Antibacterial activity and action mechanism of questin from marine *Aspergillus flavipes* HN4-13 against aquatic pathogen *Vibrio harveyi*

Lei Guo^{1,2,3} · Fei Zhang^{1,2} · Xintong Wang^{1,2} · Hui Chen² · Qianqian Wang² · Jiakai Guo² · Xi Cao² · Le Wang²

Received: 1 October 2018 / Accepted: 14 December 2018 / Published online: 2 January 2019
© King Abdulaziz City for Science and Technology 2019

Abstract

This study investigated the antibacterial activity and mechanism of questin from marine *Aspergillus flavipes* HN4-13 against aquatic pathogenic *Vibrio harveyi*. The minimal inhibitory concentration and minimal bactericidal concentration of questin against *V. harveyi* strain SZ-1 and 1.8690 were determined by Oxford cup and tube dilution methods. The mechanism of action of questin against *V. harveyi* 1.8690 was investigated by bacterial growth curve analysis, ultraviolet absorption, Mo-Sb-Vc colorimetry, alkaline phosphatase and scanning electron microscopy. Results showed that questin exhibited favourable antibacterial and bactericidal activity against *V. harveyi* by disrupting the cell wall and membrane, which caused the destruction of permeability and integrity of cell wall and membrane, resulting in the leakage of intracellular biological components and change of cell morphology. This paper is the first to report the mechanism of action of questin against the aquatic pathogen *V. harveyi*.

Keywords Questin · *Vibrio harveyi* · Antibacterial activity · Action mechanism

Introduction

In the past few decades, outbreaks of infectious diseases caused by *Vibrio harveyi* became a main economic problem to the culture and quality of aquatic animals (Morya et al. 2014). *V. harveyi* is widely distributed in the mariculture environment, thereby causing high mortality in the early larval stages of farmed and wild shrimps, resulting in a huge loss of production and marketing (Thompson et al. 2010; Guo et al. 2017). Antibiotics play a very important role in the prevention and treatment of bacterial diseases of shrimps. However, the long-term use or abuse of antibiotics causes resistance of various strains, ecological imbalance

and weakened immune system, even leading to the presence of antibiotic residues in aquatic products and thus endangering human body health (Harikrishnan et al. 2010; Cao et al. 2011). Therefore, preventing and controlling *V. harveyi* are necessary in the mariculture industry. Alternative sources of antibiotics include plant extracts, essential oils, probiotics and microbial metabolites, which have been applied to control bacterial infections (Guo et al. 2016; Kesarcodi-Watson et al. 2008; Randrianarivelo et al. 2010; Turker and Yildirim 2015; Xu et al. 2014; Yu et al. 2012).

Marine microorganisms have been proven to be a major source of marine active natural products during the past several decades (Jin et al. 2016; Wang et al. 2016). Among them, marine fungi have become a novel hotspot for the exploration of pharmacological active substances due to their complex genetic background, unique living environments and complicated physiological properties (Gomes et al. 2015; Song et al. 2014; Sun et al. 2014). During the course of discovering antimicrobial agents from marine fungi in our laboratory, *Aspergillus flavipes* strain HN4-13, which can be isolated from sea mud, has attracted our attention because of its favourable activity against the aquatic pathogen *V. harveyi*. By subjected to bioactivity-guided isolation over solvent extraction, silica gel, Sephadex LH-20 and semi-preparative high performance liquid

✉ Lei Guo
leiguoo@sina.com

¹ Jiangsu Key Laboratory of Marine Bioresources and Environment, Huaihai Institute of Technology, Lianyungang 222005, China

² Jiangsu Key Laboratory of Marine Biotechnology, Co-Innovation Center of Jiangsu Marine Bio-industry Technology, Huaihai Institute of Technology, Lianyungang 222005, China

³ Jiangsu Institute of Marine Resources Development, Lianyungang 222004, China

chromatography, an anti-*V. harveyi* compound, namely, questin was obtained from the fermentation broth of *A. flavipes* HN4-13 (Guo and Wang 2017). We were the first to report the antibacterial activity of questin against aquatic pathogenic *V. harveyi*, and the action mechanism of questin against *V. harveyi* has not been reported.

In the present study, the in vitro growth inhibitory effect of questin on *V. harveyi* was studied. Then, the action mechanism of questin against *V. harveyi* was investigated by bacterial growth curve, ultraviolet (UV) absorption, Mo-Sb-Vc colorimetry, alkaline phosphatase analysis and scanning electron microscopy.

Materials and methods

Materials and chemicals

Questin (purity > 94%, Fig. 1) was prepared from the ethyl acetate extract of the fermentation broth of *A. flavipes* HN4-13 (CCTCC AF 2015022) over silica gel, Sephadex LH-20 and semi-preparative high performance liquid chromatography according to the reference (Guo and Wang 2017). *V. harveyi* 1.8690 was purchased from China General Microbiological Culture Collection Center (Beijing, China). Streptomycin sulfate (potency > 720 U/mg) was obtained from Bio Basic Inc. (Toronto, Canada). MH broth medium was obtained from Aoboxing Biotech Co., Ltd (Beijing, China). Phosphorus detection kit was purchased from Luheng Biotech Co., Ltd (Hangzhou, China). Alkaline phosphatase (AKP) detection kit was obtained from Jiancheng Bioengineering Institute (Nanjing, China). All other chemicals were of analytical grade and obtained from Sinapharm Chemical Reagent Co., Ltd (Shanghai, China).

Oxford cup assay

The minimal inhibitory concentration (MIC) of questin against *V. harveyi* strain SZ-1 and 1.8690 was measured using Oxford cup assay according to the reference. The diameter of solid medium plate was about 90.00 mm. 15 mL of precooled (~ 50 °C) MH broth agar medium was poured into the plates and left for 20 min to solidify. 100 µL of *V.*

harveyi SZ-1 or 1.8690 suspension (2×10^6 cfu/mL) was inoculated and evenly smeared on the medium. Then, Oxford cups (outside diameter of about 8.0 mm) were placed on the plates after 20 min. Questin was dissolved in methanol and diluted by continuous two-fold dilution method to 125, 62.5, 31.25, 15.625 and 7.8125 µg/mL. Streptomycin sulfate was dissolved in distilled water and diluted to the same concentration. 100 µL of different concentrations of questin and streptomycin sulfate were added in Oxford cups, which were then cultured at 37 °C for 24 h. All the experiments were conducted in triplicates, and MIC was defined as the lowest concentration of samples that generated an inhibition zone against *V. harveyi* (Guo et al. 2017).

Tube dilution assay

MIC and minimal bactericidal concentration (MBC) of questin against *V. harveyi* 1.8690 were determined by tube dilution assay. Questin was dissolved in dimethyl sulfoxide (DMSO) and diluted by continuous two-fold dilution method to 12.5, 6.25, 3.125, 1.5625 and 0.78125 mg/mL. Streptomycin sulfate was dissolved in distilled water and diluted to the same concentration. 1.0 mL of *V. harveyi* 1.8690 suspension (2×10^6 cfu/mL) was inoculated into the test tube (20.5 × 2.5 cm) with 4 mL of MH broth medium, respectively. Then 50 µL of different concentration of sample solutions were added into the test tube separately and mixed well, 50 µL DMSO or distilled water was added into the test tube and used as the negative control. The test tubes were incubated at 37 °C with 160 rpm/min for 24 h. After that, the bacterial growth in the test tube was observed, and MIC was defined as the lowest concentration of compounds without bacterial growth (Guo and Wang 2017). On the basis of the MIC experiment, 100 µL solutions of the test tubes without bacterial growth was pipetted and then spread on the surface of MH broth agar medium plates. The plates were cultivated at 37 °C for 24–48 h, and MBC was defined as the lowest concentration of compounds without bacterial colonies. The experiments for MIC and MBC analyses were carried out in duplicates, with full agreement between both results.

Bacterial growth curve

The effect of questin on the growth of aquatic bacterial pathogens was assessed using *V. harveyi* strain 1.8690 (Patra and Baek 2016). 40 µL of 100-fold and 200-fold MIC of questin dissolved in DMSO, 40 µL of 100-fold MIC of streptomycin sulfate dissolved in distilled water, and 40 µL of DMSO were added to 4 mL of MH broth medium. After mixing, 40 µL of the bacteria culture (2×10^6 cfu/mL) was added into each tube separately. The test tubes were incubated at 37 °C with 160 r/min for 30 h. During incubation, 100 µL of bacterial samples were taken

Fig. 1 Chemical structure of questin

from each incubating tube at every 6 h, and the absorbance at 620 nm was determined using a Synergy HT spectrophotometer (BioTek, USA). Experiments were conducted in triplicates. The culture time was used as the *X*-axis, and the average absorbance was used as *Y*-axis for the growth curve.

Integrity of cell membrane

The cellular contents would leak out if the bacterial cell membrane was damaged. Bioendogenous UV absorptive substances mainly include proteins, DNA and RNA, which can be used to analyse the integrity of cell membrane by detecting the UV absorbance at 260 nm (Patra and Baek 2016). The integrity of the cell membrane of *V. harveyi* 1.8690 was determined by the UV absorption assay. 200 μ L of 100-fold and 200-fold MIC of questin dissolved in DMSO, 200 μ L of 100-fold MIC of streptomycin sulfate dissolved in distilled water, and 200 μ L of DMSO were added to 100-mL conical flask with 20 mL of MH broth medium. After mixing, 200 μ L of the bacteria culture (2×10^6 cfu/mL) was added into each conical flask separately. The conical flasks were incubated at 37 °C with 160 r/min, and the experiments were carried out in triplicates. 1.0 mL of bacterial sample was collected after 0, 4, 8, 12 and 16 h, and then placed in 4 mL of distilled water, and centrifuged at 5000 r/min for 10 min. After that, the absorbance value of the supernate was obtained at 260 nm, the zero control was prepared by adding 1.0 mL of MH broth medium to 4.0 mL of distilled water.

Leakage of phosphate ions

The leakage of phosphate ions in the bacterial suspension of *V. harveyi* 1.8690 after treatment with questin was determined using Mo-Sb-Vc colorimetry (Yuan et al. 2012). Yellow phosphomolybdenum heteropoly acid was formed under the acidic conditions by phosphoric acid and ammonium molybdate. Ascorbic acid and antimony potassium tartrate were used as the reducing agents. The yellow phosphomolybdenum heteropoly acid can be reduced to phosphomolybdenum heteropoly blue with the maximum absorption at 720 nm. 1.0 mL of the supernatant obtained after determining the absorbance at 260 nm was added in the 50-mL flask, and the absorbance at 720 nm was measured using a phosphorus detection kit. Phosphorus content was calculated by the regression equation of the standard curve. All data were reported as phosphorus content (mg/mL) in bacterial suspensions at each time interval. The effect of questin on the phosphorus leakage of *V. harveyi* 1.8690 was then evaluated.

Permeability of cell wall

The contents of extracellular alkaline phosphatase (AKP) can reflect the changes of the cell wall permeability (Li et al. 2017). An AKP detection kit was used to determine the effect of questin on the cell wall permeability of *V. harveyi* 1.8690. The principle of AKP detection kit is that AKP breaks down disodium phenyl phosphate to produce free phenol and phosphoric acid under alkaline conditions, and then phenol reacts with 4-aminoantipyrine in alkaline solution through the oxidization of potassium ferricyanide to form red quinone derivatives with the maximal absorbance value at 520 nm. 1.0 mL of the supernatant obtained after determining the absorbance at 260 nm was added in the 10-mL tubes, and the absorbance at 520 nm was determined using an AKP detection kit. AKP content was calculated by the regression equation of the standard curve. All data were reported as the AKP content (μ g/mL) in bacterial suspensions at each time interval.

Scanning electron microscopy

The effect of questin on the morphology of *V. harveyi* 1.8690 was determined by scanning electron microscopy (Qu et al. 2016). 200 μ L of 100-fold and 200-fold MIC of questin dissolved in DMSO, 200 μ L of 100-fold MIC of streptomycin sulfate dissolved in distilled water, and 200 μ L of DMSO were added to 10-mL centrifuge tubes with 2 mL of the suspension of *V. harveyi* 1.8690 (2×10^6 cfu/mL) in 50 mM phosphate buffer solution (pH 7.0). After mixing, the tubes were incubated at 37 °C for 0 and 4 h, and centrifuged at 5000 r/min for 5 min. Subsequently, the thallus was washed slowly with 50 mM phosphate buffer solution (pH 7.0) three times, and then 1 mL of glutaraldehyde (2.5%) was added and allowed at 4 °C overnight. The thallus was collected after the mixture was centrifuged at 5000 r/min for 5 min, mounted over the coverslip and dehydrated in a graded ethanol series. Finally, the specimen was critical-point dried with CO₂, sputter-coated with a thin layer of gold and observed under a JSM-6390LA scanning electron microscope (Hitachi, Japan).

Results and discussion

Antibacterial activity of questin

The antibacterial activity of questin against *V. harveyi* was measured by Oxford cup and tube dilution methods. The MIC values of questin determined by Oxford cup method against *V. harveyi* strain SZ-1 and strain 1.8690 were 31.25 μ g/mL (Table 1). Meanwhile, the MIC values of positive control streptomycin sulfate against *V. harveyi* strain

Table 1 MIC of questin against *V. harveyi* SZ-1 and 1.8690 by Oxford cup method

Compound	MIC ($\mu\text{g/mL}$)	
	<i>V. harveyi</i> SZ-1	<i>V. harveyi</i> 1.8690
Questin	31.25	31.25
Streptomycin sulfate	15.625	15.625

Table 2 MIC and MBC of questin against *V. harveyi* 1.8690 by tube dilution method

Compound	MIC ($\mu\text{g/mL}$)	MBC ($\mu\text{g/mL}$)
Questin	31.25	62.50
Streptomycin sulfate	31.25	62.50

Fig. 2 Effect of questin on the growth of *V. harveyi* 1.8690

SZ-1 and strain 1.8690 were 15.625 $\mu\text{g/mL}$. The MIC and MBC of questin measured by tube dilution method against *V. harveyi* 1.8690 were 31.25 and 62.5 $\mu\text{g/mL}$, respectively, the same inhibitory activity as streptomycin sulfate (Table 2).

Effect of questin on the growth of *V. harveyi* 1.8690

By plotting the growth curve of *V. harveyi* 1.8690 treated with or without questin (Fig. 2), it can be seen that the strain grew normally in the control group and reached the logarithmic growth stage in 12 h. However, onefold and twofold MIC of questin, and onefold MIC of streptomycin sulfate grew more slowly in the first 6 h, which indicated that questin and streptomycin sulfate can significantly inhibit the growth of *V. harveyi* at certain concentrations. *V. harveyi* 1.8690 treated with onefold and twofold MIC of questin, and onefold MIC of streptomycin sulfate began to slowly grow after 6 h and reached the logarithmic growth stage after 12 h. Thus, the inhibition of *V. harveyi* by questin and

Fig. 3 Effect of questin on the release of 260 nm absorbing materials of *V. harveyi* 1.8690

streptomycin sulfate gradually decreases with the consumption of drugs over time. The bacterial density of twofold MIC questin group was lower than that of onefold MIC of questin group. These results indicated that questin has considerable effects on the growth of *V. harveyi* with a concentration-dependent mode of action.

Effect of questin on the integrity and permeability of cell membrane of *V. harveyi* 1.8690

The integrity of cell membrane was determined by UV absorption assay and the effect of questin on the release of 260 nm absorbing materials of *V. harveyi* 1.8690 was shown in Fig. 3. The absorbance values at 260 nm in the two questin-treated groups were higher than that of onefold MIC of streptomycin sulfate at 0 h, which may be attributed to the UV absorption of questin itself at this wavelength. A significant increase in the absorbance at 260 nm was noticed in the bacterial culture treated with onefold and twofold MIC of questin, and onefold MIC of streptomycin sulfate. These finding indicated a more active release of intercellular substances, such as DNA or RNA, to the outer solution by cellular leakage and a higher concentration of questin result in more obvious leakage of the intracellular biological macromolecules.

The effect of questin on the permeability of the cell membrane of *V. harveyi* 1.8690 was determined by evaluating the leakage of phosphate ions using a phosphorus detection kit. The results shown in Fig. 4 indicated that the phosphorus contents of the two questin treated groups and the streptomycin sulfate treated group were higher than that of the control group. Thus, questin can induce leakage of phosphate ions from *V. harveyi* 1.8690 and a higher concentration of questin also has greater effect on the phosphorus leakage of *V. harveyi* 1.8690.

Fig. 4 Effect of questin on phosphorus leakage of *V. harveyi* 1.8690

Fig. 5 Effect of questin on extracellular AKP content of *V. harveyi* 1.8690

Effect of questin on the cell wall permeability of *V. harveyi* 1.8690

AKP is an enzyme that exists between the cell wall and membrane. Under normal circumstances, AKP activity cannot be detected outside the cell wall. However, the permeability of cell wall would increase after the cell wall was damaged, and then AKP is leaked out to the cellular outside (Su et al. 2012). The contents of extracellular AKP can reflect the changes of the cell wall permeability. The effect of questin on the cell wall permeability of *V. harveyi* 1.8690 was analysed using an AKP detection kit (Fig. 5). The AKP content of the control group was basically in a stable state, however, after treatment with different concentrations of questin, the AKP content rapidly increased to a higher level within 8 h and then basically levelled off. A higher concentration of questin treatment resulted in a higher content of

extracellular AKP. Thus, questin could damage the integrity of bacterial cell wall and increase the permeability of bacterial cell wall in a short time in a concentration-dependent manner.

Effect of questin on the cellular ultrastructure of *V. harveyi* 1.8690

The effect of questin on the cellular ultrastructure of *V. harveyi* 1.8690 was observed by scanning electron microscopy (Fig. 6). The cell morphology of untreated *V. harveyi* 1.8690 is short rod-shaped with elliptic at both ends, and there is no cell damage or content overflow, and the bacteria grows well without aggregation. The cells of *V. harveyi* 1.8690 treated with questin at onefold MIC concentration for 4 h showed abnormal morphology accompanied with distorted, deformed and shrivelled shapes. Some areas started to sag, and a small number of vesicles adhered to the surface of the bacteria. Furthermore, after treatment with twofold MIC questin for 4 h, the majority of cells ruptured and the cytoplasm leaked out, resulting in the blurring of cell edges. These results indicated that questin had an obvious destructive effect on the cell wall and cell membrane of *V. harveyi* 1.8690, thereby causing an efflux of cytoplasm with varying concentration. This effect was better than that of streptomycin sulfate.

Questin, also called as emodin-8-methyl ether, can be secreted by several kinds of marine-derived fungi such as *Eurotium rubrum*, *Pseudogymnoascus* sp., *Aspergillus* sp. B-F-2 and *Aspergillus* sp. F1, or isolated from plants such as *Cassia tora* and *Uvaria kurzii*. Questin exhibits antibacterial property against the plant pathogen *Fusarium oxysporium*, scavenging activity on DPPH free radical and weak cytotoxic activity (Li et al. 2009; Lin et al. 2009; Liu et al. 2006; Lv et al. 2011; Park and Kim 2011).

We have reported the optimized production and identification of questin with the antagonistic activities against *Vibrio* spp., such as *V. harveyi*, *V. anguillarum*, *V. cholerae* and *V. parahaemolyticus* (Guo and Wang 2017). In the current study, the antibacterial activity of questin on *V. harveyi* and its mechanism of action were studied. The results of MIC and MBC exhibited that questin possesses a bacteriostatic effect not only against different strains of *V. harveyi*, but also equivalent to that of streptomycin sulfate, indicating that questin has a favorable and universal activity against *V. harveyi*. Moreover, the mechanism of action was investigated by evaluating the effects of questin on the bacterial growth of *V. harveyi* 1.8690, integrity and permeability of cell membrane, permeability of cell wall and cellular morphology. One of the causes of leakage of cellular biomolecules, such as DNA, RNA, protein and phosphate ions after the action is that the antimicrobial substances destroy the cell wall, and then the cell membrane, thereby damaging the structure of

Fig. 6 Scanning electron microscope image of *V. harveyi* 1.8690 treated with questin and streptomycin sulfate

intracellular macromolecules and blocking the biochemical cascade that leads to the release of intracellular ions, nucleic acids, enzymes, as well as the hydrolysis of intracellular ATP. This phenomenon results in the morphological changes and increases the contents of nucleic acids, phosphate ions and AKP in the treated bacteria solution (Minahk et al. 2000; Patra and Baek 2016; Reddy et al. 2004; Su et al. 2012; Zhang et al. 2017). We also found that the inorganic phosphorus and intracellular biomacromolecules of *V. harveyi* 1.8690 treated by questin were leaked out, which occurred rapidly in a short time, eventually causing cellular deformation, lysis and death.

Conclusion

In conclusion, questin obtained from marine *A. flavipes* strain HN4-13 can be used as an antibacterial agent against the aquatic pathogen *V. harveyi*. The study on the mechanism of action revealed that questin can affect the permeability and integrity of the cell wall and membrane by binding to the cell surface, destroying the bacterial cell wall and cell membrane, consequently resulting in the unstable bacterial intracellular environment, cellular lysis and cell death. The results of this study provide basis for the exploitation and utilization of questin in preventing and controlling the aquatic pathogen *V. harveyi*.

Acknowledgements This work financially supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (CXKT20180216), Postgraduate Research & Practice Innovation Program of Jiangsu Province (KYCX18_2585), Natural Science Foundation of Jiangsu Province (BK20151283), Technical Plan Project of Lianyungang (CG1612) and 521 Talented Project of Lianyungang (KKC17001).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

References

- Cao H, He S, Wei R, Diong M, Lu L (2011) *Bacillus amyloliquefaciens* G1: a potential antagonistic bacterium against eel-pathogenic *Aeromonas hydrophila*. Evid Based Complement Alternat Med 2011:824104
- Gomes NG, Lefranc F, Kijjoa A, Kiss R (2015) Can some marine-derived fungal metabolites become actual anticancer agents? Mar Drugs 13:3950–3991
- Guo L, Wang C (2017) Optimized production and isolation of antibacterial agent from marine *Aspergillus flavipes* against *Vibrio harveyi*. 3 Biotech 7:383
- Guo L, Wang C, Zhu W, Xu F (2016) Bioassay-guided fractionation and identification of active substances from the fungus *Aspergillus*

- tubingensis* against *Vibrio anguillarum*. Biotechnol Biotech Eq 30:602–606
- Guo L, Guo J, Xu F (2017) Optimized extraction process and identification of antibacterial substances from Rhubarb against aquatic pathogenic *Vibrio harveyi*. 3 Biotech 7:377
- Harikrishnan R, Balasundaram C, Heo MS (2010) Effect of probiotics enriched diet on *Paralichthys olivaceus* infected with lymphocystis disease virus (LCDV). Fish Shellfish Immunol 29:868–874
- Jin L, Quan C, Hou X, Fan S (2016) Potential pharmacological resources: natural bioactive compounds from marine-derived fungi. Mar Drugs 14:76
- Kesarcodi-Watson A, Kaspar H, Lategan MJ, Gibson L (2008) Probiotics in aquaculture: the need, principles and mechanisms of action and screening processes. Aquaculture 274:1–14
- Li DL, Li XM, Wang BG (2009) Natural anthraquinone derivatives from a marine mangrove plant-derived endophytic fungus *Eurotium rubrum*: structural elucidation and DPPH radical scavenging activity. J Microbiol Biotechnol 19:675–680
- Li X, He C, Song L, Li T, Cui S, Zhang L, Jia Y (2017) Antimicrobial activity and mechanism of Larch bark procyanidins against *Staphylococcus aureus*. Acta Biochim Biophys Sin 49:1058–1066
- Lin T, Lu C, Shen Y (2009) Secondary metabolites of *Aspergillus* sp. F1, a commensal fungal strain of *Trewia nudiflora*. Nat Prod Res 23:77–85
- Liu R, Zhu W, Zhang Y, Zhu T, Liu H, Fang Y, Gu Q (2006) A new diphenyl ether from marine-derived fungus *Aspergillus* sp. B-F-2. J Antibiot (Tokyo) 59:362–365
- Lv Z, Zhang Q, Chen R, Yu D (2011) Alkaloids and anthraquinones from branches and leaves of *Uvaria kurzii*. Chin J Chin Mat Med 36:1190–1192
- Minahk CJ, Fariás ME, Sesma F, Morero RD (2000) Effect of enterocin CRL35 on *Listeria monocytogenes* cell membrane. FEMS Microbiol Lett 192:79–83
- Morya VK, Choi W, Kim EK (2014) Isolation and characterization of *Pseudoalteromonas* sp. from fermented Korean food, as an antagonist to *Vibrio harveyi*. Appl Microbiol Biotechnol 98:1389–1395
- Park YB, Kim SB (2011) Isolation and identification of antitumor promoters from the seeds of *Cassia tora*. J Microbiol Biotechnol 21:1043–1048
- Patra JK, Baek KH (2016) Antibacterial activity and action mechanism of the essential oil from *Enteromorpha linza* L. against foodborne pathogenic bacteria. Molecules 21:388
- Qu L, She P, Wang Y, Liu F, Zhang D, Chen L, Luo Z, Xu H, Qi Y, Wu Y (2016) Effects of norspermidine on *Pseudomonas aeruginosa* biofilm formation and eradication. Microbiologyopen 5:402–412
- Randrianarivelo R, Danthu P, Benoit C, Ruez P, Raherimandimby M, Sarter S (2010) Novel alternative to antibiotics in shrimp hatchery: effects of the essential oil of *Cinnamosma fragrans* on survival and bacterial concentration of *Penaeus monodon* larvae. J Appl Microbiol 109:642–650
- Reddy KV, Yedery RD, Aranha C (2004) Antimicrobial peptides: premises and promises. Int J Antimicrob Agents 24:536–547
- Song F, Ren B, Chen C, Yu K, Liu X, Zhang Y, Yang N, He H, Liu X, Dai H, Zhang L (2014) Three new sterigmatocystin analogues from marine-derived fungus *Aspergillus versicolor* MF359. Appl Microbiol Biotechnol 98:3753–3758
- Su Y, Zhang C, Wang Y, Li P (2012) Antibacterial property and mechanism of a novel Pu-erh tea nanofibrous membrane. Appl Microbiol Biotechnol 93:1663–1671
- Sun K, Li Y, Guo L, Wang Y, Liu P, Zhu W (2014) Indole diterpenoids and isocoumarin from the fungus, *Aspergillus flavus*, isolated from the prawn. *Penaeus vannamei* Mar Drugs 12:3970–3981
- Thompson J, Gregory S, Plummer S, Shields RJ, Rowley AF (2010) An in vitro and in vivo assessment of the potential of *Vibrio* spp. as probiotics for the Pacific white shrimp, *Litopenaeus vannamei*. J Appl Microbiol 109:1177–1187
- Turker H, Yıldırım AB (2015) Screening for antibacterial activity of some Turkish plants against fish pathogens: a possible alternative in the treatment of bacterial infections. Biotechnol Biotech Eq 29:281–288
- Wang C, Guo L, Hao J, Wang L, Zhu W (2016) α -Glucosidase inhibitors from the marine-derived fungus *Aspergillus flavipes* HN4-13. J Nat Prod 79:2977–2981
- Xu HM, Rong YJ, Zhao MX, Song B, Chi ZM (2014) Antibacterial activity of the lipopeptides produced by *Bacillus amyloliquefaciens* M1 against multidrug-resistant *Vibrio* spp. isolated from diseased marine animals. Appl Microbiol Biotechnol 98:127–136
- Yu M, Wang J, Tang K, Shi X, Wang S, Zhu WM, Zhang XH (2012) Purification and characterization of antibacterial compounds of *Pseudoalteromonas flavipulchra* JG1. Microbiology 158:835–842
- Yuan YY, Liu QM, Zhong YS, Liao FP, Lin JR (2012) Mechanism of CP7 antibacterial protein against *Aeromonas hydrophila*. Microbiology China 39:949–957
- Zhang L, Tian X, Kuang S, Liu G, Zhang C, Sun C (2017) Antagonistic activity and mode of action of phenazine-1-carboxylic acid, produced by marine bacterium *Pseudomonas aeruginosa* PA31x, against *Vibrio anguillarum* in vitro and in a zebrafish in vivo model. Front Microbiol 8:289