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Inhibition of quorum sensing, biofilm, and spoilage potential in *Shewanella baltica* by green tea polyphenols

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We investigated the quorum sensing (QS) system of *Shewanella baltica* and the anti-QS related activities of green tea polyphenols (TP) against spoilage bacteria in refrigerated large yellow croaker. Autoinducer-2 (AI-2) and the diketopiperazines (DKPs) cyclo-(L-Pro-L-Leu) and cyclo-(L-Pro-L-Phe) were detected in the culture extract of *S. baltica* XH2, however, no N-acylhomoserine lactones (AHLs) activity was observed. Green TP at sub-inhibitory concentrations interfered with AI-2 and DKPs activities of *S. baltica* without inhibiting cell growth and promoted degradation of AI-2. The green TP treatment inhibited biofilm development, exopolysaccharide production and swimming motility of *S. baltica* in a concentration-dependent manner. In addition, green TP decreased extracellular protease activities and trimethylamine production in *S. baltica*. A transcriptional analysis showed that green TP repressed the *luxS* and *torA* genes in *S. baltica*, which agreed with the observed reductions in QS activity and the spoilage phenotype. Epigallocatechin gallate (EGCG)-enriched in green TP significantly inhibited AI-2 activity of *S.baltica*. These findings strongly suggest that green TP could be developed as a new QS inhibitor for seafood preservation to enhance shelf life.

Keywords: *Shewanella baltica*, AI-2, diketopiperazines, green tea polyphenols, biofilm, fish spoilage

Introduction

The genus *Shewanella* belongs to the family *Vibrionaceae* within the gamma subdivision of proteobacteria. *Shewanella* species are Gram-negative, oxidative, H_2S-producing rod bacteria. To date, numerous *Shewanella* species have been recovered from fish, marine animals, and seawater. Much attention has been paid to *Shewanella* due to their important role in fish spoilage (Gram *et al.*, 1987; Gram and Dalgaard, 2002; Vogel *et al.*, 2005). *Shewanella* have been described as specific spoilage organisms (SSO) in temperature waterfish species stored in ice due to their psychrotrophic nature and ability to reduce trimethylamine-N-oxide (TMAO) to trimethylamine (TMA), which causes a "fishy" off-odor (Vogel *et al.*, 2005). Additionally, *S. baltica* was also identified as an SSO involved in the spoilage of large yellow croaker (*Pseudosciaena crocea*) (Gu *et al.*, 2013) and shrimp (*Litopenaeus vannamei*) (Zhu *et al.*, 2015) stored at 4°C.

Bacteria communication by producing and responding to small diffusible molecules named autoinducers in a cell-density-dependent manner has been called quorum sensing (QS). The most widely studied autoinducers of QS signals contain N-acylhomoserine lactones (AHLs) produced by Gram-negative bacteria, peptides exploited by Gram-positive bacteria, and autoinducer-2 (AI-2) used for interspecies communication between Gram-negative and Gram-positive bacteria. Additionally, diketopiperazines (DKPs) are the smallest cyclic peptides known and represent a new class of QS interspecies or interkingdom signal molecules (de Carvalho and Abraham, 2012). Recently, AHLs and AI-2 signal molecules were detected in certain microbial community in food, mainly by the genera of the family *Enterobacteriaceae*, *Pseudomonadaceae*, and lactic acid bacteria (LAB), which contributed the food spoilage of meat and vegetables (Skandamis and Nychas, 2012). Four DKPs were reported as autoinducers in *S. baltica,* the SSO in the large yellow croaker (Gu *et al.*, 2013). Numerous studies have shown the possible role of QS signal molecules in microbial spoilage, suggesting that QS inhibitors or antagonists could be used as food preservatives to enhance food shelf life.

Phytochemicals from plant and fruit extracts have gained much interest as a source of anti-QS activities against pathogens and spoilage bacteria without toxic side effects (Girennavar *et al.*, 2008; Truchado *et al.*, 2012). Various plant food extracts and phytochemicals have been highlighted as QS inhibitors, including furocoumarins (Girennavar *et al.*, 2008), limonoids (Vikram *et al.*, 2011), curcumin (Packiavathy *et al.*, 2013), pomegranate extract, resveratrol, ellagic acid, and rutin (Truchado *et al.*, 2012). Green tea polyphenols (TP) are natural food additives with potential uses as preservatives and anti-oxidants in the food industry due to antioxidant and antimicrobial properties and enzyme inhibitory activities (Perumalla and Hettiarachchy, 2011). Many studies have investigated the antimicrobial activities of green TP against major food-borne pathogenic and spoilage bacteria (Lee *et al.*, 2009; Yi *et al.*, 2010), however, the effects of green TP on QS activity and biofilm formation are poorly understood. Therefore, we investigated the QS system in *S. baltica* isolated from refrigerated large yellow croaker, and inhibition

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of QS by green TP was further evaluated, as well as biofilm formation and spoilage potential of *S. baltica*.

Materials and Methods

Strains and growth conditions

S. baltica XH2 was isolated previously from spoiled *Pseudosciaena crocea* stored at 4°C and identified as a SSO. The AI-2 reporter strain *Vibrio harveyi* BB170 (AI-1 sensor negative and AI-2 sensor positive) and the AHLs biosensor strains *Chromobacterium violaceum* CV026 and *Agrobacterium tumefaciens* A136 were a kind gift from Dr. Yang, Zhejiang A&F University. All bacterial strains were cultivated in Luria-Bertani (LB) broth and maintained at 30°C, except *V. harveyi* BB170, which was cultured in modified autoinducer bioassay (AB) medium (Bodor *et al.*, 2008).

Determination of minimum inhibitory concentration (MIC) of green TP

Green TP (purity≥98%) was purchased from the Tea Institute of Zhejiang University (Hangzhou, China). A stock solution was prepared by dissolving 10 mg green TP in 1 ml sterile MilliQ water. The solution was filtered through a 0.22 μm Millipore filter and stored at -20°C until use. Serial dilutions of green TP (3.00, 2.00, 1.00, 0.75, 0.50, and 0.25 mg/ml) were made in 1 ml LB broth. For each concentration, one percentage of overnight *S. baltica* XH2 culture (0.4 OD at 600 nm) was inoculated into the prepared LB containing green TP and incubated at 30°C for 24 h. The MIC of the green TP suspension was the lowest concentration that inhibited visible bacteria growth. Furthermore, the cell density of *S. baltica* in the presence of green TP at the concentration of 0, 0.25, 0.50, 0.75 mg/ml was monitored by plate counting every 3 h for 24 h.

Autoinducer assay

Cell-free suspensions of *S. baltica* were obtained from cultures sampled every 3 h after centrifugation at 12,000 × *g* for 10 min at 4°C, and were analyzed for the QS autoinducers, including AI-2, AHLs, and DKPs. An overnight culture of *V. harveyi* BB170 was diluted (1:5,000) in fresh AB medium. The diluted cells (90 μl each) were dispensed into each well of a 96-well microplate (BD Biosciences). Aliquots of the cell-free culture of *S. baltica* (10 μl) were added to microplates, and incubated at 30°C for 6 h with shaking to start the AI-2 activity assay. Luminescence was measured by using a luminometer (Perkin-Elmer Victor X).

The biosensor strains *C. violaceum* CV026 and *A. tumefaciens* A136 were used to detect the AHLs of *S. baltica* in a semi-quantitative well diffusion bioassay, as described by Ravn *et al.* (2001).

DKPs in the cell-free culture supernatant were extracted and detected by gas chromatography-mass spectrometry (GC-MS), according to Gu *et al.* (2013), using a Agilent 5979 GC-MS instrument (Agilent Technologies) with a 30 m × 0.25 mm × 0.25 μm HP-5 ms capillary column (Agilent Technologies) and the NIST (V05a) MS library. The extracted samples were injected when GC inlet temperature was 260°C and transfer line temperature was 260°C. Column temperature was maintained at 50°C for 2 min and then programmed to reach 260°C at a rate of 15°C/min, which was maintained for 8 min. Mass spectra were acquired by electron ionization under normal conditions: ion-source temperature at 230°C, electron energy of 70 eV, scan rate on 2 scans/sec, and mass range at 35–600 amu. Selected ion monitor was chosen to quantify DKPs content.

Autoinducer degradation assay

The degrading percentage of AI-2 and DKPs was measured based on the interactions between green TP at 0.25, 0.50, 0.75 mg/ml and cell-free culture of *S. baltica* XH2. Sterile water was used as the positive control. AI-2 and DKPs activities were determined in the mixture according to the above methods after 9 h at 30°C.

Biofilm assay

The effect of TP (0, 0.25, and 0.50 mg/ml) on *S. baltica* biofilm formation was determined by quantifying biofilm biomass, exopolysaccharide (EPS) and scanning electron microscope (SEM) analyses. Biofilm biomass was assessed by crystal violet assay (Packiavathy *et al.*, 2013), and EPS content of biofilm was determined following a previous procedure (Jung *et al.*, 2013) with a slight modification. *S. baltica* XH2 biofilm was grown on glass coverslips in 6-well microtiter plates with LB medium in the absence or presence of 0.50 mg/ml TP at 30°C for 48 h for the SEM observations (Jung *et al.*, 2013). Then, the coverslips with biofilm were washed with PBS and transferred to 2.5% glutaraldehyde, and incubated for 1 h at room temperature. The samples were serially dehydrated in alcohol, fixed in a critical-point drier (Samdri-790, Tousimis), coated with gold-palladium and viewed with a TM-1000 (Hitachi) scanning electron microscope.

Swimming motility assay

Swimming motility was performed by following a method described previously (Rashid and Kornberg, 2000). About 3 μl of overnight cultures of *S. baltica* XH2 (0.5 OD at 600 nm) were point inoculated at the center of a swimming agar plate consisting of 1% tryptone, 0.5% NaCl, and 0.3% agarose supplemented with green TP at 0, 0.25, and 0.50 mg/ml. After 48h incubation at 30°C, the reduction in swimming migration was recorded by measuring the swim zones of the bacterial cells.

Extracellular protease activity assay

Extracellular protease activities of *S. baltica* were qualitatively analyzed according to the method of Periasamy (Anbu, 2013). Five microlitre of *S. baltica* XH2 overnight culture was added as droplets to the center of skim milk agar plates containing TP (0, 0.25, and 0.50 mg/ml). The plates were incubated at 30°C for 72 h, and a clear zone in the center indicated the presence of extracellular protease activities.

TMA determination

S. baltica XH2 was cultured in LB broth containing 10 mM

Table 1. Primer used in this study

Primer name	Sequence (5′→3′) description
luxSF	5′-TGGTATTCACACTCTGGAGC-3′
luxSR	5′-TAAGCACATCTTCCATCGCA-3′
torAF	5′-ACAAACGCTTACACTCGCAAATG-3′
torAR	5′-TTGCGTCGACAGGGCTGATA-3′
16S rRNAF	5′-CGGCGGACGGGTGAGTAAT-3′
16S rRNAR	5′-CCGTAGGGCGTATGCGGTATTA-3′

TMAO and green TP (0, 0.25, and 0.50 mg/ml) at 30°C for 24 h. After samples were taken every 3 h and centrifuged at 5,000 × *g* for 10 min, TMA content in the supernatant was assayed spectrophotometrically using colorimetric formation of the picric acid salt of TMA (Dyer, 1945).

luxS and *torA* genes expression assay

The bacterial strain was cultured in LB broth supplemented with green TP (0, 0.25, and 0.50 mg/ml) at 30°C for 12 h. Total RNA was isolated using the RNA Isolated Kit (Sangon Biotech). A quantitative reverse-transcription-polymerase chain reaction (qRT-PCR) analysis was performed as described by Balestrino *et al.* (2005) and Zhu *et al.* (2015) with some modifications. Specific probes and primers were designed according to the known sequences in the GenBank sequence database using Premier 5.0 software (Table.1). Relative expression was measured by one-step quantitative RT-PCR (Taqman system, Applied Biosystems) on the ABI 7500 system following manufacturer's conditions in a 25 µl total reaction volume. Gene expression levels were normalized to levels of the housekeeping 16S rRNA gene utilizing a standard curve for relative quantification.

Assay of active components in green TP

Total polyphenol and total flavonoid concentration in green TP were analyzed according to the previous methods (Zhang *et al.*, 2014). Polyphenols in TP was analyzed using a high-performance liquid chromatography (HPLC) system (Agilent 1100) with a BDS-Hypersil C18 column, 4.6 × 250 mm, 5 µm particle size (Thermo Scientific) as described by Zhang *et al.* (2014). The gallic acid and tea catechins, catechin, epicatechin (EC), epigallocatechin (EGC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG) standards were purchased from Sigma-Aldrich.

Six of the polyphenolic compounds in green TP were dissolved in dimethyl sulfoxide (DMSO). After a 9 h incubation at 30°C, bioluminescence intensity in cell-free supernatant of *S. baltica* treated with 5 µg/ml of each of these components was measured using the above methods. Cell density was measured at 600 nm using a spectrophotometer (UV-2550, Shimadzu).

Statistical analysis

Three replicate trials were done for each sample, and all ex-

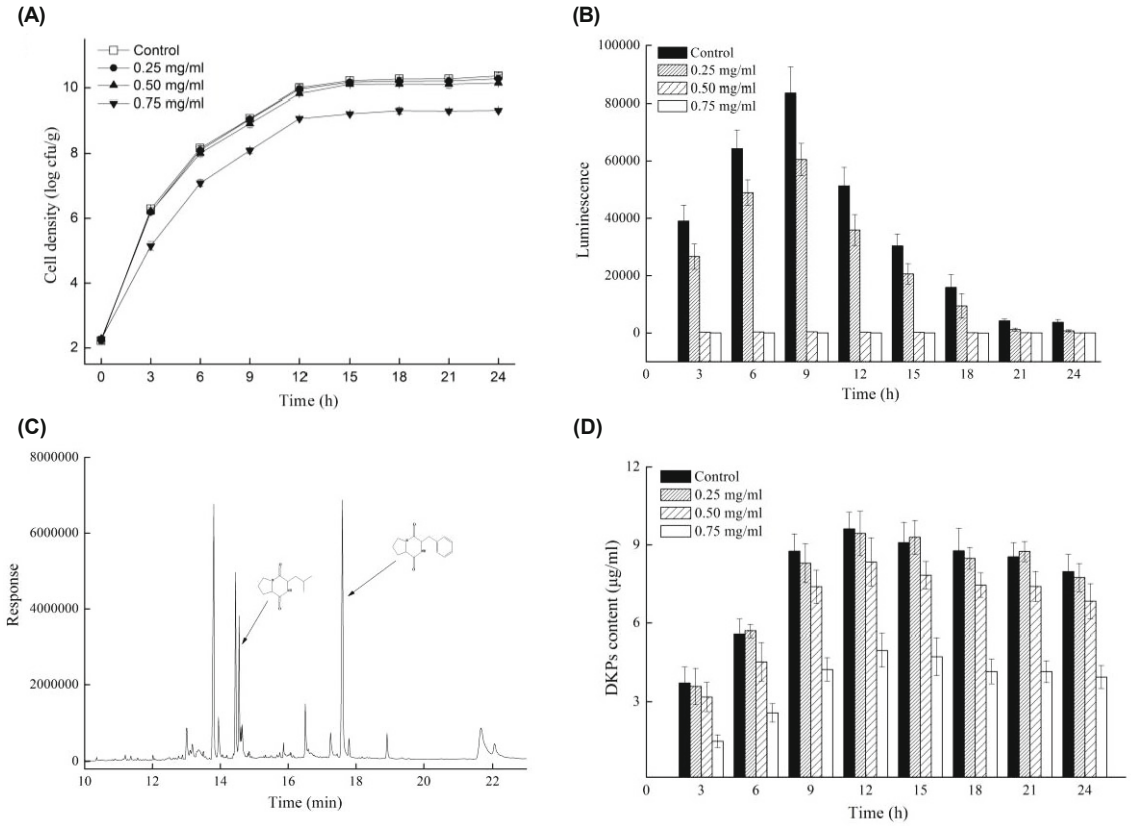

Fig. 1. Effects of green TP on the growth curve and two QS signal molecules in *S. baltica* XH2. (A) Growth curve, (B) AI-2 activity, (C) GC-MS chromatogram of DKPs, (D) DKPs content.

periments were repeated for three times. Results were expressed as mean ± standard deviation (SD) and analyzed by one-way analysis of variance (ANOVA) using SPSS 16.0 software (SPSS Inc.). Significant different were expressed at the $p<0.05$ level.

Results and Discussion

Identification of QS signal molecules in *S. baltica* XH2

Growth curves and QS system of *S. baltica* XH2 were shown in Fig. 1. *S. baltica* XH2 grew rapidly in LB broth at 30°C and was in the stationary phase after 15 h of incubation. The AI-2 signal molecule was produced by *S. baltica* XH2 after 3 h, and reached a maximum level during the late exponential phase (at 9 h), then decreased rapidly during the early stationary phase ($p<0.05$) (Fig. 1B). The results of AI-2 activity in *S. baltica* agreed with those of Bodor *et al.* (2008), who first reported that *Shewanella* produced a pronounced AI-2. Fig. 1C presented the GC/MS chromatogram of DKPs in cell-free supernatant of *S. baltica*. Of all the peaks, the two peaks with retention times of 14.56 and 17.64 min were identified as cyclo-(L-Pro-L-Leu) and cyclo-(L-Pro-L-Phe), respectively. DKPs increased gradually in the *S.baltica* supernatant and reached a maximum level of 9.61 μg/ml after 12 h, then decreased slowly during the stationary phase (Fig. 1D). Gu *et al.* (2013) also found that cyclic dipeptides isolated from *S. baltica* were involved in the regulation of spoilage capability of *S. baltica*. No color was formed by *C. violaceum* CV026 or *A. tumefaciens* A136, indicating that *S. baltica* had weak AHLs activities. Though the AHLs signal molecules are the most intensive autoinducers in Gram-negative bacteria, relative reports on AHLs production in *Shewanella* are scarce.

Recently, Zhu *et al.* (2015) found that AI-2 and DKPs, rather than AHLs, were detected in *S. baltica* and *S. putrefaciens*, which were the SSO in the shrimp (*Litopenaeus vannamei*). DKPs have attracted considerable interest as a potential new class of QS signal molecules, however, very little is known about their functions *in vivo*. It was reported that DKPs diffused through the cell membranes and modulated LuxR-mediated QS systems of bacteria instead of AHLs (de Carvalho and Abraham, 2012), suggesting that these DKP compounds may influence cell-cell signaling in *S. baltica*. Our results indicated that QS system in *S. baltica* contained AI-2 and DKPs autoinducers, which were probably involved in the regulation of the spoilage phenotype of the SSO.

Effects of green TP on microbial growth and QS signal molecules

The effects of green TP on the growth curve and QS signal molecules in *S. baltica* were shown in Fig. 1. The MIC of green TP suspension was 0.75 mg/ml against *S. baltica* XH2. Cell densities did not differ between the control and TP-treated (0.25 and 0.50 mg/ml) samples ($p>0.05$), whereas green TP at 0.75 mg/ml significantly inhibited cell growth of *S. baltica* ($p<0.05$) (Fig. 1A). A significant decrease in AI-2 activity was observed in response to all treated concentrations of green TP at tested ($p<0.05$), particularly the 0.50 and 0.75 mg/ml treatments (Fig. 1B). After 9 h of incubation, luminescence intensities were inhibited 27.7% and 100% by green TP at concentration of 0.25 and 0.50 mg/ml or more ($p<0.05$), respectively. The supplement of green TP also decreased DKPs activity of *S. baltica* during the entire growth period, except that no significant difference was detected between the control and 0.25 mg/ml TP treatment ($p>0.05$) (Fig.1D). After 12 h of incubation, DKPs production was inhibited 13.2% and 51.7% when TP at 0.50 and 0.75 mg/ml were added, respectively. In two DKPs, cyclo-(L-Pro-L-Leu) decreased by 7.3% and 20.8%, whereas cyclo-(L-Pro-L-Phe) decreased 20.8% and 36.2% in the presence of 0.50 and 0.75 mg/ml green TP, respectively. Our results indicated that green TP quench AI-2 and DKPs activities in *S. baltica*, particularly those of AI-2, without inhibiting cell growth. Some authors also found that phytochemical compounds, such as cinnamaldehyde (Niu *et al.*, 2006) and curcumin (Packiavathy *et al.*, 2013), significantly inhibited AI-2 activity. Few reports have explored the inhibition of DKPs production; however, green TP had anti-DKPs activity against *S. baltica*.

In the present study, the degradation of QS autoinducers by green TP were further evaluated (Fig. 2). The degradation rates of AI-2 in a mixture of green TP and endogenous autoinducer in the cell-free supernatant of *S. baltica* were 13.4%, 37.6%, and 75.2% in the presence of TP at 0.25, 0.50, and 0.75 mg/ml respectively, however, no significant degradation of DKPs was observed. Many phytochemicals are believed to inhibit QS by interfering with AHLs activity and altering their synthesis (Vattem *et al.*, 2007). Green TP in the supernatant of *S. baltica* resulted in a significant deg-

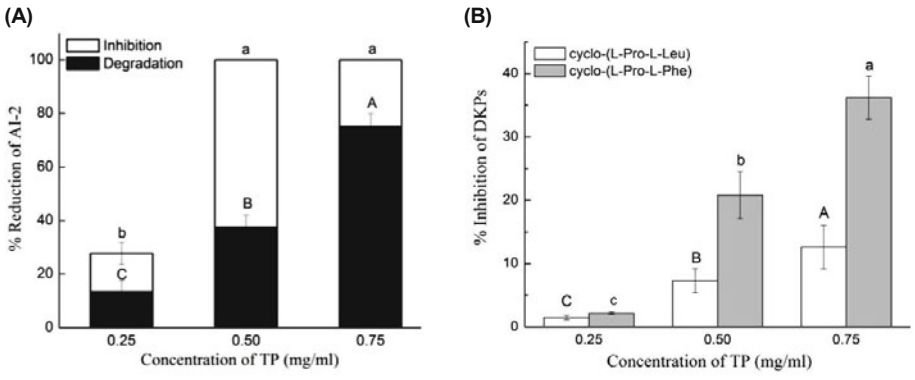

Fig. 2. The percentage of inhibition and/or degradation of AI-2 (A) and two DKPs (B) in *S. baltica* XH2 by green TP at concentration of 0, 0.25, 0.50, and 0.75 mg/ml. Bars labeled with different letters indicated significant differences at $p<0.05$.

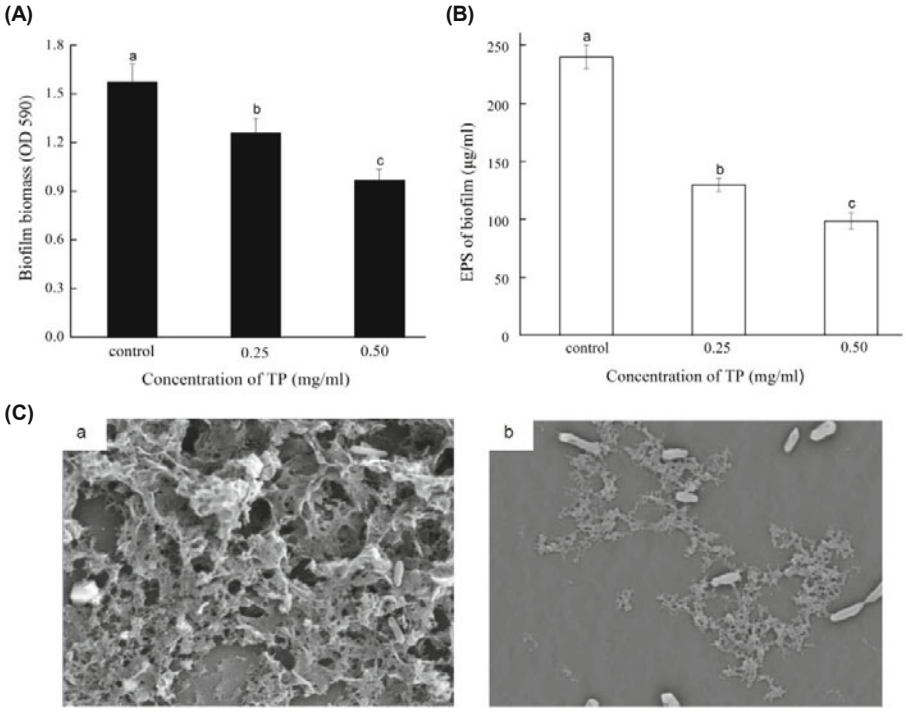

Fig. 3. Inhibition on biofilm development of *S. baltica* XH2 by TP at concentration of 0, 0.25, and 0.50 mg/ml. (A) Biofilms biomass, (B) EPS of biofilm, (C) SEM imaged *S. baltica* XH2 biofilms after 48 h culture on glass coverslips by TP at concentration of 0 (a) and 0.50 (b) mg/ml. Bars labeled with different letters indicated significant differences at $p<0.05$.

radation of AI-2 activity, but had no effect on DKPs. Our results went well with the findings of Truchado *et al.* (2012), wherein selected phytochemicals exhibited different degradation-transformation rates of autoinducer. It was indicated that plant polyphenols quench bacteria QS-related mechanisms in different ways depending on the bacterial species and autoinducers produced. Clearly, the decreased AI-2 activity in *S. baltica* by green TP was associated with the combined effects of degradation and inhibition of synthesis, whereas TP may have mainly affected synthesis of DKPs.

Effect of green TP on biofilm formation

Many bacteria adhere to and colonize surfaces by producing biofilm, and biofilm formation of spoilage bacteria usually results in food contamination and shortened shelf-life. The antibiofilm activity of green TP against *S. baltica* XH2 revealed a concentration dependent reduction (Fig. 3). Compared with the control, 0.25 and 0.50 mg/ml green TP decreased biofilm biomass by 20% and 38.6%, respectively (Fig. 3A). Consistent with our findings, an extract of *Rosa rugosa* tea polyphenols specifically inhibited QS-controlled biofilm formation by *E. coli* K-12 and *Pseudomonas aeruginosa* PAO1 (Zhang *et al.*, 2014). QS-based EPS production is essential for the development of biofilm structure and maturation (Jung *et al.*, 2013). Green TP at the concentrations of 0.25 and 0.50 mg/ml effectively decreased the EPS production of *S. baltica* by 44.3% and 56.2%, respectively (Fig. 3B). Packiavathy *et al.* (2013) observed the decreased production of EPS by *Vibrio* spp. after curcumin treatment.

Effect of green TP on swimming motility

SEM was used to correlate the results of biofilm biomass and the EPS assay with visual observations on biofilm development structure. As shown in Fig. 3C(a), *S. baltica* XH2 on the surface of glass coverslips in LB broth produced a mature biofilm consisting of a dense group of cells enmeshed with a large amount of polysaccharide. *S. baltica* failed to form a three-dimensional structure of biofilm treated by 0.5 mg/ml TP (Fig. 3C(b)). The extracellular matrix and attached bacteria decreased significantly on the surface of coverslips after treatment with TP, suggesting that the active compound in green TP possibly interfered with the expression of genes responsible for biofilm formation. A *luxS* mutation in *S. oneidensis* resulted in the development of loosely bound biofilms (Bodor *et al.*, 2011).

As shown in Fig. 4, green TP significantly inhibited QS-dependent swimming migration by *S. baltica* XH2 in a concentration-dependent manner. After treatment with green

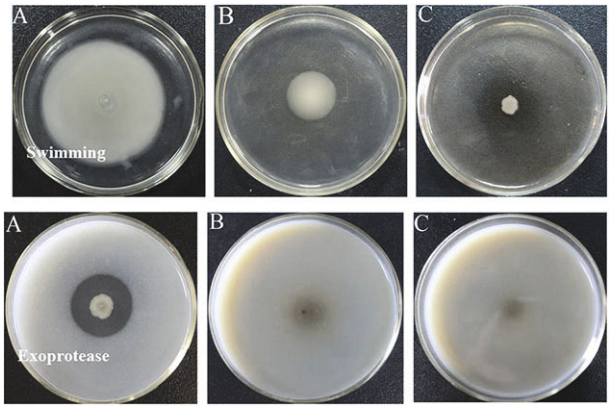

Fig. 4. Inhibition of swimming motility and exoprotease activity of *S. baltica* XH2 by TP at concentration of 0 (A), 0.25 (B), 0.5 (C) mg/ml.

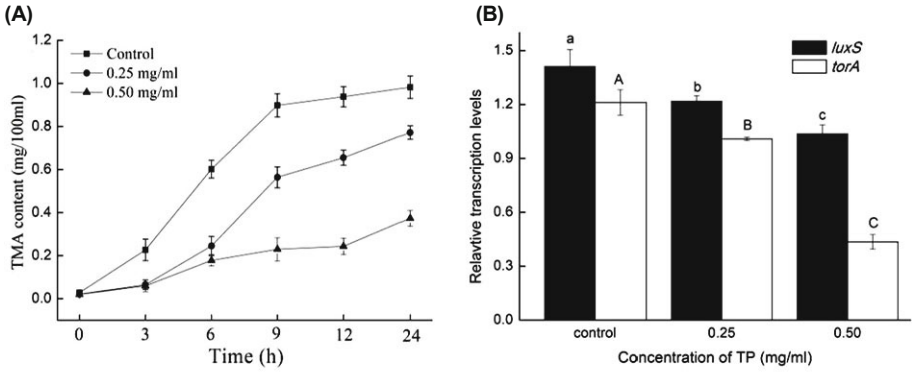

Fig. 5. Inhibition of TMA production from TMAO (A) and relative expression *luxS* and *torA* (B) of *S. baltica* XH2 in LB broth supplemented with 10 mM TMAO by TP at concentration of 0, 0.25, 0.50 mg/ml. Bars labeled with different letters indicated significant differences at $p<0.05$.

TP at 0.25 and 0.50 mg/ml, swimming motility were inhibited 87.7% and 93.5%, respectively, indicating that swimming motility of *S. baltica* treated by green TP was comparatively weaker than that of the control. Flagellar-dependent swimming motility is related to biofilm formation, which are coordinately regulated by signal-mediated QS (Pratt and Kolter, 1998). Hence, interfering with synthesis of the flagellar could affect the bacterial motility and subsequent colonization. These results were in accordance with the findings of Lee *et al.* (2009), wherein EGCG inhibited biofilm formation and swarming motility of *E. coli* O157:H7.

Effect of green TP on extracellular protease activity

Extracellular proteases are secreted from spoilage bacteria and decompose food proteins into small peptides and amino acids, which are further metabolized into end products of volatile nitrogenous, sulfur, or acidic compounds with offensive odors (Gram *et al*., 2002). The clear zone was formed around colonies in skim milk agar, indicating high activity of extracellular proteases in *S. baltica* XH2 (Fig. 4A). The diameter of the clear zone was 29.5 mm in the control, whereas weaker clear zones were observed in samples treated by TP at 0.25 and 0.50 mg/ml (Fig. 4). From the obtained results, the green TP treatment significantly inhibited extracellular protease activity in *S. baltica* XH2.

Effect of green TP on TMA production

TMA is one of the most common indicators to assess spoilage in aquatic products. As presented in Fig. 5A, effect of green TP was examined for its efficacy to inhibit the reduction from TMAO to TMA in *S. baltica* XH2. TMA was produced continuously at the early exponential growth phase, and reached maximal content at the late exponential growth phase in *S. baltica*. The accumulation of TMA decreased significantly with the increase in TP concentration. Green TP inhibited TMA production by 21.4% and 62.0% at 12 h after 12 h of exposure to 0.25 and 0.50 mg/ml, respectively.

Effect of green TP on *luxS* and *torA* expression

LuxS expression and AI-2 production are known to occur in *Shewanella* species (Bodor *et al*., 2008). The TMAO reductase encoded by *torA* is a large periplasmic protein containing a molybdenum confactor in its catalytic site (Dos Santos *et al*., 1998). Transcription of *luxS* and *torA* in *S. baltica* was repressed significantly by green TP ($p<0.05$) (Fig. 5B). Green TP at concentrations of 0.25 and 0.50 mg/ml reduced *luxS* expression by 13.7% and 26.6%, and inhibited *torA* expression by 16.7% and 64.3%, respectively compared with the control. These results revealed that green TP supplementation at sub-inhibitory concentrations repressed expression of *luxS* and *torA* genes, which was in accordance with the decrease in AI-2 activity and TMA production. AI-2 was demonstrated to regulate proteolytic activities in *Vibrio anguillarum* (Brackman *et al*., 2008). Additionally, the inhibition of protease activities and TMA production in *S. baltica* showed a clear response to AI-2 decrease by green TP, suggesting that the signal molecule may induce the regulation of some spoilage factors in *S. baltica*.

Green TP components and anti-QS activities of the compounds

Green TP was rich in total polyphenols (614.0 mg GA/g, 61.40%) but low in flavonoids (159.4 mg rutin/g, 15.94%) (Table 2). Six kinds of main polyphenols presented in

Table 2. Green TP components and anti-AI-2 activity of the compounds

Peak number	Polyphenolic	compounds Rt (min)	TP (%)	Inhibition ofAI-2 (%)
1	Gallic acid (GA)	5.440	0.1	4.3
2	Epigallocatechin (EGC)	17.578	10.2	6.7
3	Catechin (C)	19.975	0.8	7.3
4	Epicatechin (EC)	28.812	5.2	5.6
5	Epigallocatechin gallate (EGCG)	29.125	32.7	23.2
6	Epicatechin gallate (ECG)	30.629	9.3	12.5
	Total polyphenol	614.0 (mGAE/g)		-
	Total flavonoid	159.4 (mg rutin/g)		-

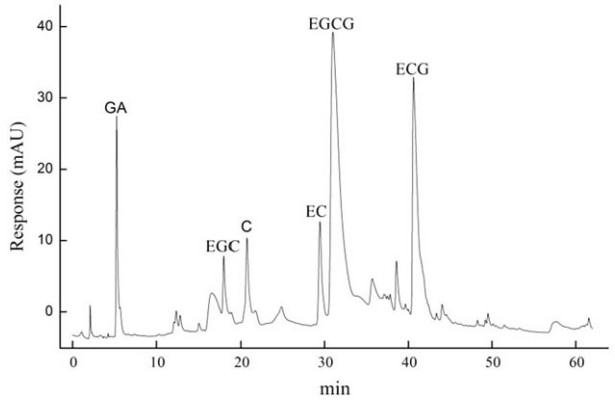

Fig. 6. The active compounds in green TP identified by HPLC analysis. Six peaks were gallic acid (GA), epigallocatechin (EGC), catechin (C), epicatechin (EC), epigallocatechin gallate (EGCG), and epicatechin gallate (ECG).

green TP, including gallic acid, EGC, catechin, EC, GCG, and ECG at approximately 0.1%, 10.2%, 0.8%, 5.2%, 32.7%, and 9.3%, respectively (Fig. 6 and Table 2). EGCG showed the strongest AI-2 inhibitor compared to other catechins (Table 2) at low concentration without inhibiting bacterial growth (data not shown). EGCG also resulted in the reduced AI-2 production by *E. coli* O157:H7 (Lee *et al.*, 2009). Green tea catechins are natural compounds with Generally Recognized as Safe, and have potential applications in seafood preservation (Li *et al.*, 2012). Our results showed that anti-QS activity of green TP was attributed to the pronounced inhibitory effects of the catechins, particularly EGCG.

Conclusion

The present study indicated that AI-2 and DKPs signal molecules were produced in *S. baltica* XH2, as SSO of large yellow croaker. Green TP interfered with QS system in *S. baltica*, and inhibited biofilm formation, swimming motility, extracellular protease and TMAO reductase activities without impairing its growth. The anti-QS activity of green TP was associated with the significant inhibitory effect of EGCG. These results provide the evidence for potential application as a QS inhibitor in seafood preservation. Further studies need to clarify the pathways that quench QS and control the spoilage potential of *S. baltic* by polyphenols.

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