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ORIGINAL ARTICLE

Quorum sensing system and influence on food spoilage in *Pseudomonas fluorescens* from turbot

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Abstract The spoilage of aquatic products is mainly caused by the bacterial growth, and the specific spoilage organism (SSO) plays an important role. Quorum sensing (QS) is a microbial cell–cell communication system which is coordinated with the population density, and is controlled by *N*-acyl-homoserine lactone (AHLs) as the Gram-negative bacteria communication signals. In this study, the SSO was *Pseudomonas fluorescens* (PF-04), isolated from the turbot (*Scophthalmus maximus L.*) during aerobically refrigerated storage. The supernatant extract of PF-04 tested the AHLs activities utilizing biosensor *Chromobacterium violaceum* CV026. AHL production was influenced by the environment temperature, and AHL production reduced obviously at 10 °C compare with 25 °C. In Luria-Bertani (LB) supplemented with 0.5–1.0% NaCl, AHL production reached the maximum. The AHL production was also regulated by pH of culture medium, acidic condition was conducive to persistent existence of the AHL molecules, but the alkaline environment would cause chemically unstable of AHL molecules. QS system in *P. flurosecens* played an imperative role in biofilm formation, protease and siderophore production. AHLs could regulate above three factors in PF-04. In summary, this study showed that (1) the influence of different environmental conditions (temperature, NaCl and pH) on AHL production revealed the correlation of QS in foods and (2) that proved the effect of external AHLs to regulate the biofilm formation, protease and siderophore production in PF-04.

Keywords *Pseudomonas fluorescens* · Quorum sensing · Biofilm formation · Protease activity · Siderophore

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Introduction

Food is an impressionable commodity bound to decompose timely (Gram et al. 2002). Usually, apart from the concerned factors (e.g., physicochemical alterations, variety of food, environment, etc.), microbial growth is critical for food spoilage. At the onset of spoilage, the product contains some bacteria that are involved in spoilage, the so-called specific spoilage organism, which produce a variety of extracellular enzymes (proteases, lipases, etc.) and other metabolites role in the food matrix. The SSOs are not only involved in the food spoilage, but also are the organisms responsible for the food spoilage to occur (Loureiro 2000; Ragaert et al. 2007). *Pseudomonas* is a sort of Gram negative bacterium, a psychrotroph bacterium which causes the spoilage of high-protein food, and it plays a key role in aquatic products spoilage (Lund et al. 2000). Various Gram negative bacteria have been reported to result in the decay

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of foodstuffs, and *N*-acylhomoserine lactones (AHLs) have been found in decomposed food (Bruhn et al. 2004; Pinto et al. 2007).

Quorum sensing (QS), intercellular communication system in bacteria, was used as a tool to monitor microbial population density and to control their physiological process (Ferkinghoff-Borg and Sams 2014). The supervision of specific behaviors depends on the production of the signaling molecule (Autoinducer, AI). In the present research, some kinds of AI have been reported. Gram negative bacteria usually utilize AHLs as AI-1 whereas the oligopeptide is the most common AI-2 in Gram positive bacteria (Chen et al. 2012; Kalia 2013; Papenfort and Bassler 2016), and the AI-3/epinephrine/norepinephrine and diketopiperazines (DKPs) signaling system. Various biological habits and physiological processes are controlled by QS system, including biofilm formation, bioluminescence, production of antibiotics and expression of virulence factors (Kim et al. 2008; Reading and Sperandio 2006; Wang et al. 2013).

The autoinducers-1(AHLs) system was the first time to be discovered in the bioluminescence in *Vibrio fischeri* (Nealson et al. 1970). AHL signal molecule has a conserved homoserine lactone ring whereas the acyl side chain length varies from four to eighteen carbons and the substituent pattern (carbonyl group, hydroxyl or fully reduced) at the third position in the acyl side chain. AHL system is regulated by two proteins, LuxI, which is an AHL synthase, LuxR, which is the AHL receptor protein (Li Ying Tan and Chan 2012). AHL is a small molecular mass which can penetrate the cytomembrane and accumulate in the environment. When the density of signal molecules reaches a critical threshold, it combines with the LuxR-Type proteins and regulates the transcription genes in a variety of phenotypes (Reading and Sperandio 2006). These actions include anti-resistance, virulence factors in *Pseudomonas aeruginosa*, and swarming migration in *Rhizobium*, *Bacillus subtilis* and *Serratia liquefaciens* (Daniels et al. 2004; Schuster and Greenberg 2006; Høiby et al. 2010).

Pseudomonas is an important microbial species that can cause decomposition of high-protein foodstuffs, which forms biofilm, synthesize siderophore and protease (Bahari et al. 2017; Chen et al. 2017). The formation of biofilm increases a risk for food contamination with spoilage. The prominent characteristic is metallic sheen and offensive odours in food spoilage process. Some proteinaceous foods contain a small number of carbohydrates, but the free-amino-acid is very rich. These characteristics show that *Pseudomonas* will secrete a large number of proteolytic enzymes in these products, and hydrolysis protein can cause losses of original flavor and odor of products. At the same time, the growth of other microorganisms will be restrained by the iron deficiency. Because *Pseudomonas* spp. can produce a large number of siderophores, which will chelate the iron in the environment, and thus the iron of environment are deficient. Finally, *Pseudomonas* spp. can cause food spoilage as specific spoilage organism (Gram et al. 2002). In this study, the main purpose is to study the AHL production by PF-04 and acquire evidence for AHL-based QS system regulating biofilm formation, protease activity and siderophore production.

Materials and methods

Material and bacterial strains

Live turbot were purchased from Jinzhou-linxi Aquatic Market (Jinzhou, China) and transported to laboratory. *N*-Butyryl-DL-homoserine lactone (C_4-HSL), *N*-Hexanoyl-DL-homoserine lactone (C_6-HSL), *N*-Decanoyl-DL-homoserine lactone (C_{10}-HSL), Kanamycin were purchased from Sigma-Aldrich (Munich, Germany). Other reagents were analytical grade and purchased from Shanghai Chemical Reagent Co., Ltd (Shanghai, China) and Nanjing Chemical Reagent Co., Ltd (Nanjing, China). Clove oil (70%) and aspirin (acetylsalicylic acid, 99%) were purchased from Aladdin Industrial Corporation (Shanghai, China). Aspirin was dissolved by DMSO.

Chromobacterium violaceum CVO26 (a mini-Tn5 mutant of the wild strain *C. violaceum* ATCC 31532, kanamycin resistance (20 μg/mL), unable to synthesize AHLs, produce purple to respond exogenous AHLs) was kind gifts from Dr. Zhu (Zhejiang Gongshang University, Hangzhou, China). The test bacteria *P. fluorescens* PF-04 and *Hafnia alvei* were preserved at our laboratory.

Extraction and detection of AHLs

The test bacterial cultures were centrifuged (10,000 r/min, 10 min, 4 °C) to acquire supernatant. The supernatant was extracted twice in equal volume of acidified ethyl acetate (0.1% acetic acid) and evaporated in vacuum at 35 °C. The AHLs were dissolved by appropriate methanol and stored at − 20 °C for the following experiment (Vattem et al. 2007).

Chromobacterium violaceum CVO26 and *P. fluorescens* PF-04 were grown in a rotary shaker at 160 r/min at 28°C using Luria-Bertani (LB) medium (1% peptone, 0.5% yeast extract, 1% NaCl, pH 7.0 ± 0.2), and supplemented with seemly antibiotics (*C. violaceum* CV026 kanamycin 20 μg/mL). The CV026 culture medium and LB ager medium were mixed at 40–50 °C and then poured into glass garden that the central placed an oxford cup (Φ 8 mm). After the ager was solidified, AHLs extract or methanol (200 μL) was added to the hole. The medium was cultured at 28 °C

for 48 h. The diameter of purple was measured by vernier caliper (0.02 mm).

Temperature factors

Overnight culture (100 μL) of *P. fluorescens* PF-04 (adjusted to 1.0 at 595 nm) was added into LB broth (100 mL) and incubated in the rotary shaker at different temperature (10 and 25 °C). Sampling time interval was 4–6 h at 25 °C and 4 days at 10 °C. The sample was used to test AHLs activity, OD_{595nm} and pH. The test was implemented in triplicate.

NaCl factors

Overnight culture (100 μL) of *P. fluorescens* PF-04 (adjusted to 1.0 at OD_{595nm}) was inoculated into LB medium (peptone 10 g/L, yeast extract 5 g/L, NaCl, pH 7.0 ± 0.2) that NaCl concentration was 2.5, 5, 10, 15, 20, 25, 30 g/L respectively and incubated at 25 °C for 48 h. Culture was extracted in ethyl acetate by QS analysis using a rotary evaporators. The test was implemented in triplicate.

pH factors

Hafnia alvei was isolated from turbot that could produce a large number of signaling molecules. It grew in LB medium at 28 °C for 48 h, and then culture was centrifuged (10,000 r/min, 10 min, 4 °C) in order to acquire supernatant. Ten milliliter supernatant that containing kanamycin (50 μg/mL) was used to inhibit the growth of microorganism, which was sub-packaged in the seven centrifuge tube. The pH of supernatant was adjusted to 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 without shaking at 28 °C. The supernatant was assayed for AHLs activity every 4 h. The test was implemented in triplicate.

Preparation of sterile fish muscle juice

Sterile fish muscle juice (SFJ). Five hundred gram fish flesh of newly killed turbot was mixed with 500 mL tap water, and the mixture was boiled for 5 min. Boiled mixture was placed for 10 min at room temperature and filtered with gauze. 0.1 mol/L phosphate buffer was added and pH of the leachate was adjusted to 6.60. The filtrate was heated to 105 °C for 15 min and stored at room temperature. 40 mg L-cysteine, 40 mg L-methionine and 1600 mg trimethylamine oxide were added per litre of fish juice in order to compensate attenuation and heat decomposition.

Biofilm formation assay

The biofilm formation was experimented by an alteration of the method reported by (Chong et al. 2016). An overnight culture of *P. fluorescens* PF-04 was diluted (1:100) in 0.5 mL LB medium containing 0, 40, 80, 120, 160, 200 μg/mL C_4-HSL in a germfree centrifuge tube (1.5 mL) and further grown for 24 h without shaking at 28 °C. Planktonic bacteria were removed, and the tubes were softly rinsed thrice by deionized water and adherent cells were stained with 1 mL of 0.1% (w/v) crystal violet for 15 min. The stained biofilms were washed thoroughly with deionized water. Retained crystal violet was solubilized in 1 mL glacial acetic acid (33%, v/v), 200 μL of above solution was transferred to a microplate and determined at 595 nm. The test was implemented in triplicate.

Detection for extracellular protease activity

Production of extracellular protease in *P. fluorescens* PF-04 was detected on skim milk agar plates (15% (w/v) skim milk powder, 1.5% (w/v) agar). The central part of plates was punched two holes (Φ 8 mm). The assay sample including LB, LB + AHLs extracts (1:100 v/v), LB + C_6-HSL (10 mg/mL), LB + Aspirin (3 mg/mL) and LB + Clove oil (8 μL/mL) was added in the hole and incubated at 28 °C for 24 h. The test was implemented in triplicate.

Assay for siderophore

Siderophore production by *P. fluorescens* PF-04 was detected on Chrome azurol S (CAS) plate (Shin et al. 2001). The plate was punched with 8-mm-diameter holes. Each hole was filled with 100 μL of the sample solution, the culture of strain was inoculated into LB, SFJ + AHLs extracts (1:100 v/v), SFJ + C_6-HSL (10 mg/mL), SFJ + Aspirin (3 mg/mL) and SFJ + Clove oil (8 μL/mL). The plate was incubated at 28 °C for 4–8 h. The test was implemented in triplicate.

Results and discussion

Effect of temperature on the production of AHLs

The production of AHLs derived from *P. fluorescens* PF-04 was analyzed based on the bioassay method in the agar plate, which could be induced to produce violacein of *C.violaceum*. The output of violacein was based on the AHLs activity of *P. fluorescens* PF-04. Figure 1 showed that *P. fluorescens* PF-04 could secrete AHLs at 10 and 25 °C, and 25 °C was beneficial to produce signaling molecules. Before the bacterial-density reached stationary

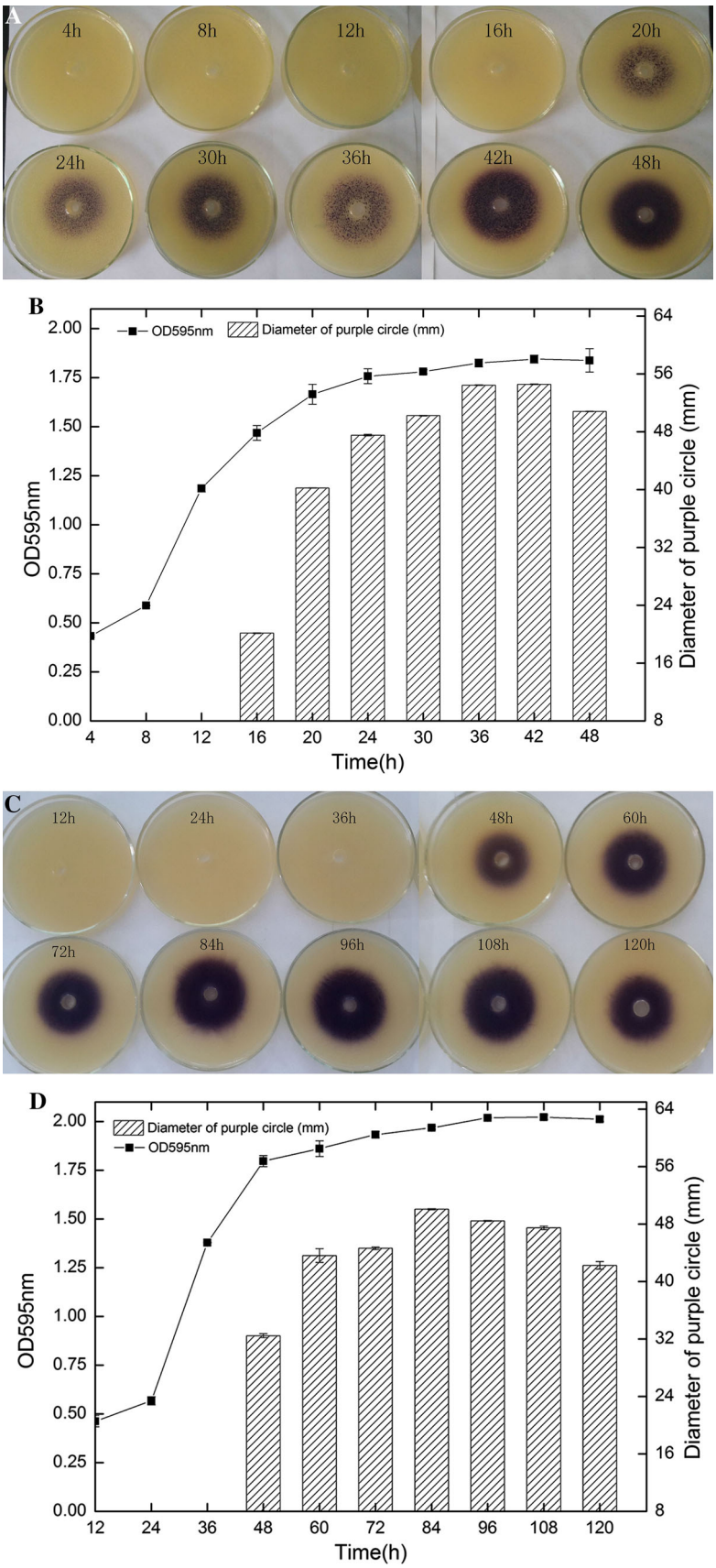

Fig. 1 N-acyl homoserine lactone induction of *P. fluorescens* PF-04 incubated at **a** 25 °C and **c** 10 °C, determined using *C. violaceum* 026 biosensor strain. OD_{595nm} and induction diameter in mm of *P. fluorescens* PF-04 incubated at **b** 25 °C and **d** 10 °C

phase, the amount of AHLs increased with the accretion of bacterial density. As shown in Fig. 1a, b, the AHLs activity was detected from exponential growth phase (16 h) and reached the maximum activity at mid-stationary phase (36 h), then decreased with cell growth. The variation trend of purple circle diameter and bacterial-density (OD_{595nm}) showed the consistency ($p < 0.05$). When *P. fluorescens* PF-04 was incubated at 10 °C, the lag-phase of bacteria became longer and reached 24 h (Fig. 1d), the reason might be that the low temperature could retard the biochemical reaction rate and growth rate of cell, especially the enzyme activity and mobility of the bacteria. The AHLs activity reached the maximum at mid-stationary phase (84 h). As could be shown on Fig. 1, the AHLs activity decreased at death phase in 10 and 25 °C, which was caused by internal bunching consumption of *P. fluorescens* PF-04 or the degradation of AHLs due to increase of pH of the culture medium (Fig. 3a). Temperature play an important factor in AHL production by *P. fluorescens*, Hypothermia could reduce the activity of LuxI-protease or inhibit the bacterial growth, which lead to the decrease of AHLs production.

Effect of NaCl concentration on the production of AHLs

The AHL production by *P. fluorescens* PF-04 at different NaCl concentrations was shown in Fig. 2a. The high concentration of NaCl was associated with a lesser bacterial population density, which induced the production of AHL. Notably, there was a little AHL production secreted in response to the accretion of 3.0% NaCl. The maximal level of AHLs was detected in response to 0.5% NaCl treatment. In this study, The optimal NaCl concentration for secrete of autoinducer-1 was 0.5–1.0%. With the increasing of NaCl concentration, the production of AHL decreased.

Effect of pH on the production of AHLs

The production of AHL by *P. fluorescens* PF-04 in death phase began to reduce when microbial strain was incubated at 10 and 25 °C (Fig. 1). As showed in Fig. 3a, the pH of bacteria cultured at 10 and 25 °C increased with the bacterial growth, pH reached weakly-alkaline values (pH $\geq$ 7.5) after incubation 72 and 30 h respectively, and pH was 7.89 and 8.21 at death phase, respectively. These findings indicated that the molecular structure or synthesis of AHLs was regulated by the pH media. The acidic condition is conducive to the persistent existence of AHL molecules, but the alkaline environment would cause chemically unstable of AHL molecules.

Figure 3b verified the chemical stability of signal molecules related with pH. *H. alvei* could release large amounts of signaling molecules, so AHLs activity was detected by utilizing the supernatant of culture medium, which was adjusted with different pH. The result showed that the AHLs activity decreased faster when pH of the supernatant was higher and standing time was longer. When treatment time was 8 h and the pH were 8.0, 8.5 and 9.0, the AHLs activity of *H. alvei* were decreased by 81.96, 42.80 and 35.39%, respectively. Notably, there was no AHLs activity detected at pH 8.5 and 9 after 12 h of incubation. The pH value affects the chemical stability of AHL molecules, strong alkaline solution can inactivate AHL molecules by opening the lactonic ring of AHLs.

Biofilm Formation of *Pseudomonas fluorescens* strain PF-04

To investigate whether AHLs can modulate biofilm formation, we tested *P. fluorescens* strains PF-04 in the presence of various concentrations of exogenous AHLs. As seen in Fig. 4, biofilm production increased by adding C_4-HSL, C_6-HSL and C_{10}-HSL to young culture media. Biofilm formation reached the maximum with the presence of C_6-HSL. C_4-C_{10} increased the biofilm production of *P. fluorescens* PF-04 ($p < 0.05$) significantly. This result agreed well with the previous study, which could have better correlation between QS system and biofilm formation. sensing system can regulate biofilm formation of *P. fluorescens*.

Protease activity of *Pseudomonas fluorescens* strain PF-04

Figure 5 aimed to analyze the correlation between the exogenous signaling molecules or quorum sensing inhibition (QSI) and proteolytic activity. Protease hydrolysis analyses revealed that AHLs extracts and C_6-HSL could promote the proteolytic activity significantly ($p < 0.05$), and the QSI reduced the proteolytic activity significantly ($p < 0.001$) in *P. fluorescens* PF-04. The minimal inhibitory concentration (MIC) values of clove oil and aspirin against *P. fluorescens* PF-04 were 8 μL/mL and 3 mg/mL, respectively (data not shown). When the concentrations of clove oil and aspirin were 1/2MIC (4 μL/mL) and 1MIC (3 mg/mL), rate of proteolytic activity inhibition of *P. fluorescens* PF-04 were 12.22 and 11.57%, respectively. At the concentration of 8 μL/mL, clove oil showed a maximum of 20.92% reduction on proteolytic activity of *P. fluorescens* PF-04 (Fig. 5). Quorum sensing system can regulate protease activity of *P. fluorescens.*

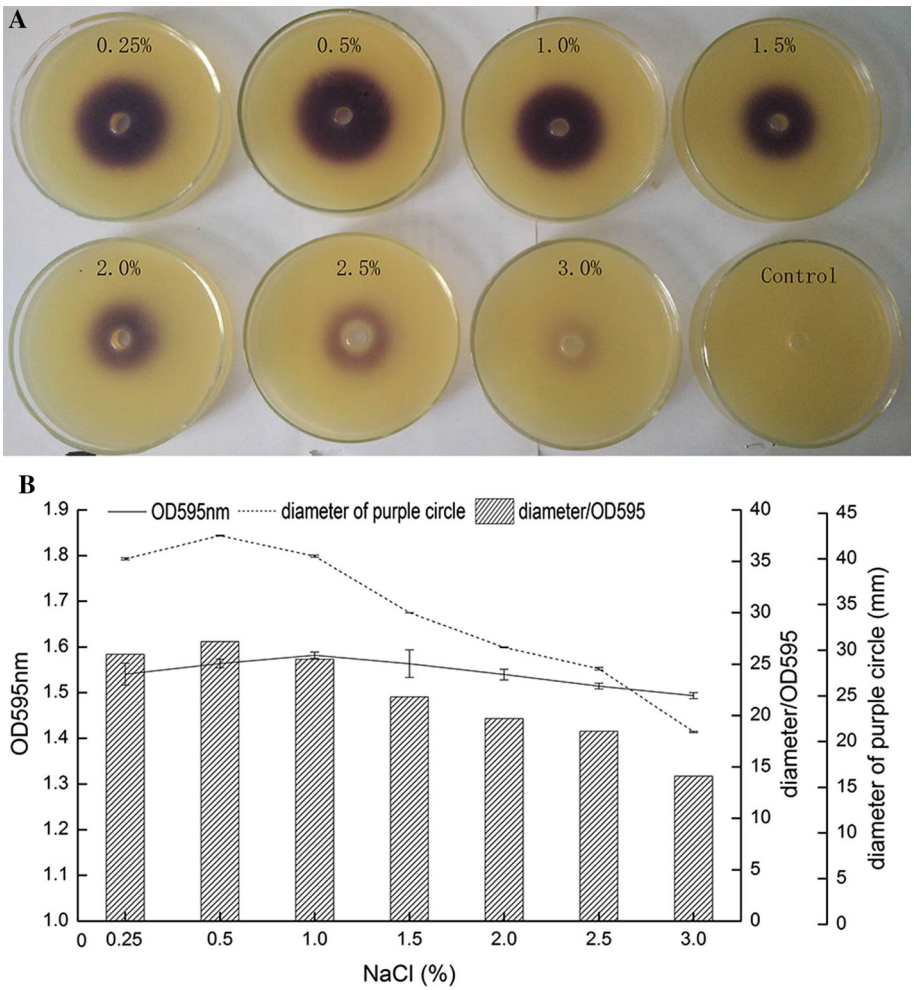

Fig. 2 **a** *N*-acyl homoserine lactone induction of *P. fluorescens* PF-04 incubated in different concentration of NaCl, determined using *C. violaceum* 026 biosensor strain. **b** OD_{595nm}, diameter/OD_{595nm} and induction diameter in mm of *P. fluorescens* PF-04 incubated at 25 °C. (diameter/OD_{595nm}: influence coefficient: the greater the influence coefficient, the greater the influence of the concentration of NaCl.)

Siderophore synthesis of *Pseudomonas fluorescens* strain PF-04

Siderophore synthesis was tested in sterile fish muscle juice (SFJ), which could be controlled by exogenous AHLs. Siderophore can chelate the iron ion in Chrome azurol S (CAS) plate, and there will be an orange halo formation around the hole. As shown in Fig. 6, Chrome azurol S (CAS) agar diffusion assay revealed that AHL extracts and C_6-HSL could promote the siderophore synthesis significantly ($p < 0.05$). When additive was quorum sensing inhibition, the siderophore synthesis was down-regulated by the addition of aspirin and clove oil significantly ($p < 0.001$). When the concentrations of aspirin was 3 mg/mL, rate of siderophore synthesis inhibition of *P. fluorescens* PF-04 was 20.96%. At the concentration of 8 μL/mL, clove oil showed a maximum of 19.16% reduction on siderophore synthesis of *P. fluorescens* PF-04 (Fig. 6). To evaluate whether QS could influence siderophore synthesis of *P. fluorescens* PF-04, we examined content of AHLs in SFJ, and the result showed that the content of AHLs in SFJ + QSI was significantly lower than in control sample (data not shown). Quorum sensing system can regulate siderophore synthesis of *P. fluorescens*.

Prokaryotic microorganisms have evolved complicated information communication system to convey information, and then to accommodate environmental conditions containing changes in temperature, NaCl (osmotic pressure), pH and growth substrate. Refrigerated storage was an effective method to prevent the deterioration of the quality on aquatic products. We detected the production of AHLs by PF-04 during incubation at 10 and 25 °C. The production of AHLs reduced at 10 °C when compared to incubation at 25 °C (Fig. 1b, d). As seen in Fig. 3a, we knew that pH of culture increased during the growth period of PF-04, and this was might be caused by secrete of ammonia from degradation of polypeptide as energy sources. Moreover, pH of culture at 10 °C was higher than 25 °C during the whole incubation process, and alkaline environment would cause chemically unstable of AHL molecules, which might be a reason to cause reduce of AHLs. Some studies also discovered that the content of C_6-HSL

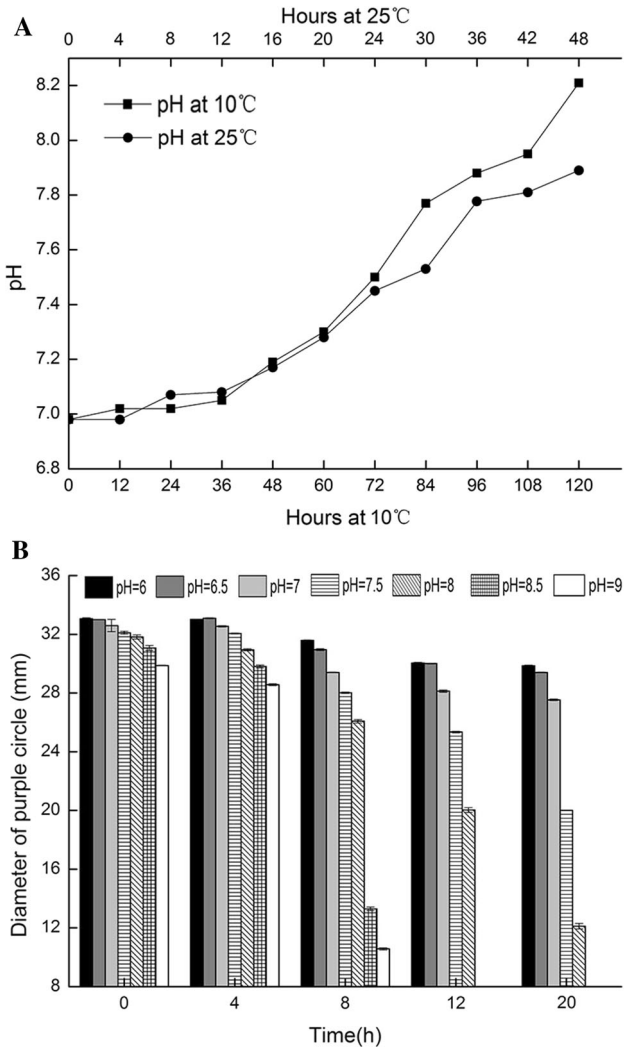

Fig. 3 **a** Changes in pH of *P. fluorescens* culture incubated at 10 °C (down x axis) and 25 °C (up x axis). **b** Effect of standing time of induction diameter in mm of different pH values

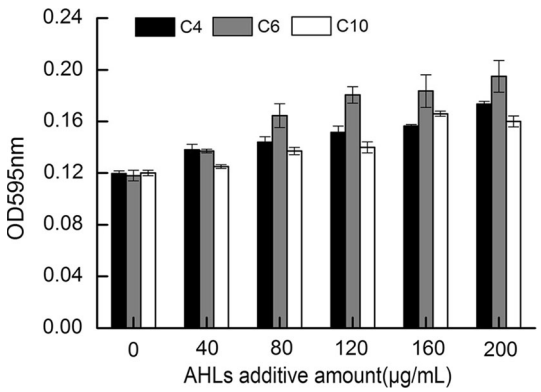

Fig. 4 Biofilm formation expressed as OD595 nm of *P. fluorescens* PF-04 growth in presence of 0, 40, 80, 120, 160, 200 μg/mL exogenous AHLs

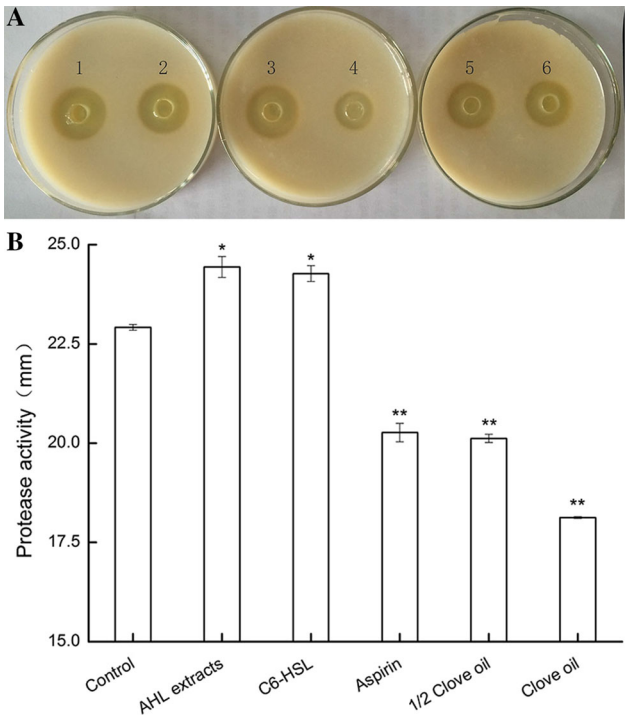

Fig. 5 Protease production of *P. fluorescens* PF-04 incubated 24 h in presence of different additive. The results were the mean (n = 3) (standard deviation). Additive used: AHLs extract (1), control (2), C_6-HSL (3), 1/2 MIC of clove oil (4), clove oil (5), aspirin (6). (*represented $p < 0.05$,**represented $p < 0.01$)

increased and then decreased when the pH varied from 5 to 9, and it was not detected in culture medium incubated at pH 9 (Wang et al. 2012). (Yates et al. 2002) explored the hydrolysis of the AHLs by a chemical approach utilizing ^{13}C nuclear magnetic resonance to research the pH influence on the ring opening and closing, the ring of N-Butyrylhomoserine remained mostly complete until pH 5 to 6 and was entirely opened at pH 8. Therefore, the pH of growth culture medium must be regulated to acidity to quantify AHL production.

Several studies had shown that food ingredient and environment condition like pH or temperature could affect the QS system. Under low temperature conditions the AHLs reduced gradually (Medinamartínez et al. 2007) and the AHLs stability decreased as the temperature rose from 22 to 37 °C (Yates et al. 2002). (Medinamartínez et al. 2007) also showed that eight *Aeromonas* strains could produce C_4-HSL at 12 °C after 96 h of incubation. (Blana and Nychas 2014) reported that the AHL signals expressed as induction diameter of *A. tumefaciens* A136 began to increase after 224, 110 and 60 h of minced beef stored aerobically at 5, 10 and 15 °C, respectively, whereas AHLs were detected after 650 h at 0 °C, which testified that the low-temperature could inhibit the synthesis of AHLs.

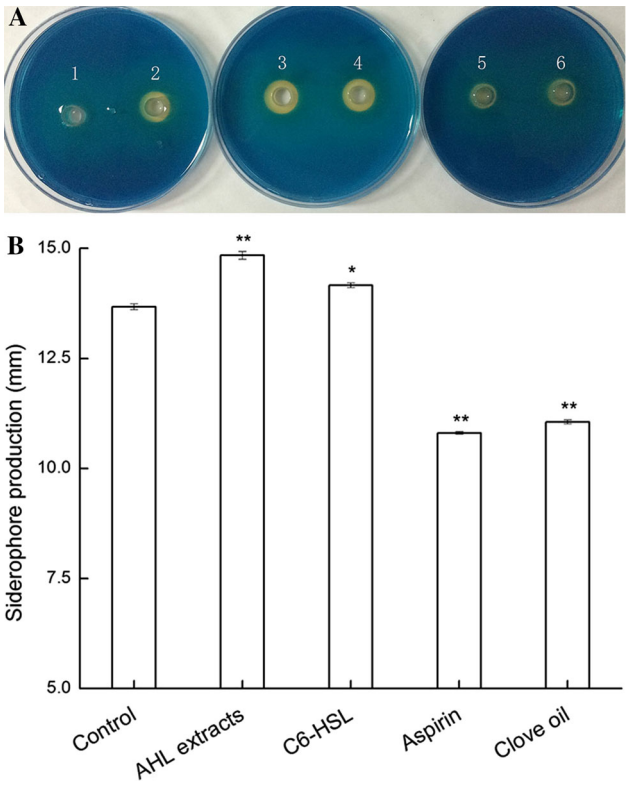

Fig. 6 Siderophore synthesis of *P. fluorescens* PF-04 incubated 4–8 h in presence of different additive. The results were the mean (n = 3) (standard deviation). Additive used: LB (1), control (2), AHLs extract (3), C_6-HSL (4), clove oil (5), aspirin (6). (*represented $p < 0.05$,**represented $p < 0.01$)

Several studies had showed that the concentration of NaCl could affect the QS system. (Yarwood and Schlievert 2003) reported that the release of AHLs was inhibited under the low or high salt stress. Results from the recent studies confirmed the production of three signal molecules by *pseudomonas* sp. from 0.2 to 2.5%, similar results were also reported by (Wang et al. 2012), who reported the production of AHL increased in the initial period and then decreased. The optimal NaCl concentration for secrete of autoinducer-1 was 0.5–1.0%.

One of the most common QS regulated phenotypes is biofilm development which often initiates with microbial cells adhesion, followed by biofilm formation, and finally maturation of biofilm structure. The ability of biofilm formation is often linked to decayed traits of food and pathogenic characteristics of chronic infections (Solano et al. 2014). Quorum sensing system is useful in a spoilage process of food by obtaining a competitive-advantage for the specific spoilage organisms.

Biofilms always exist in a variety of surfaces and different ecological niches, and are the common microbial lifestyle in different environments. Biofilms not only can cause serious infections in medical aspects, but also can increase the risk of food spoilage or pathogenic microflora(Wang et al. 2016). QS signaling molecule has been extensively studied in the biofilm formation of *P. aeruginosa*. (Davies et al. 1998) provided the first evidence to verify the relationship between biofilm formation and *P. aeruginosa* QS system, which showed that the LasI/R system was prerequisite for the biofilm differentiation process. QS system could induce massive release of extracellular DNA which played an important role in providing structural stability of biofilm, the result was showed by Allesenholm et al. 2006. Zhu et al. 2016 reported that the DKPs (cyclo-($_L$-Pro-$_L$-Leu)) could accelerate the production of biofilm significantly. Meanwhile, it also catalyzed the synthesis of trimethylamine oxide (TMA) and putrescine during fish spoilage process.

Many studies had reported that *Pseudomonas* spp. were the dominate spoilage species of proteinaceous fresh foods stored aerobically under low temperature. Aquatic products like turbot contain high concentrations of soluble protein and low-molecular-weight compounds (LMWC), bacterial early growth consumed LMWC until it was depleted, and then protein was degraded by extracellular proteolytic enzymes which caused the certain spoilage organismal growth and accelerated food spoilage (Nychas et al. 1998). *Pseudomonas* spp. as the SSO in aerobically cold-stored foods could produce extracellular proteolytic enzymes. Several strains of *Pseudomonas* spp. employed quorum sensing to regulate gene expression containing the production of elastase, alkaline protease and metalloprotease (Juhas et al. 2005).

In this study, QS system was verified to can regulate the proteolytic enzyme activity of *P. fluorescens* PF-04. Many researches had reported that many bacteria of producing the extracellular proteases were regulated by QS system (Christensen et al. 2003; Juhas et al. 2005). (Christensen et al. 2003) reported that *Serratia proteamaculans* B5a of producing extracellular proteolytic enzymes was regulated by an AHL-based QS system, and it confirmed that quorum sensing was involved in the milk spoilage. (Liu et al. 2006) suggested that extracellular proteolytic enzymes as an important spoilage characteristic of *P. fluorescens* was regulated at least partially by an AHL-based QS system. In another study, Zhu and his colleagues verified that the effects of various types of AHLs on extracellular proteases were different. C14-HSL, O-C6-HSL, O-C8-HSL and cyclo-(L-Pro-L-Leu) increased the proteolytic activity of Shewanella baltica 02 isolated from Litopenaeus vannamei. However, C6-HSL, C8-HSL, C10-HSL reduced the proteolytic activity of Shewanella baltica 02 (Zhu et al. 2015).

The iron is required for growth of all facultative anaerobic and aerobic bacteria. Under iron-limiting conditions, most bacteria have synthesized siderophore, an low-molecular-weight Fe(III)-specific sequestering

compounds. Siderophore is synthesized by pathogenic and nonpathogenic bacteria, and nonpathogenic bacteria may play an important role in quality of food products (Gram 1993). *Pseudomonas* spp. is an aerobic bacterium that needs iron for growth. Particularly, the siderophore and protease of *Pseudomonas* spp. increases in iron-deficient environment. Other bacteria will not grow in the iron-deficient environment and thus *Pseudomonas* spp. will become dominant spoilage bacterium in food spoilage process (Ams et al. 2002; Brown and Luke 2010).

The siderophore synthesis was a way of the existence and competition of microorganism, which was first proved by (Kloepper et al. 1980). From earlier studies, culture medium components would affect the synthesis of siderophore. For example, (Gram 1996) reported that *Shewanella putrefaciens* could synthesize siderophore only grown in fish muscle juice, and could not synthesize siderophore in basal culture medium. In this study, the siderophore was not detected in LB medium, but a large number of siderophore were detected in SFJ (Fig. 6). Some research also reported that QS system could regulate siderophore production in *Pectobacterium* spp. and *Burkholderia cepacia* (Rasch et al. 2005).

Conclusion

In the present study, the biosensor assays was an obviously semiquantitative method for detection of AHL production. AHL production was influenced by the different environment conditions. AHL production were reduced at 10 °C when compared with 25 °C. AHL production could reach maximum at 0.5–1.0% NaCl in LB supplemented. acidic conditions was conducive to the stable existence of the AHL molecules, but the alkaline environment was diametrically opposite. AHL-based quorum sensing system could up-regulate biofilm formation, production of protease and siderophore. Our group was currently building the LuxI/R genes deletion of *P. fluorescens* strain PF-04 to further demystify the correlation between quorum sensing and turbot spoilage.

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