

Inhibition of Quorum Sensing-mediated Bioluminescence in *Vibrio harveyi* by a Recombinant AHL-lactonase from *Bacillus cereus*

Nguyen Thi Ngoc Tinh*, Nguyen Viet Dung,
Van Thi Thuy and Nguyen Thao Suong

Research Institute for Aquaculture No. 2, Hochiminh City, Vietnam.

(Received: 12 April 2014; accepted: 09 May 2014)

Quorum sensing is a cell-to-cell communication process in bacterial kingdom, in which the expression of genetic information is controlled via the production and detection of “signal molecules” in a population density-dependent manner. Quorum sensing system has been discovered and shown to control virulence factors in several Gram-negative bacterial pathogens in aquaculture, including *Vibrio harveyi*, a harmful bacterium in shrimp. It has been shown that the ability to inactivate AHL signal molecules may be useful in controlling the AHL-mediated virulence in many Gram-negative pathogenic bacteria. AHL-lactonase, one of the three AHL-disrupting enzyme classes, attracts significant interest of researchers due to its high substrate specificity toward AHL signals. In this study, a recombinant and purified AHL-lactonase (AiiA_{N26.2} protein) was verified for its ability to inhibit bioluminescence of *V. harveyi* cultures. AiiA_{N26.2} protein at a concentration of 15 µg ml⁻¹ could significantly attenuate the luminescence intensity of *V. harveyi* cultures over a 6-hour period. The findings of the study are important for future research as it was proven that recombinant AHL-lactonase can be used to control infectious diseases in aquaculture which are related to the AHL-mediated quorum sensing system.

Key words: AHL-lactonase; AHL-mediated quorum sensing; bioluminescence; *Vibrio harveyi*.

Quorum sensing is well-known as a cell-to-cell communication process in bacterial kingdom, in which the expression of genetic information is controlled via the production and detection of so-called “signal molecules” in a population density-dependent manner¹. In Gram-negative bacteria, this communication is mediated by N-acyl homoserine lactones (AHLs). Quorum sensing system has been discovered in several Gram-negative bacterial pathogens in aquaculture, including *Vibrio harveyi*, an important pathogen

which causes luminous disease in shrimp from post-larval to grow-out stages². Quorum sensing in *Vibrio harveyi* utilizes three cell-signalling systems that function in parallel and are regulated by three types of signal molecules: HAI-1 (harveyi autoinducer 1, which is an AHL), AI-2 (autoinducer 2), and CAI-1 (cholerae autoinducer 1). These quorum sensing systems regulate several virulence factors in *Vibrio harveyi* such as bioluminescence³⁻⁵, metalloprotease⁶, siderophore, and exopolysaccharide production⁷.

The finding that many pathogens rely on quorum sensing mechanism to synchronize microbial activities essential for infection and survival in the host suggests a promising disease control strategy, i.e. quenching microbial quorum sensing or quorum quenching. The naturally occurring quorum-quenching mechanisms appear to play important roles in microbe-microbe and

* To whom all correspondence should be addressed.
Tel.: +84-908-216101;
E-mail: tinhntn.ria2@mard.gov.vn

pathogen-host interactions, and have been used in developing and formulating a new generation of antimicrobial substances⁸.

It has been shown that the ability to inactivate AHL signal molecules may be useful in controlling the AHL-mediated virulence in many Gram-negative pathogenic bacteria. Among the various naturally occurring AHL-inactivating substances, the most effective have been found to be the AHL-inactivating enzymes of bacterial origin. The reaction of disruption of AHL molecules can be catalyzed by three types of bacterial enzymes, namely AHL-acylase, AHL-lactonase and paraoxonase^{9,10}. Recently, AHL-oxidoreductase, a novel type of AHL-inactivating enzyme, was described¹¹. AHL-lactonases, which belong to the metallo-beta-lactamase superfamily and catalyze the reaction of lactone ring opening, attract significant interest of researchers due to their high substrate specificity toward AHL signals¹². The ability to produce AHL-lactonase is widely inherent in bacterial genera such as *Agrobacterium*, *Acinetobacter*, *Arthrobacter*, *Bacillus*, *Klebsiella*, and *Pseudomonas*^{8,13,14}, with the highest frequency of AHL-lactonase genes (*aiiA* gene) being recorded in the *Bacillus* group. In a study conducted by Nusrat et al. (2011)¹⁵, among 800 bacterial strains isolated from different sources, gene encoding AHL-lactonase was present in 42 strains. These strains were all identified as *Bacillus* strains, predominantly *B. cereus*. A survey of the available databases shows that, around 100 sequences of AHL-lactonase gene have been submitted to GeneBank, of which 88 belong to *Bacillus* sp. (<http://www.ncbi.nlm.nih.gov/>). It is also revealed that, since AHL-lactonase activity is independent of the length and substitutions in the AHL signal molecule¹⁶, AHL-lactonase is likely to be more effective in controlling quorum-sensing dependent bacterial infections¹⁷.

Two studies concerning the application of recombinant AHL-lactonase in aquaculture have been reported recently. An AHL-lactonase originating from *Bacillus* sp. B546 was produced extracellularly in the yeast *Pichia pastoris*. This recombinant AHL-lactonase was able to decrease the mortality rate and to delay the mortality time in common carp, when it was co-injected with *Aeromonas hydrophila* in the fish¹⁸. Recently, the same research group has reported on the use of

another *Bacillus*-originated recombinant AHL-lactonase. This AHL-lactonase, when orally administered via fish feed, significantly attenuated *A. hydrophila* infection in zebrafish¹⁹.

In the present study, a purified *aiiA* gene product from a *Bacillus cereus* strain N26.2, called *aiiA*_{N26.2} (GenBank accession number KC128668), was verified for its capacity to attenuate the bioluminescence production in *V. harveyi* strain BB120 and its double-mutant JMH606.

MATERIALS AND METHODS

Bacterial strains

Bacillus cereus strain N26.2 was isolated in striped catfish pond water in Dong Thap Province, Vietnam. This strain was identified by API 20 biochemical kit and 16S rRNA sequence analysis. *E. coli* strain BL21(DE3)pLysS was purchased from Promega (USA). *V. harveyi* strain BB120 and its mutant strain JMH606, which is dysfunctional in AI-2 and CAI-1 synthase, were obtained from the Department of Molecular Biology, Princeton University, New Jersey, USA. *Chromobacterium violaceum* strain CV026 was used as a reporter strain for a commercial HHL (N-Hexanoyl homoserine lactone) molecule (Sigma, USA). CV026 is a mini-Tn5 mutant derived from the *C. violaceum* strain ATCC 31532²⁰. This strain cannot produce AHL, but can detect and respond to a range of AHL molecules (with acyl side chain of four to eight carbons) by inducing the synthesis of the purple pigment violacein.

Culture media

V. harveyi strains were grown in Marine Broth (Difco, Detroit, USA). *E. coli* strain and CV026 strain were grown in Luria-Bertani (LB) medium (1% peptone, 0.5% yeast extract, 0.5% NaCl) supplemented with appropriate antibiotics (ampicillin 100 µg ml⁻¹ or kanamycin 20 µg ml⁻¹, respectively). All these strains were incubated for 24 h at 30°C.

Amplification of *aiiA* gene

Total DNA from *Bacillus cereus* strain N26.2 was extracted as described by Bai et al. (2008)²¹ and used as template for polymerase chain reaction (PCR) amplification. The forward primer was *aiiA*-F2 (5'-GCATGCCATGGGCATGACAGTAAAGAAGCTTTA-3') and reverse primer was *aiiA*-R2 (5'-GCAGGTGACCTATATATATTC

CGGGAAC-3') with a product size of 773 bp bearing *Nco*I (underlined in forward primer) and *Sal*I (underlined in backward primer) recognition sites at each of end sites²¹. The full-length *aiiA* gene was amplified in 50 µl of PCR reaction contained 1 µl of DNA template, 0.6 mM of each primer, 0.2 mM of dNTPs and 1.25 U of *Pfu* DNA polymerase in 1X of PCR buffer (Promega, USA). PCR reaction was carried out in a thermal cycler (Icycler, Biorad, USA) programmed for 95°C for 2 min; 35 cycles at 94°C for 30 s, 55°C for 45 s, and 72°C for 90 s, followed by 72°C for 10 min. The resulting *aiiA* gene was separated by electrophoresis in a 1.5 % (w/v) agarose gel and recovered by using Wizard® SV Gel and PCR Clean-Up Kit (Promega, USA).

Construction of expression vector and sequencing

The recovered *aiiA* gene was double digested by *Nco*I and *Sal*I (New England Biolabs, USA) and ligated into pMAL-c5x vector (New England Biolabs, USA) previously digested with the same restriction enzymes. The recombinant pMAL-c5x-*aiiA* vector was then transformed into *Escherichia coli* JM109 chemical competent cells as manufacturer's instructions (Promega, USA) for analysis of recombinant vector. The presence of the insert in the recombinant vector was confirmed by PCR with primers *aiiA*-F2 and *aiiA*-R2, followed by double digestion at respective restriction enzyme sites and sequencing with primer vector pMAL-5R (5'-TGTCCTACTCAGGA GAGCGTTCAC-3') on 3130 Genetic Analyzers device (Applied Biosystems, USA). The good construct of pMAL-c5x-*aiiA* vector from transformed *E. coli* JM109 was transformed into *E. coli* BL21(DE3)pLysS (Promega, USA) chemical competent cells for expression as manufacturer's instructions. PCR cloning analysis with primer pair *aiiA*-F2 and *aiiA*-R2 was used to confirm the positive transformed *E. coli* BL21(DE3)pLysS.

Expression of recombinant AHL-lactonase

The transformed *E. coli* strain BL21(DE3)pLysS carrying pMAL-c5x-*aiiA* vector was cultured at 37°C in 100 ml Luria-Bertani Broth (LB) medium containing 50 µg ml⁻¹ ampicillin and 2 % glucose in shaking condition until the culture reached an optical density of 0.5-0.6 at 600 nm in wavelength. To stimulate the expression of recombinant protein, the culture was supplemented with 0.01 mM CoCl₂ and 0.05 mM isopropyl thio-2-D-galactopyranoside (IPTG) and was further

incubated with shaking at 16°C overnight. The control experiment was performed similarly to *E. coli* BL21(DE3)pLysS carrying vector pMAL-c5x (without *aiiA* gene). The purification protocol for recombinant AHL-lactonase was done with Amylose Magnetic Beads (New England Biolabs, USA), using column-binding buffer and elution buffer without EDTA. The purified recombinant AHL-lactonase was re-named as AiiA_{N26.2} protein and was evaluated on 12% SDS-PAGE gel and stained with Coomassie Brilliant Blue G250 (Biorad). **Effect of AiiA_{N26.2} protein on the luminescence intensity of *Vibrio harveyi***

V. harveyi strains were cultivated overnight in Marine Broth (MB) medium at 30°C with shaking until OD₆₀₀ reached approximately 1.0, and further diluted 1/5000 in fresh MB medium. Then 50 µl aliquots were mixed with 50 µl of fresh medium in 96-well microplate. Purified AiiA_{N26.2} protein was added to the wells at three different concentrations (5 µg ml⁻¹, 15 µg ml⁻¹ and 25 µg ml⁻¹, in six replicates). The luminescence intensity of *V. harveyi* cultures in the absence and presence of AiiA_{N26.2} protein was monitored for up to 6 h after the addition of protein and with one-hour interval, by means of TECAN Multireader apparatus Infinite M200.

RESULTS

Amplification and sequence analysis of *aiiA* gene

The bioassay using AHL-reporter strain *C. violaceum* CV026 showed that N26.2 strain has a high capacity for degradation of HHL signal molecule (data not shown). PCR product was amplified from genomic DNA extraction of *B. cereus* strain N26.2 by using primers pair *aiiA*-F2 and *aiiA*-R2. The sequence analysis confirmed that this amplicon is 753 bp in length, was named as *aiiA*(N26.2) (deposited GenBank accession number KC128668) and shares high identity of the AHL-lactonase-encoding gene sequence with those from other *Bacillus* species. The putative *aiiA*(N26.2) gene encodes a protein of 250 amino acid residues which contains no leader peptide and has a predicted molecular weight of 27 kDa. Amino acid sequence alignment indicates that it contains a ¹⁰⁴HXHXDH¹⁰⁹ - H¹⁶⁹ zinc-binding motif that is conserved in some AHL-lactonases and several groups of metallo-hydrolases (Fig. 1).



Fig. 1. Predicted amino acid sequence alignment of AiiA_{N26.2} protein of our study (44262) with *Bacillus* AiiA-like proteins and with AHL-lactonases from other species. *Bacillus cereus* E33L (GenBank accession number YP_084761), *Bacillus anthracis* strain Ames (NP_845794), *Bacillus thuringiensis* (ABD93926), *Bacillus amyloliquefaciens* (ACX55096), *Bacillus* sp. AI96 (ADK91097), *Bacillus subtilis* (AAY51611), *Arthrobacter* sp. strain IBN110 (AAP57766) (AhlD), *Klebsiella pneumoniae* (AAO47340) (AhlK), *Agrobacterium tumefaciens* C58 (AAK91031) (AiiB), *Agrobacterium fabrum* strain C58 (AAD43990). The similar and identical residues are shaded in blue and red, respectively

Expression and purification of recombinant AHL-lactonase

The transformed *E. coli* strain BL21(DE3) pLysS/pMAL-c5x-aiiA was induced overnight with 0.05mM IPTG, at 16°C and in a CoCl₂-supplemented medium. The construct vector of pMAL-c5x-aiiA allowed to express AHL-lactonase as a fusion protein with MBP tag at its N-terminal. Amylose Magnetic Beads and modified buffers were used to purify recombinant MBP-aiiA lactonase in

induced cell lysate. The SDS-PAGE analysis showed that the recombinant MBP-AHL-lactonase enzyme was expressed mostly as a soluble protein with a molecular mass approximately 71 kDa, which is consistent with the predicted molecular mass (71.4 kDa, including molecular mass of MBP tag and AHL-lactonase) (Fig. 2). The purity of the obtained recombinant AHL-lactonase (AiiA_{N26.2} protein) should be more than 95 %.

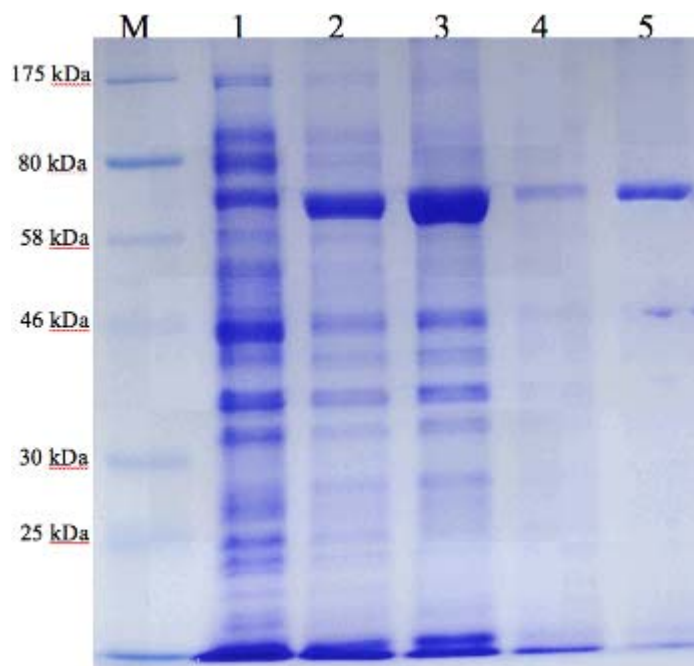


Fig. 2. SDS-PAGE analysis of the recombinant MBP-AHL-lactonase enzyme. M: marker; 1: total protein of non-induced *E. coli* BL21(DE3)pLysS/pMAL-c5x-aiiA cell lysate; 2: total protein of IPTG-induced *E. coli* BL21(DE3)pLysS; 3: total soluble protein fraction; 4: insoluble protein fraction; 5: purified AiiA_{N26.2} protein.

Inhibition of luminescence intensity of *Vibrio harveyi* by AiiA_{N26.2} protein

In this series of experiments, the optical density and luminescence intensity (LI) of *V. harveyi* cultures (BB120 and JMH606 strains), with and without the addition of AiiA_{N26.2} protein, were

monitored over a 6-hour period. As shown in Fig. 3, the log phase of both *V. harveyi* cultures started about 4 h after inoculation. Besides, the addition of AiiA_{N26.2} protein at different concentrations apparently did not affect the growth of *V. harveyi* cultures.

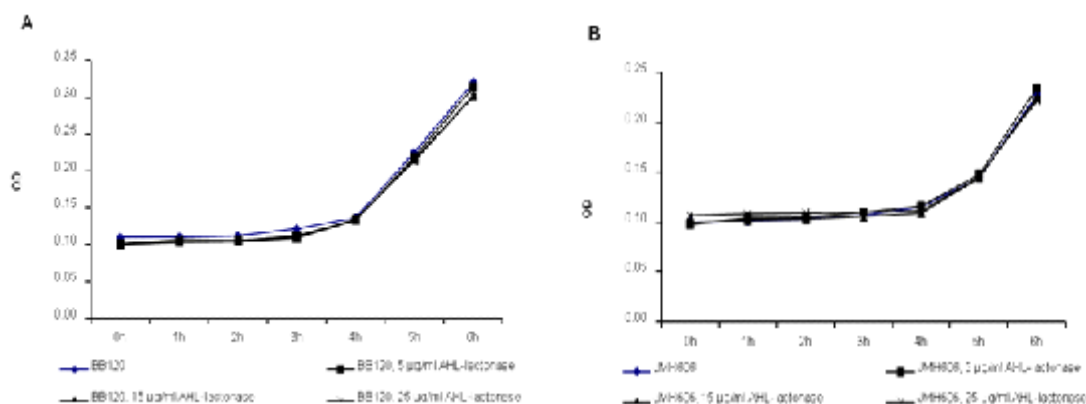


Fig. 3. Optical density (OD₆₀₀) of *Vibrio harveyi* wild-type BB120 culture (A) and double-mutant JMH606 culture (B) over 6-h period, with and without the addition of AiiA_{N26.2} protein.

On the other hand, different results were obtained considering the luminescence intensity of *V. harveyi* cultures over the same period. The luminescence intensity of BB120 culture was significantly attenuated ($p < 0.05$) from 2 h onwards, when AiiA_{N26.2} protein was added at 15 $\mu\text{g ml}^{-1}$ or 25 $\mu\text{g ml}^{-1}$ (Fig. 4A). The luminescence intensity of JMH606 culture was also significantly inhibited (p

< 0.05) at the same concentrations of AiiA_{N26.2} protein, but 1 h later (Fig. 4B). In all cases, the level of attenuation of luminescence intensity increased with protein concentration. Luminescence intensity of *V. harveyi* cultures was attenuated by 40-44 % at 5 $\mu\text{g ml}^{-1}$ of AiiA_{N26.2} protein, by 84-89 % at 15 $\mu\text{g ml}^{-1}$ of AiiA_{N26.2} protein, and by 97-99 % at 25 $\mu\text{g ml}^{-1}$ of AiiA_{N26.2} protein.

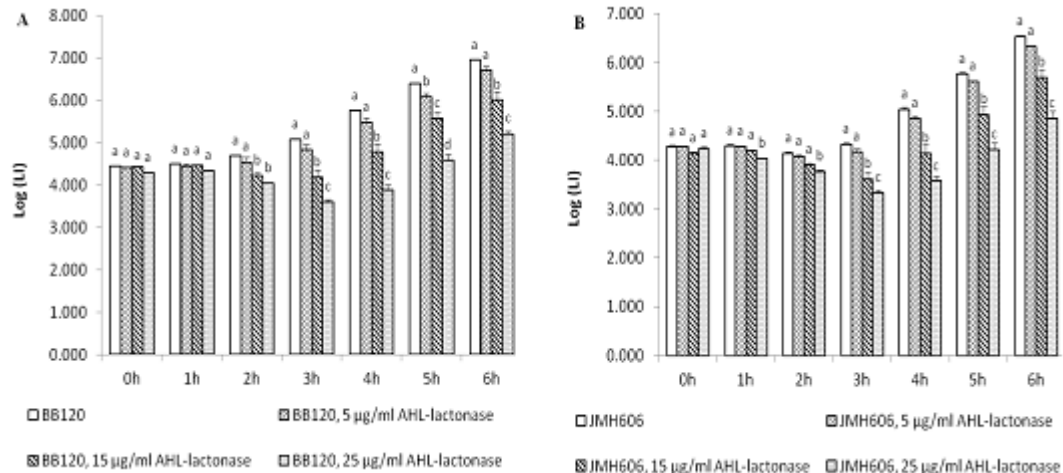


Fig. 4. Log luminescence intensity (LI) of *Vibrio harveyi* wild-type BB120 culture (A) and double-mutant JMH606 culture (B) over 6-h period, with and without the addition of AiiA_{N26.2} protein

A second experiment was conducted to confirm the inhibiting effect of AiiA_{N26.2} protein at the concentration of 15 $\mu\text{g ml}^{-1}$ (the lowest concentration that showed a significant effect) towards the luminescence intensity of *V. harveyi* cultures. The results shown in Fig. 5 confirmed those from the previous experiment. Hence, it can

be concluded that, AiiA_{N26.2} protein at the concentration of 15 $\mu\text{g ml}^{-1}$ was able to inhibit the luminescence intensity of both *V. harveyi* cultures (wild-type strain BB120 and mutant strain JMH606) during the early log phase (3 h until 6 h after inoculation).

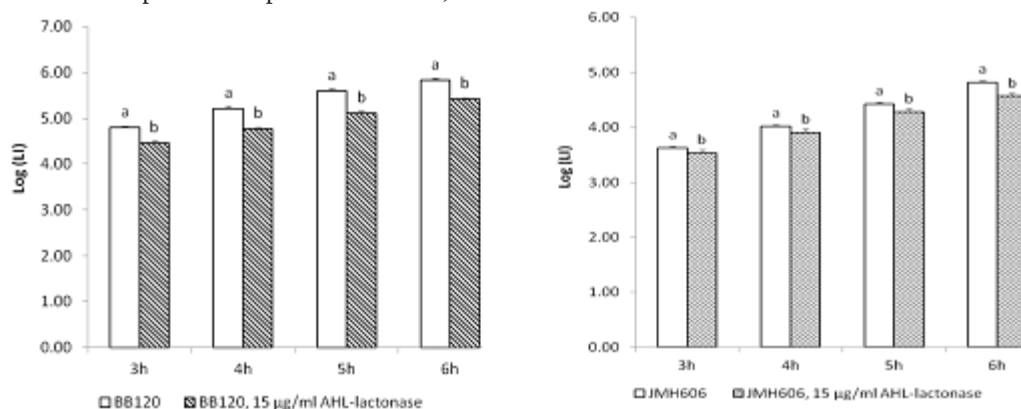


Fig. 5. Log luminescence intensity (LI) of *Vibrio harveyi* wild-type BB120 culture (A) and double-mutant JMH606 culture (B) with the addition of AiiA_{N26.2} protein (at 15 $\mu\text{g ml}^{-1}$) and of control cultures (no AiiA_{N26.2} protein added), over 6-h period

DISCUSSION

We evaluated the activity of AiiA_{N26.2} protein via the ability to attenuate the AHL-mediated bioluminescence intensity in *V. harveyi*. This bacterium has three quorum-sensing systems which act as a three-way coincidence detector and regulate genes responsible for a variety of phenotypes, including bioluminescence⁵, which can be quantified by means of a multireader apparatus. Two strains of *V. harveyi* were used in this study, wild-type strain (BB120) and a mutant strain (JMH606) which has only the HAI-1 channel functional. This mutant strain was used to ensure the specificity of AiiA_{N26.2} protein through the AHL-dependent channel.

In a similar study done previously by Bai et al. (2008)²¹, a recombinant AiiA protein was over-expressed from the *aiiA* gene of a *Bacillus thuringiensis* strain, and the activity of this protein based on its ability to inhibit the luminescence intensity in *V. harveyi* was investigated. These authors studied the activity of AiiA protein in a short duration of time (2 min) and at the early stationary phase of *V. harveyi* culture (OD = 0.8). Moreover, since the cell lysate of the recombinant *E. coli* strain BL21(DE3)pLysS was used, the target protein was unquantified, and it is difficult to correlate the protein's activity to its concentration.

In this study, the pattern of luminescence intensity in *V. harveyi* wild-type strain and its mutant was followed in the early log phase with one-hour interval since the bioluminescence in *V. harveyi* is most intensively expressed in this period of growth. Besides, since AiiA_{N26.2} protein was used in the purified form and other components in the *E. coli*'s cell lysate were excluded, any observed bioluminescence-inhibiting effect can be inferred solely to the target protein. The level of bioluminescence attenuation in this study was 84-89 % (at a protein concentration of 15 µg ml⁻¹), which was comparable with that from the study of Bai et al. (2008)²¹. However, since the luminescence intensity of *V. harveyi* culture increases as it grows, it is suggested for further application that recombinant AHL-lactonase is added regularly during the whole growth period in order to see a sustained effect.

From the work of Henke and Bassler (2004)⁵, it can be concluded that bioluminescence

and other virulence factors in *V. harveyi* are both regulated by quorum sensing. Since the results of this study showed that AiiA_{N26.2} protein can greatly inhibit the bioluminescence intensity of *V. harveyi* cultures, it might be concluded that this type of protein can block the AHL-dependent quorum sensing channel in *V. harveyi*, and therefore, can also inhibit the virulence factor production which are controlled by the AHL-mediated channel. Recently, the study of Ruwandepika *et al.*, (2011)²² showed that there is a relationship between bioluminescence in *V. harveyi* and its virulence towards gnotobiotic *Artemia*, revealing that the non-luminescent *V. harveyi* strains are less virulent than their luminescent isogenic counterparts.

A functional quorum sensing system, and AHL-mediated channel in particular have been shown to be essential for the virulence of several pathogenic species^{23, 24}. Thus, the results of this study are important for future research on the application of recombinant AHL-lactonase as a measure to control infectious diseases caused by *V. harveyi* as well as other aquatic pathogens which regulate virulence factors via the AHL-mediated quorum sensing system.

ACKNOWLEDGEMENTS

This study was supported by the Vietnamese National Foundation for Science and Technology Development (NAFOSTED), agreement No. 05/2010/HD-KHTN.

REFERENCES

1. Schauder, S., Bassler, B.L. The languages of bacteria. *Genes & Development*, 2001; **15**: 1468-1480.
2. Manefield, M., Harris, L., Rice, S.A., de Nys, R., Kjelleberg, S. Inhibition of luminescence and virulence in the black tiger prawn (*Penaeus monodon*) pathogen *Vibrio harveyi* by intercellular signal antagonists. *Appl. Environ. Microbiol.*, 2000; **66**: 2079-2084.
3. Bassler, B.L., Wright, M., Showalter, R.E., Silverman, M.R. Intercellular signalling in *Vibrio harveyi*: sequence and function of genes regulating expression of luminescence. *Mol. Microbiol.*, 1993; **9**: 773-786.
4. Bassler, B.L., Wright, M., Silverman, M.R. Multiple signalling systems controlling expression of luminescence in *Vibrio harveyi*:

- sequence and function of genes encoding a second sensory pathway. *Mol. Microbiol.*, 1994; **13**: 273-286.
5. Henke, J.M., Bassler, B.L. Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. *J. Bacteriol.*, 2004; **186**: 6902-6914.
 6. Mok, K.C., Wingreen, N., Bassler, B.L. *Vibrio harveyi* quorum sensing: a coincidence detector for two autoinducers controls gene expression. *EMBO J.*, 2003; **22**: 870-881.
 7. Lilley, B.N., Bassler, B.L. Regulation of quorum sensing in *Vibrio harveyi* by LuxO and Sigma-54. *Mol. Microbiol.*, 2000; **36**: 940-954.
 8. Dong, Y.H., Wang, L.-H., Zhang, L.-H. Quorum-quenching microbial infections: mechanisms and implications. *Philosoph. Trans. Royal Soc. B-Biol. Sci.*, 2007; **362**(1483): 1201-1211.
 9. Lin, Y.H., Xu, J.L., Hu, J., Wang, L.H., Ong, S.L., Leadbetter, J.R., Zhang, L.H. Acyl-homoserine lactone acylase from *Ralstonia* strain XJ12B represents a novel and potent class of quorum-quenching enzymes. *Mol. Microbiol.*, 2003; **47**: 849-860.
 10. Draganov, D.I., Teiber, J.F., Speelman, A., Osawa, Y., Sunahara, R., La Du, B.N. Human paraoxonases are lactonases with overlapping and distinct substrate specificities. *J. Lipid Res.*, 2005; **46**: 1239-1247.
 11. Czajkowski, R., Jafra, S. Quenching of acyl-homoserine lactone-dependent quorum sensing by enzymatic disruption of signal molecules. *Acta Biochimica Polonica*, 2009; **56**(1): 1-16.
 12. Wang, L.H., Weng, L.X., Dong, Y.H., Zhang, L.H. Specificity and enzyme kinetics of the quorum-quenching N-Acyl homoserine lactone lactonase (AHL-lactonase). *J. Biol. Chemistry*, 2004; **279**: 13645-13651.
 13. Chu, W., Lu, F., Zhu, W., Kang, C. Isolation and characterization of new potential probiotic bacteria based on quorum-sensing system. *J. Appl. Microbiol.*, 2010; **110**: 202-8.
 14. Kalia, V.C., Raju, S.C., Purohit, H.J. Genomic analysis reveals versatile organisms for quorum quenching enzymes: Acyl-homoserine lactone-acylase and -lactonase. *Open Microbiol. J.*, 2011; **5**: 1-13.
 15. Nusrat, H., Shankar, P., Kushwah, J., Bhushan, A., Joshi, J., Mukherjee, T., Raju, S.C., Purohit, H.J., Kalia, V.C. Diversity and polymorphism in AHL-lactonase gene (*aiiA*) of *Bacillus*. *J. Microbiol. Biotechnol.*, 2011; **21**(10): 1001-1011.
 16. Lee, S.J., Park, S.Y., Lee, J.J., Yum, D.Y., Koo, B.T., Lee, J.K. Genes encoding the N-acyl homoserine lactone-degrading enzyme are widespread in many subspecies of *Bacillus thuringiensis*. *Appl. Environ. Microbiol.*, 2002; **68**: 3919-3924.
 17. Kalia, V.C., Purohit, H.J. Quenching the quorum sensing system: Potential antibacterial drug targets. *Crit. Rev. Microbiol.*, 2011; **37**: 121-140.
 18. Chen, R., Zhou, Z., Cao, Y., Bai, Y., Yao, B. High yield expression of an AHL-lactonase from *Bacillus* sp. B546 in *Pichia pastoris* and its application to reduce *Aeromonas hydrophila* mortality in aquaculture. *Microb. Cell Fact.*, 2010; **9**: 39-48.
 19. Cao, Y., He, S., Zhou, Z., Zhang, M., Mao, W., Zhang, H., Yao, B. Orally administered thermostable N-acyl homoserine lactonase from *Bacillus* sp. strain AI96 attenuates *Aeromonas hydrophila* infection in zebrafish. *Appl. Environ. Microbiol.*, 2012; **78**(6): 1899-1908.
 20. McClean, K.H., Winson, M.K., Fish, L., et al. Quorum sensing and *Chromobacterium violaceum*: exploitation of violacein production and inhibition for the detection of N-acyl homoserine lactones. *Microbiology*, 1997; **143**: 3703-3711.
 21. Bai, F., Han, Y., Chen, J., Zhang, X.H. Disruption of quorum sensing in *Vibrio harveyi* by the AiiA protein of *Bacillus thuringiensis*. *Aquaculture*, 2008; **274**: 36-40.
 22. Ruwandeepika, H.A.D., Defoirdt, T., Bhowmick, P.P., Karunasagar, I., Bossier, P. Expression of virulence genes in luminescent and nonluminescent isogenic vibrios and virulence towards gnotobiotic brine shrimp (*Artemia franciscana*). *J. Appl. Microbiol.*, 2011; **110**(2): 399-406.
 23. Zhang, M., Sun, K., Sun, L. Regulation of autoinducer-2 production and luxS expression in a pathogenic *Edwardsiella tarda* strain. *Microbiology*, 2008; **154**: 2060-9.
 24. Defoirdt, T., Verstraete, W., Bossier, P. Luminescence, virulence and quorum sensing signal production by pathogenic *Vibrio campbellii* and *Vibrio harveyi* isolates. *J. Appl. Microbiol.*, 2008; **104**: 1480-7.