Development of pharmabiotics as antibiotic alternatives for seafood security and marine aquaculture health: two cases of study in Vietnam

Van Duy Nguyen[1*]

ABSTRACT: Pharmabiotics are any biological materials of gut microbes, including probiotics, bacteriocins, bacteriophages and bioactive molecules. They have been introduced in food, agriculture and clinical settings to replace the use of traditional antibiotics, which led to current threat posed by multi-drug resistant bacteria. This research aims to develop bacteriocins or bacteriocin-producing bacteria as new pharmabiotics for improving seafood security and marine aquaculture health. We screened bacteria isolated from Vietnamese traditional fermented foods and marine animals of interest to the aquaculture industry (lobster, tiger shrimp, otter clam, snubnose pompano and cobia) for antimicrobial and bacteriocin-like activities in order to uncover biodiversity of bacteriocin producers, and explore the potential application in seafood preservation and marine aquaculture. In total, 32 screened isolates showed antimicrobial activities and 15 of these exerted bacteriocin-like activities. Sequencing of 16S rRNA genes identified the isolates as members of the nine genera *Lactobacillus, Bacillus, Proteus*, *Providencia*, *Klebsiella*, *Alcaligenes*, *Enterococcus, Enterobacter* and *Cronobacter.* The bacteriocinogenic isolates showed a wide antimicrobial spectrum against foodborne and animal pathogens, which open the way to their potential use as drugs and probiotics in food, aquaculture, livestock and clinical settings. As the first case of study, two strains *Lactobacillus plantarum* T8 and T13 were found to produce bacteriocins Class I (Lantibiotics), which remained active at 121°C for 15 min, at pH 4-10 and with proteinase K but deactivated by α-chymotrypsin treatment. The application of culture extract from the strain T13 with cell concentration of 10^{10} CFU/ml or crude bacteriocin extract from the strain T8 with bacteriocin activity of 800 AU/ml was shown to prolong the chilling preservation of fresh cobia meat compared to control within first 7 days. As the second case of study, the protective effect of bacteriocinogenic *Bacillus* and *Lactobacillus* isolates was tested in aquaculture-raised spiny lobster (*Panulirus ornatus*) juveniles. Lobsters in the probiotic treatments displayed increased growth and reduced feed conversion rates after 60 days, and increased survival rate after pathogen *Vibrio owensii* DY05 challenge relative to the control. This study represents the first evidence of the use of bacteriocins or bacteriocin producers as biopreservatives for fresh cobia meat and as probiotics for lobsters.

Keywords: antimicrobials, aquaculture, bacteriocins, pharmabiotics, probiotics

Introduction

According to Alimentary Pharmabiotic Centre (APC) at University College Cork in Ireland, pharmabiotics are defined as any biological entity 'mined' from the gastrointestinal microbiota, including probiotics, bacteriocins, bacteriophages and bioactive molecules. They are believed to make significant impacts in the pharmaceutical, medical food and functional food sectors to adapt with the current challenge posed by multi-drug resistant bacteria. Among them, bacteriocins, or ribosomally synthesized antibiotic peptides or proteins, have been looking for a positive health benefit to the host including human, livestock, and aquaculture animals. While bacteriocin and probiotic therapies for humans and livestock were focused on, the application of bacteriocins for aquatic animals has not been considered following the increasing development of aquaculture farming. Therefore, an alternative approach to disease prevention in aquaculture is

[1] Institute of Biotechnology and Environment, Nha Trang University, Nha Trang, Khanh Hoa, Vietnam

* Corresponding author: duy.1981@yahoo.com

the use of pharmabiotics (bacteriocins, bacteriocin-producing bacteria) with at least dual role of anti- and pro-biotic activity (Gillor et al., 2008).

Bacteriocins were first identified almost 100 years ago as a heat-labile product present in *Escherichia coli* V and toxic to *E. coli* S. By now bacteriocins have been found in all major lineages of Bacteria and some members of the Archaea. Two main features distinguish the majority of bacteriocins from classical antibiotics: bacteriocins are ribosomally synthesized (Desriac et al., 2008) and have a broad or narrow killing spectrum (Cotter et al., 2005). To date, more than 400 bacteriocins have been characterized and classified mainly based on producer bacterial family, their molecular weight, their amino acid sequence homologies and/or gene cluster organization. Traditionally, bacteriocins were mainly isolated from lactic acid bacteria in food products for their applications in food preservation. Bacteriocins can be introduced into food in at least three different ways: produced *in situ* by bacterial hosts as starter cultures in fermented food; purified or semi-purified then directly added to food; or an ingredient based on a fermentate of a bacteriocin-producing bacterium can be used (Cotter et al., 2005). Bacteriocins have been shown to have potential in the biopreservation of meat, dairy products, canned food, fish, alcoholic beverages, salads, egg products, high-moisture bakery products, and fermented vegetables. Their effects are either alone, in combination with other methods of preservation, or through their incorporation into packaging film/food surfaces (Chen and Hoover, 2003).

Besides, marine bacteria are a wealthy source of diverse types of potentially useful antimicrobial compounds including bacteriocins and bacteriocin-like substance (BLIS). The high biodiversity in marine ecosystems with novel antimicrobial substances is, however, waiting to be discovered. Study on marine animal-associated microorganisms has shown that *Vibrio, Pseudoalteromonas, Aeromonas, Alteromonas*, and to the Cytophaga-Flavobacterium- Bacteroides group are the most dominant bacteria (Desriac et al., 2008). However, there are relatively few reports on bacteriocin or BLIS producing marine bacteria, even only a few studies have focused on marine bacterium isolation from marine animals and the search for their ability to produce bacteriocins. Currently, Wilson and his co-workers (Wilson et al., 2010) have isolated eight marine bacteria which produced proteinceous antibacterial substances from diverse marine invertebrates.

To screen bacteriocinogenic bacteria as probiotics in marine aquaculture, bacteria must firstly be isolated from marine sources to facilitate change in temperature and salinity in aquaculture farms. Secondly, potentially probiotic bacteria are screened under an *in vitro* test for antagonistic activities against the selected target pathogens (Sahu et al., 2008). Then two ways are possible: the use of inhibitory compounds as antibiotics or that of bacteria as probiotics. To be used as antibiotics, inhibitory compounds have to be defined the nature, mode of action and genetic aspects but purified bacteriocins do not seem to be cost-effective for aquaculture application, especially in Southeast Asian developing countries. Thus, the use of bacteriocinogenic bacteria as dual-functional probiotics becomes a more feasible approach.

In this conference report, we summary and update results from our research group on pharmabiotics in recent years including data

published previously (Nguyen et al., 2014a; Pham et al., 2014; Nguyen et al., 2014b).

Materials and Methods

Bacteria isolation

For marine probiotic isolation, total 29 samples of five species of marine animals were collected in Nha Trang and Cam Ranh Bay including cobia (*Rachycentron canadum)*, snubnose pompano (*Trachinotus blochii*), ornate spiny lobster (*Panulirus ornatus*), black tiger shrimp (*Penaeus monodon*) and otter clam (*Lutraria philippinarum*). Gut samples were homogenized with an appropriate volume of sterile 1% peptone water at 30°C for 24 h and serial dilutions plated onto TSB (Difco) supplemented with 1.5% agar and 1% NaCl. The plates will then be incubated at 30°C for 24-48 h. *(*Todorov and Dicks, 2009*)*. For bacteriocin-producing lactic acid bacteria isolation, ten samples of traditional Vietnamese fermented cabbage in some markets in Nha Trang, Vietnam were selected. Lactic acid bacteria were isolated as described above but MRS medium (Difco) without NaCl addition was used.

Assay for biological activities

Antibacterial activity was determined by agar-well diffusion method as described by Todorov and Dicks (2009) with plates overlaid with 3 ml soft agar containing 1.0 ´ 10^6 cells of selected indicator bacteria (**Table 1**). Bacteriocin production activity was defined as described above with some exceptions. After cell-free supernatants were obtained, pH of the supernatant fluid was adjusted to 7.0 with 1N NaOH and 1N HCl to remove the effect of organic acids, then treated with catalase (Promega, USA) at the final concentration of 0.5 mg/ml at 37°C for 30 mins. to remove the effect of hydrogen peroxide. To check the protein nature of bacteriocin, proteinase K and α-chymotrypsin (Promega, USA) at the final concentration of 1 mg/ml were treated with supernatant fluid at 50°C for 3 h.

Bacterial identification

The DNA of bacterial strains was extracted by alkaline lysis method using the kit Wizard®SV Genomic DNA Purification System (Promega, USA). Purified DNA samples were used as templates for amplification of 16S rDNA gene segments using eubacterial universal primers (Integrated DNA Technologies, USA), namely forward primer 16S-27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and reverse primer 16S-1492R (5'-ACG GCT ACC TTG TTA CGA CT-3'). The PCRs and gene sequencing were performed as described previously *(*Nguyen et al., 2004b*)*.

Fresh cobia meat preservation and quality evaluation

Fresh cobia (*Rachycenton canadum*) was washed and immersed into culture extracts of lactic acid bacteria (cell density ≥ 10^{10} CFU/ml) for 2 mins. or into crude bacteriocin extracts (bacteriocin activity 800 AU/ml) for 5 mins., packed in polyethylene bags, and stored at the temperatures of 0-4°C. Samples without bacteriocin or culture extracts immersion were used as controls. For sensory evaluation, samples were assessed on the basis of color, texture, flavor, elasticity, and taste. Five people were selected and trained during three 1-h sessions with this type samples. Chill stored cobia samples were compared with control which was taken as a reference value (five points for each parameter).

To define physicochemical change, total volatile basic nitrogen (TVB-N) was used by Conway's diffusion method (Conway, 1950). To determine microbiological quality, a portion of chilled cobia samples was inoculated with either *Salmonella typhimurium* or *Vibrio cholera* (10^7 CFU/ml) for 2 minutes. Then, survival was monitored by enumeration on selected RV (Difco) and TCBS (Difco) medium, respectively.

In vivo probiotic trials

Lobster juveniles (12.66 ± 1.12 g), supplied by a local lobster juvenile farm at Nha Trang Bay and shipped to Nha Trang University, Vietnam were merged and then randomly redistributed at a density of 10 animals per 75 L-tank. Experimental design and conditions were done as described previously *(*Nguyen et al., 2004b*)*. Freeze-dried probiotics (5% of weight of daily feeds ration) were mixed with one third of milled feeds for daily diets, then naturally dried in a sterile incubator for 30 to 60 mins. and finally covered by squid oil (10 - 20 ml/kg feed). Lobsters were fed firstly with the probiotic-supplemented feeds followed by the remaining two thirds of the daily feeds ration. Probiotics supplemented feeds were provided every third day for first 60 days only. From day 61, a 10-day pathogen challenge trial was started, in which juveniles in all tanks were challenged with *Vibrio owensii* DY05 at 1×10^7 CFU/ml. The urvival rate (SR), specific growth rates (SGR) and food conversion ratio (FCR) was defined as described previously *(*Nguyen et al., 2004b*)*. All statistical analyses were performed using the statistical software SPSS (IBM) with post-hoc Duncan's tests standardized at significance level of a= 0.05.

Results and discussion

Screening of bacteriocinogenic bacteria strains of Vietnam origin

Results have shown that total 69 strains of lactic acid bacteria were isolated from 10 samples of traditional Vietnamese fermented cabbage. Among 69 strains, 19 strains were found to express their antagonistic effect on at least two of food borne pathogenic and spoilage microorganisms as selected indicators with the diameter of inhibitory zone ≥ 5 mm. Among them, two strains T8 and T13, identified as *Lactobacillus plantarum* have the broadest, strongest and supplemented inhibitory spectra (**Table 1**). This may because they secrete not only organic acids but also bacteriocins. Their bacteriocins belong to Class I (Lantibiotic), which are stable at 121°C for 15 mins., at pH 4-10 and with proteinase K but deactivated by α-chymotrypsin *(*Nguyen et al., 2004a*)*.

From 29 samples of five animal species, total 228 bacterial isolates were isolated. The results have shown that 50 isolates were found to inhibit the growth of at least one of indicator microorganisms (**Table 1**). Among of them, three isolates D9, D10 and D15 from snubnose pompano, two isolates CT1.1 and G1 from cobia, B3.10.2B from black tiger shrimp, L5B from ornate spiny lobster, and six isolates H9, H18, H51, H61, H77 and H108 from otter clam showed relatively stable inhibitory zone diameters after their supernatants were neutralized and treated with catalase. As the cell-free supernatants of all these isolates were completely inactivated by proteinase K treatment, they were likely bacteriocin-like producers and would be referred to as bacteriocinogenic bacteria hereafter.

For bacterial identification, the 16S rRNA genes of all 15 bacteriocinogenic strains were amplified and sequenced. Following the sequence alignment, members of five different genera were identified, of which 10 were Gram-negative bacteria and only 5 were Gram-positive. Out of the 15 isolates identified, 5

belonged to the genus *Cronobacter*, 4 to *Proteus*, 2 to *Lactobacillus*, 2 to *Bacillus*, 1 to *Enterococcus,* and 1 to *Enterobacter*. *Proteus* strains were found in three different animal species whereas representatives of the other genera were unevenly distributed among the different host species. It is not surprised that almost all strains belong to the family Enterobacteriaceae, a large family of Gram-negative bacteria of many harmless symbionts and even more familiar pathogens. Members of this family are a normal part of the gut flora found in the intestines of humans and other animals.

Table 1 Source, identification and antimicrobial activity of 15 bacteriocinogenic bacteria strains of Vietnam origin

No.	Isolate	Source	16S rRNA gene	Identification	Sensitive indicator bacteria
1	D15	Snubnose pompano	KC213810	*Proteus mirabilis*	*Proteus mirabilis, Bacillus pumilus, Staphylococcus aureus, Vibrio owensii*
2	D10	Snubnose pompano	KC213809	*Proteus mirabilis*	*P. mirabilis, B. pumilus, S.aureus, V. owensii*, *B. cereus*
3	CT1.1	Cobia	KC213808	*Proteus* genomo-species 4	*B. cereus*
4	G1	Spiny lobster	KC213811	*Proteus* genomo-species 4	*P. mirabilis*, *E. coli, V. owensii, B. cereus*
5	H77	Otter clam	KC894669	*Enterobacter cloacae*	*Vibrio owensii, V. parahaemolyticus*
6	H9	Otter clam	KC894665	*Cronobacter sakazakii*	*V. parahaemolyticus, V. alginolyticus, Enterococcus* sp.
7	H18	Otter clam	KC894666	*C. sakazakii*	
8	H51	Otter clam	KC894667	*C. sakazakii*	
9	H61	Otter clam	KC894668	*C. sakazakii*	
10	H108	Otter clam	KC894670	*C. sakazakii*	
11	T8	Fermented cabbage	KC213804	*Lactobacillus plantarum*	*B. cereus, E. coli, Clostridium perfringens, Salmonella enterica, S.aureus, Vibrio* spp.
12	T13	Fermented cabbage	KC213805	*L. plantarum*	
13	L5B	Spiny lobster	KC894671	*Enterococcus faecalis*	*E. coli, P. mirabilis*
14	D9	Snubnose pompano	KC213798	*Bacillus cereus*	*P. mirabilis, V. owensii, B. pumilus, S. Enteric*
15	B3.10.2B	Black tiger shrimp	KC894663	*B. pumilus*	*P. mirabilis, V. owensii*

Source: (Nguyen et al., 2004a; Pham et al., 2004; Nguyen et al., 2004b).

Case of study 1: Development of bacteriocins as antibiotic alternatives for cobia meat preservation

This research aims to screen bacteriocin-producing lactic acid bacteria and investigate their application for the preservation of chilled fresh cobia meat. Therefore, we firstly determine the favourite culture conditions and maximum bacteriocin activity of two strains T8 and T13 identified above. The results showed that their favourite culture conditions for maximum bacteriocin production are on the MRS medium with glucose as carbon source and peptone as nitrogen source, pH of 6.4, and the temperature at 31°C. The maximum bacteriocin activity was obtained at the end of the logarite growth phase after 12 h of culture, followed by a centrifugation at 6000 rpm for 20 mins. In this condition, the yield for obtaining bacteriocin by the T8 and T13 was approximate 92.1% and 81.5%, and the bacteriocin activity was determined as 6000 AU/ml and 8000 AU/ml, respectively.

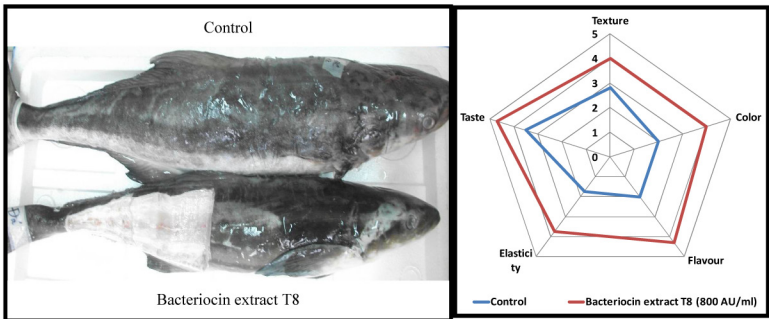

Figure 1 Fish picture (left) and sensory analysis (right) at the 7th day of fresh cobia preservation using the bacteriocin extract from *L. plantarum* T8 *(*Nguyen et al., 2004a*)*.

At low temperatures of 0-4°C, the application of culture extract from lactic acid bacteria strain T13 (cell concentration of 10^{10} CFU/ml) or crude bacteriocin extract from the strain T8 (bacteriocin activity of 800 AU/ml) was shown to prolong the preservation time of fresh cobia meat compared to control. This was revealed that the sensory quality (**Figure 1**), physicochemical change and microbiological counts of fresh cobia meat were well kept within first 7 days. Finally, the procedures of fresh cobia meat preservation using the culture extract from lactic acid bacteria strain T13 or crude bacteriocin extract from the strain T8 were successfully set up.

In this research, experiments on fresh cobia preservation using bacteriocins or their starter cultures were also carried out. Traditionally, a popularly-used and effective method of preserving the freshness of seafood in Vietnam and other countries is to chill with ice (Nhu et al., 2011; Shakila et al., 2012), which requires a large mass of ice, large ice container and transporter leading to a high preservation cost. Moreover, the recent development in cobia production in Vietnam made this nation becomes the 3rd largest producer of farmed cobia in the world, which requires the advanced technologies for cobia processing and preservation (Nhu et al., 2011).

Despite the recent progress in food preservation technologies and safety concepts, the problem of seafood safety and security remains to be solved. We report here the application of culture extract from lactic acid bacteria strain T13 (cell density of 10^{10} CFU/ml) or crude bacteriocin extract from the strain T8 (bacteriocin activity of 800 AU/ml) was shown to prolong the chilling preservation time of fresh cobia meat. It was reported for the first time in Vietnam about some characteristics of bacteriocin Class I (Lantibiotic). Study on using bacteriocin-producing lactic acid bacteria or their bacteriocin extract for the preservation of aquatic food products has been just progress. It is the first research on this topic applied in cobia meat.

Case of study 2: Development of potential probiotics for lobster health management

In vitro tests showed that *Vibrio owensii* DY05, a pathogenic agent of ornate spiny lobster larvae and juveniles, was inhibited by cell-free supernatants of *Proteus* spp. (D10, D15, T9, T14), *Enterococcus faecalis* L5B, *Bacillus cereus* D9, *Bacillus pumilus* B3.10.2B and *Lactobacillus plantarum* T8 and T13 (**Table 1**), indicating the potential of these bacteria for the development as probiotics in lobster culture. While further study on the pathogenicity of *Proteus* and *Enterococcus* isolates is required, *Lactobacillus, B. pumilus* and many other *Bacillus* species have been considered as safe probiotics for human and animals. Therefore, these strains were formulated into candidate probiotics BioLobster 1 and Biolobster 2 as below. BioLobster 1 was a single-strain probiotic of *Bacillus pumilus* B3.10.2B at 1.0×10^9 CFU/ml whereas BioLobster 2 was a multi-strain probiotic of *Bacillus pumilus* B3.10.2B, *Bacillus cereus* D9 and *Lactobacillus plantarum* T13, all at 1.0×10^9 CFU/ml

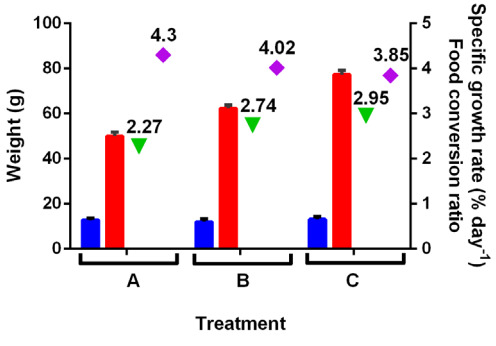

Figure 2 Average individual mass of *P. ornatus* juveniles after pathogen challenge and probiotic admistration. The body weight at the initial time (1st column) and 60 days after probiotic supplementation (2nd column). Specific growth rate (3rd column) and food conversion ratio (4th column) calculated and given. A: Control without any probiotic supplementation, B: Supplemented with probiotic BioLobster 1, C: Supplemented with probiotic BioLobster 2. Weight expressed as Means ± SD (Nguyen et al., 2014b).

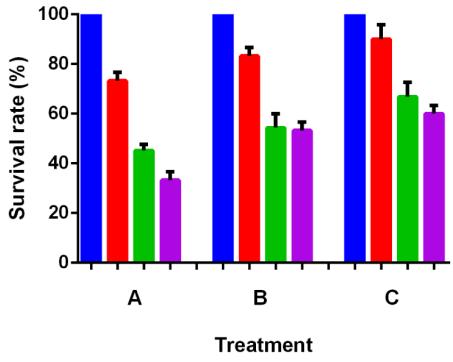

Figure 3 Survival rate of *P. ornatus* juveniles after pathogen challenge and probiotic admistration. Survival rate of lobsters at the initial time (1st column), 60 days after probiotic supplementation (2nd column) and 10 days after *Vibrio owensii* DY05 infection at 107 CFU/ml (3rd column). Challenge-specific survival rate (4th column) was calculated based on survival rate after pathogen infection divided by survival rate after probiotic supplement. A: Control without any probiotic supplementation, B: Supplemented with probiotic BioLobster 1, C: Supplemented with probiotic BioLobster 2. Survival rate expressed as Means ± SD (Nguyen et al., 2014b).

The results showed that weight gain was significantly enhanced in juveniles fed with BioLobster 1 (Duncan's test $P<0.05$) and significantly higher with BioLobster 2 (Duncan's test $P<0.05$) compared to the non-supplemented control after 60 days (**Figure 2**). The specific growth rate of juveniles supplemented with BioLobster 2 was the highest, followed by the BioLobster 1 supplemented group and finally the control. The food conversion ratio was reduced after the probiotic administration in comparison with the control. In addition, survival rate of juveniles was recorded after probiotic administration for 60 days and after challenge with *V. owensii* DY05 at 10^7 CFU/ml for 10 days (**Figure 3**). The challenge specific survival rate of juveniles administrated with either BioLobster 1 (60%) or BioLobster 2 (53.3%) was significantly enhanced relative to the control (33.3%) (Duncan's test $P<0.05$), though there was no significant difference between the two probiotic treatments (Duncan's test $P>0.05$).

It has also previously been shown that niche specialization can contribute to the additive protection of certain probiont combinations (Goulden et al., 013). It would therefore be interesting to explore whether addition of probiotic strains to the tank water could provide additional protection of *P. ornatus* juveniles against *V. owensii* DY05, especially because immersion challenge appeared to cause little or no colonization of lobster tissues and disease was likely caused by production of *Vibrio* exoenzymes. In a previous study, a two-strain probiotic combination of *Vibrio* sp. PP05 and *Pseudoalteromonas* sp. PP107 provided efficient protection of *P. ornatus* phyllosoma larvae against experimental *V. owensii* DY05 infection (Goulden et al., 2012). The bacteriocinogenic probiotics developed in this study provide additional options for sustainable management of vibriosis in *P. ornatus* and provides further evidence that probiotics should be part of a strategy to develop a sustainable aquaculture industry for *P. ornatus*.

Conclusions

Novel bacteriocinogenic bacteria or bacteriocins could potentially be used as pharmabiotics in aquaculture or food additives. Potential application of the present study for the development of antibiotics, probiotics and food biopreservatives will be expected to reduce bacterial diseases in marine aquaculture and prolong the preservation time of foods. The success of further study would surely contribute into sustainable development of aquaculture for food security and bring to regional farmers a hope to recoup their losses, improve their life quality and protect regional environment.

References

Chen, H., and D. G. Hoover. 2003. Bacteriocins and their food applications. Comp. Rev. Food Sci. Food Safety. 2: 82–100.

Conway, E. J. 1950. Microdiffusion analysis and volumetric error (p.342). Lockwood and Son Ltd. Crosby, London.

Cotter, P.D., C. Hill, and P. R. Ross. 2005. Bacteriocins: developing innate immunity for food. Nat. Rev. Microbiol. 3(10): 777–788.

Desriac, F., D. Defer, N. Bourgougnon, B. Brillet, P. Le Chevalier, and Y. Fleury. 2010. Bacteriocin as Weapons in the Marine Animal-Associated Bacteria Warfare: Inventory and Potential Applications as an Aquaculture Probiotic. Mar. Drugs. 8: 1153-1177.

Goulden, E. F., M. R. Hall, L. L. Pereg, and L. Hoj. 2012. Identification of an antagonistic probiotic combination protecting ornate spiny lobster (*Panulirus ornatus*) larvae against *Vibrio owensii* infection. PLoS. One. 7: e39667.

Goulden, E. F., M. R. Hall, L. L. Pereg, B. K. Baillie, and L. Hoj. 2013. Probiont niche specialization contributes to additive protection against *Vibrio owensii* in spiny lobster larvae. Environ. Microbiol. Rep. 5: 39-48.

Nguyen, V.D., T. T. Pham, and N. M. Q. Pham. 2014a. Two novel strains of bacteriocin-producing *Lactobacillus plantarum* and their application as biopreservative in chill-stored fresh cobia meat. J. Pure Appl. Microbiol. 8(2): 1547-1557.

Nguyen, V. D., T. T. Pham, T. H. T. Nguyen, T. T. X. Nguyen, and L. Hoj. 2014b. Screening of marine bacteria with bacteriocin-like activities and probiotic potential for ornate spiny lobster (*Panulirus ornatus*) juvenile. Fish Shellfish Immunol. 40(1): 49-60.

Nhu, V. C., H. Q. Nguyen, T. L. Le, M. T. Tran, P. Sorgeloos, K. Dierkens, H. Reinertsen, E. Kjørsvik, and N. Svennevig. 2011. Cobia *Rachycentron canadum* aquaculture in Vietnam: Recent developments and prospects. Aquaculture. 315(1-2): 20-25.Gillor, O., A. Etzion, and M. A. Riley. 2008. The dual role of bacteriocins as anti- and probiotics. Appl. Microbiol. Biotechnol. 81(4): 591–606.

Pham, T.T., T. H. N. Ho, and V. D. Nguyen. 2014. Screening for bacteriocin-like antimicrobial activity against shrimp pathogenic vibrios and molecular identification of marine bacteria from otter clam Lutraria philippinarum. Thai J. Vet. Med. 44(3): 345-353.

Sahu, M., N. Swarnakumar, K. Sivakumar, T. Thangaradjou, and L. Kannan. 2008. Probiotics in aquaculture: importance and future perspectives. Indian J. Microbiol. 48: 299–308.

Shakila, R. J., B. E. Raj, and N. Felix. 2012. Quality and safety of fish curry processed by sous vide cook chilled and hot filled technology process during refrigerated storage. Food Sci. Technol. Int. 18(3): 261-9.

Todorov, S. D., and L. M. Dicks. 2009. Bacteriocin production by Pediococcus pentosaceus isolated from marula (Scerocarya birrea). Int. J. Food Microbiol. 132(2-3): 117-26.

Wilson, G. S., D.A. Raftos, S. L. Corrigan, and S.V. Nair. 2010. Diversity and antimicrobial activities of surface-attached marine bacteria from Sydney Harbour, Australia. Microbiol. Res.165(4): 300-11.