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SHORT COMMUNICATION

In Vitro Characterization of a Recombinant AHL-Lactonase from *Bacillus cereus* Isolated from a Striped Catfish (*Pangasianodon hypophthalmus*) Pond

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Abstract *aiiA* gene encoding AHL-lactonase was isolated from *Bacillus cereus* strain N26.2, originating from a striped catfish (*Pangasianodon hypophthalmus*) pond in Vietnam. This gene, abbreviated as *aiiA*(N26.2), was cloned and expressed in a competent *Escherichia coli* strain BL21(DE3)pLysS. The resulting protein, abbreviated as $AiiA_{N26.2}$, was highly active in the pH range of 6–8 and could retain 80 % of the maximum activity under storage for 5 days at 4 °C or for 3 days at 20 °C. These properties of $AiiA_{N26.2}$ protein confers its future application via feed supplementation, with the purpose of controlling aquaculture pathogens which regulate the virulence via a quorum sensing system.

Keywords AHL-lactonase activity · AHL-mediated quorum sensing · *aiiA* gene · *Bacillus cereus* · Recombinant AHL-lactonase

Introduction

Quorum sensing (QS) is known as a cell-to-cell communication process widely observed among prokaryotes, when bacteria ensure an appropriate coordination by communicating through signal molecules [1–3]. More than 70 species of Gram-negative bacteria have been shown to produce acylated homoserine lactones (AHLs), which play a very important role in gene regulation, including biofilm formation and virulence factors in many pathogenic bacteria [4–8]. Efforts to disrupt the QS-based biofilms and virulence factors have enabled the identification of molecules with ability to quench the QS system [3, 9–11].

Two groups of enzymes, namely acyl-homoserine lactone lactonase (AHL-lactonase) and acyl-homoserine lactone acylase (AHL-acylase), are known to degrade AHL molecules by hydrolyzing the lactone ring and the amide linkage, respectively. Acyl-homoserine lactonase is a metallo-enzyme found in numerous *Bacillus* spp. and other species [9, 12–14]. Genetically-modified *Erwinia carotovora* and *Pseudomonas aeruginosa* expressing AHL-lactonase showed decreased production of virulence factors and attenuated virulence [15, 16]. AHL-lactonase has been over-expressed successfully in *E. coli* and *Pichia pastoris* expression system as a soluble heterologous protein [17, 18]. The purified recombinant AHL-lactonase originating from *Bacillus* sp. strain B546 showed optimal activity at pH 8.0 and 20 °C, exhibited excellent stability at pH 8.0–12.0, and thermal stability at 70 °C [18]. Recombinant AHL-lactonases, which allowed the blocking of the bacterial QS by hydrolyzing the AHL molecules, can be used as potential drugs to treat QS-regulated diseases or as supplement to antibiotic based treatments.

In this study, the activity of a recombinant AHL-lactonase ($AiiA_{N26.2}$ protein) was evaluated at different temperature ranges, based on which practical application via feed supplementation is recommended.

Materials and Methods

Recombinant AHL-Lactonase ($AiiA_{N26.2}$ Protein)

aiiA gene was cloned from a *B. cereus* N26.2 strain which was isolated from striped catfish (*P. hypophthalmus*) pond water in Dong Thap Province, Vietnam. The resulting

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recombinant AHL-lactonase ($AiiA_{N26.2}$ protein) was over-expressed in the *E. coli* BL21(DE3)pLysS competent cells.

Effect of pH on the AHL-Lactonase Activity of $AiiA_{N26.2}$ Protein

The reaction tubes containing $AiiA_{N26.2}$ protein (5 μg ml^{-1}) in 0.1 M phosphate buffer were treated at different pH values ranging from 4.0 to 8.0 at 30 °C. *N*-hexanoyl homoserine lactone (HHL) was added to the reaction tubes at 5 μg ml^{-1} and the mixtures were further incubated with shaking at 30 °C for 60 min. Then the reaction was stopped by heat treatment at 90 °C for 5 min. The AHL-lactonase activity of $AiiA_{N26.2}$ protein after pH treatment was determined in a bioassay under standard conditions.

Effect of Temperature on the AHL-Lactonase Activity of $AiiA_{N26.2}$ Protein

The reaction tubes containing $AiiA_{N26.2}$ protein (5 μg ml^{-1}) in 0.1 M phosphate buffer (pH 7.0–7.5) were pre-incubated at different temperatures ranging from 4 °C to 90 °C for different time intervals. Then, HHL was added to the reaction tubes at 5 μg ml^{-1} and the mixtures were further incubated with shaking at 30 °C for 60 min. The reaction was stopped by heat treatment at 90 °C for 5 min. The AHL-lactonase activity of $AiiA_{N26.2}$ protein after temperature treatment was determined in a bioassay under standard conditions.

AHL-Lactonase Activity Bioassay

The AHL-lactonase activity of $AiiA_{N26.2}$ protein was quantified by bioassay analysis using *Chromobacterium violaceum* CV026 strain as an AHL-reporter. Briefly, 10 μl of the supernatant from each reaction tube was dropped on an LB agar plate, which was previously spread plated with 50 μl of CV026 culture. This was done in triplicate for each treatment. The LB plates were incubated at 30 °C for 24 h. Subsequently, the diameter of purple violacein zones appearing on the plates was measured. The residual HHL concentration can be extrapolated based on a standard curve relating the HHL concentration with the diameter of the violacein zone induced by CV026 culture. One unit (U) of AHL-lactonase activity was defined as the amount (in milligram) of $AiiA_{N26.2}$ that hydrolyzed 1 mM of HHL molecule per minute under the assay conditions.

Results

$AiiA_{N26.2}$ protein had the optimum pH of 6–8, where it retained more than 80 % of the maximum activity. Its activity reduced significantly when pH dropped below 6, and very little activity remained at pH 4 (Fig. 1). HHL molecule was chemically degraded at pH above 8, as indicated by the disappearance of violacein halo on the control plates (data not shown). Therefore, the AHL-lactonase activity was not investigated at alkaline pH values.

$AiiA_{N26.2}$ protein could maintain its AHL-lactonase activity well at temperatures below room temperature. It retained more than 80 % of the maximum activity for up to 5 days at 4 °C and for up to 3 days at 20 °C. Whereas, at 30 °C, the activity dropped below 80 % of the maximum activity after 24 h of treatment (Fig. 2). The protein was unstable at high temperatures. When treated with temperature ranging from 70 °C to 90 °C, its relative activity decreased sharply within 5 min of treatment (Fig. 3).

Discussion

The aim of this study is to characterize the activity of a recombinant AHL-lactonase ($AiiA_{N26.2}$ protein), which originated from a *Bacillus* sp. isolated from aquaculture environment, under different pH and temperature ranges. The experiments investigating the AHL-lactonase activity of $AiiA_{N26.2}$ protein showed that its optimum pH was in the range of 6–8. Its activity declined markedly at pH values below 6 and was completely lost at pH below 4. It was also shown that $AiiA_{N26.2}$ protein was relatively stable at the temperature ranges below room temperature. $AiiA_{N26.2}$ protein can be stored at 4 °C for up to 5 days or at 20 °C for up to 3 days, maintaining its activity at least 80 % of the maximal level. Our results are consistent with those reported by other authors [12, 18, 19]. These properties of $AiiA_{N26.2}$ protein can facilitate its future oral administration via supplementation into fish/shrimp feed ingredients for efficient control of QS-regulated aquaculture pathogens.

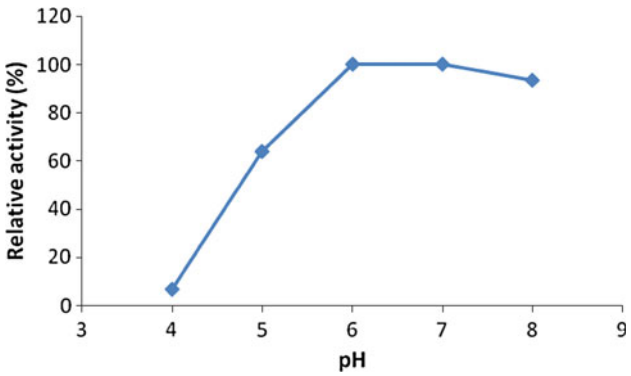

Fig. 1 Effect of pH on the AHL-lactonase activity of $AiiA_{N26.2}$ protein. Values represent the mean activity ($n = 3$) relative to the untreated control samples

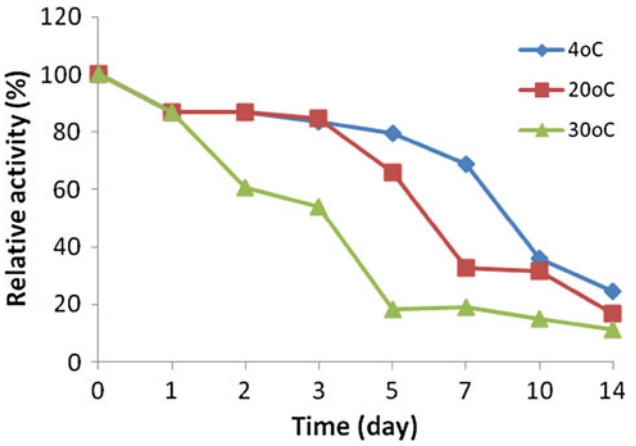

Fig. 2 Effect of room temperature (30 °C) and low temperatures (4 and 20 °C) on the AHL-lactonase activity of $AiiA_{N26.2}$ protein. $AiiA_{N26.2}$ protein was pre-incubated in phosphate buffer (pH 7.0–7.5) at different temperatures, and aliquots were removed at different time points for the measurement of residual activity at 30 °C. Values represent the mean activity ($n = 3$) relative to the untreated control samples

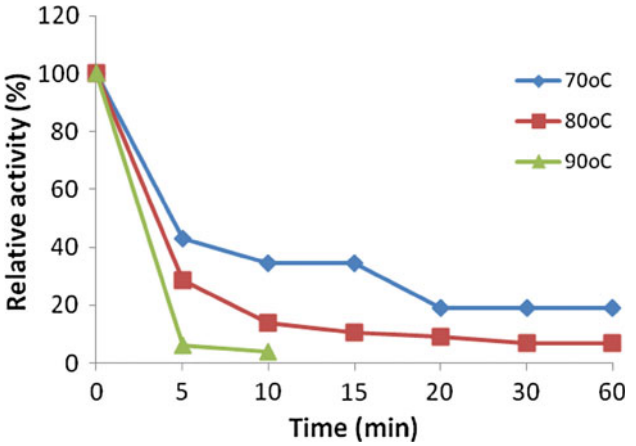

Fig. 3 Effect of high temperatures (70, 80 and 90 °C) on the AHL-lactonase activity of $AiiA_{N26.2}$ protein. $AiiA_{N26.2}$ protein was pre-incubated in phosphate buffer (pH 7.0–7.5) at different temperatures, and aliquots were removed at different time points for the measurement of residual activity at 30 °C. Values represent the mean activity ($n = 3$) relative to the untreated control samples

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