone Quorum Sensing in Tomato Phytopathogenic Pseudomonas spp. Is Involved in the Regulation of Lipodepsipeptide Production.

Journal of Biotechnology, **159**, 274-282.
https://doi.org/10.1016/j.jbiotec.2011.07.036

[13] Chow, J.Y., Yang, Y., Tay, S.B., Chua, K.L. and Yew, W.S. (2014) Disruption of Biofilm Formation by the Human Pathogen *Acinetobacter baumannii* Using Engineered Quorum-Quenching Lactonases. *Antimicrobial Agents and Chemotherapy*, **58**, 1802-1805. https://doi.org/10.1128/AAC.02410-13

[14] Natrah, F.M.I., Alam, M.I., Pawar, S., Harzevili, A.S., Nevejan, N., Boon, N. and Defoirdt, T. (2012) The Impact of Quorum Sensing on the Virulence of *Aeromonas hydrophila* and *Aeromonas salmonicida* towards Burbot (*Lota lota* L.) Larvae. *Veterinary Microbiology*, **159**, 77-82. https://doi.org/10.1016/j.vetmic.2012.03.014

[15] Carter, G.P., Awad, M.M., Hao, Y., Thelen, T., Bergin, I.L., Howarth, P.M. and Lyras, D. (2011) TcsL Is an Essential Virulence Factor in *Clostridium sordellii* ATCC 9714. *Infection and Immunity*, **79**, 1025-1032. https://doi.org/10.1128/IAI.00968-10

[16] Frederix, M. and Downie, J.A. (2011) Quorum Sensing: Regulating the Regulators. *Advances in Microbial Physiology*, **58**, 23-80.
https://doi.org/10.1016/B978-0-12-381043-4.00002-7

[17] Bhargava, N., Sharma, P. and Capalash, N. (2010) Quorum Sensing in Acinetobacter: An Emerging Pathogen. *Critical Reviews in Microbiology*, **36**, 349-336.
https://doi.org/10.3109/1040841X.2010.512269

[18] Lupp, C. and Ruby, E.G. (2005) *Vibrio fischeri* Uses Two Quorum-Sensing Systems for the Regulation of Early and Late Colonization Factors. *Journal of Bacteriology*, **187**, 3620-3629. https://doi.org/10.1128/JB.187.11.3620-3629.2005

[19] Nasser, W. and Reverchon, S. (2007) New Insights into the Regulatory Mechanisms of the LuxR Family of Quorum Sensing Regulators. *Analytical and Bioanalytical Chemistry*, **387**, 381-390. https://doi.org/10.1007/s00216-006-0702-0

[20] McClean, K.H., Winson, M.K., Fish, L., Taylor, A., Chhabra, S.R., Camara, M. and 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.
https://doi.org/10.1099/00221287-143-12-3703

[21] Mukherji, R., Varshney, N.K., Panigrahi, P., Suresh, C.G. and Prabhune, A. (2014) A New Role for Penicillin Acylases: Degradation of Acyl Homoserine Lactone Quorum Sensing Signals by *Kluyvera citrophila* Penicillin G Acylase. *Enzyme and Microbial Technology*, **56**, 1-7. https://doi.org/10.1016/j.enzmictec.2013.12.010

[22] Uroz, S., Chhabra, S.R., Camara, M., Williams, P., Oger, P. and Dessaux, Y. (2005) *N*-acylhomoserine Lactone Quorum-Sensing Molecules Are Modified and Degraded by *Rhodococcus erythropolis* W2 by both Amidolytic and Novel Oxidoreductase Activities. *Microbiology*, **151**, 3313-3322.
https://doi.org/10.1099/mic.0.27961-0

[23] Uroz, S., Oger, P.M., Chapelle, E., Adeline, M.T., Faure, D. and Dessaux, Y. (2008) A *Rhodococcus* qsdA-Encoded Enzyme Defines a Novel Class of Large-Spectrum Quorum-Quenching Lactonases. *Applied and Environmental Microbiology*, **74**, 1357-1366. https://doi.org/10.1128/AEM.02014-07

[24] Carlier, A., Uroz, S., Smadja, B., Fray, R., Latour, X., Dessaux, Y. and Faure, D. (2003) The Ti Plasmid of *Agrobacterium tumefaciens* Harbors an *attM*-Paralogous Gene, *aiiB*, Also Encoding *N*-acyl Homoserine Lactonase Activity. *Applied and Environmental Microbiology*, **69**, 4989-4993.
https://doi.org/10.1128/AEM.69.8.4989-4993.2003

[25] Park, S.Y., Lee, S.J., Oh, T.K., Oh, J.W., Koo, B.T., Yum, D.Y. and Lee, J.K. (2003)

AhlD, an *N*-acylhomoserine Lactonase in Arthrobacter sp., and Predicted Homologues in Other Bacteria. *Microbiology*, **149**, 1541-1550. https://doi.org/10.1099/mic.0.26269-0

[26] Lee, S.J., Park, S.Y., Lee, J.J., Yum, D.Y., Koo, B.T. and Lee, J.K. (2002) Genes Encoding the *N*-acyl Homoserine Lactone-Degrading Enzyme Are Widespread in Many Subspecies of *Bacillus thuringiensis*. *Applied and Environmental Microbiology*, **68**, 3919-3924. https://doi.org/10.1128/AEM.68.8.3919-3924.2002

[27] Zhang, H.B., Wang, L.H. and Zhang, L.H. (2007) Detection and Analysis of Quorum-Quenching Enzymes against Acyl Homoserine Lactone Quorum-Sensing Signals. *Current Protocols in Microbiology*, **5**, 1C-3. https://doi.org/10.1002/9780471729259.mc01c03s05

[28] Yates, E.A., Philipp, B., Buckley, C., Atkinson, S., Chhabra, S.R., Sockett, R.E. and Williams, P. (2002) *N*-acylhomoserine Lactones Undergo Lactonolysis in a pH-, Temperature-, and Acyl Chain Length-Dependent Manner during Growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infection and Immunity*, **70**, 5635-5646. https://doi.org/10.1128/IAI.70.10.5635-5646.2002

[29] Liu, D., Momb, J., Thomas, P.W., Moulin, A., Petsko, G.A., Fast, W. and Ringe, D. (2008) Mechanism of the Quorum-Quenching Lactonase (*AiiA*) from *Bacillus thuringiensis*. 1. Product-Bound Structures. *Biochemistry*, **47**, 7706-7714. https://doi.org/10.1021/bi800368y

[30] Zhang, J.W., Xuan, C.G., Lu, C.H., Guo, S., Yu, J.F., Asif, M. and Zhang, L.Q. (2019) AidB, a Novel Thermostable *N*-acylhomoserine Lactonase from the Bacterium Bosea sp. *Applied and Environmental Microbiology*, **85**, e02065-19. https://doi.org/10.1128/AEM.02065-19