# ANTIMICROBIAL ACTIVITY AND CHARACTERIZATION OF CRUDE BACTERIOCIN PRODUCED BY MARINE BACTERIA ISOLATED FROM VIETNAM

**Nguyen Thi Hai Thanh, Nguyen Thi Hong Mai, Nguyen Van Duy**
*Institute of Biotechnology and Environment, Nha Trang University*

## SUMMARY

Bacteriocins are antimicrobial peptides or proteins synthesized ribosomally by a bacterium that inhibits the growth of other bacteria, especially closely relative strains. As a result, bacteriocins can be used as potential drugs for human and animal health. In this study, we assessed the bacteriocinogenic potential and antimicrobial spectrum of 12 marine bacteria strains isolated from cobia (*Rachycentron canadum*), snubnose pompano (*Trachinotus blochii*), black tiger shrimp (*Penaeus monodon*) and ornate spiny lobster (*Panulirus ornatus*) in Nha Trang Bay and Cam Ranh Bay against together themselves and against food-borne and animal pathogens. The indicator bacteria, which were shown to be sensitive to at least one of crude bacteriocins in our study, included *E. coli*, *Proteus mirabilis*, *Clostridium perfringens*, *Salmonella enterica*, *Vibrio owensii*, *V. parahaemolyticus*, *Staphylococcus aureus*, and *Bacillus cereus*. In addition, the effect of temperature, pH and enzymes on the stability and activity of crude bacteriocin produced by these strains was also determined. The results have indicated that they were completely inactivated when treated with proteinase K, but stable to trypsin and catalase treatment. Most of bacteriocin was resistent to the treatment at 60°C in 15 min, but lost 75% their activity after 30 min at 60°C and completely inactivated at 100°C for 30 min. However, they remained active after incubation at pH 4-10. The determination of antimicrobial activity and characterization of crude bacteriocin contribute into studying on bacteriocin diversity in marine bacteria isolated from VietNam and further their applications in medicine and agriculture.

*Keywords*: antimicrobial activity, bacteriocin, marine bacteria, pathogen, physico-chemical characteristic

## INTRODUCTION

The invasion development of aquaculture farming at the industrial scale, addition to climate changes increases associated infectious epizootes (Desriac *et al.*, 2010). Prophylactic antibiotics can control epidemic diseases, but may induce an antibiotic-resistant mechanism in bacteria and an accumulation of unused residues of drugs in the environment which, in turn, results in longterm negative effects on human and animal health (Gillor *et al.*, 2008). That's why scientific communities have proposed friendly alternatives such as vaccines, antibiotic substitutes or use of probiotics. Bacteriocinogenic bacteria strains appear to be an excellent candidate for a sustainable alternative since bacteriocin would be used as an antibiotic substitute, whereas bacteria would be a potential probiotic (Gillor *et al.*, 2008).

Bacteriocins are antimicrobial peptides or proteins synthesized ribosomally by a bacterium that inhibits the growth of other bacteria, especially closely relative strains. They have a relatively narrow killing spectrum (Riley, 2009). However, inhibitory spectra of bacteriocins or bacteriocin-like substance (BLIS) produced by Gram - positive bacteria are often much more broadly than most of bacteriocins produced by Gram - negatives. In general, they tend to be active against a wide range of Gram-positive bacteria, and sometime inhibit Gram-negative species. Moreover, the degree of activity of bacteriocin-like agents against sensitive bacteria can sometimes be substantially increased by testing either at particular pH values or in the presence of chemical agents that weaken cell wall integrity (Abee *et al.*, 1995; Gillor *et al.*, 2008).

Bactenocins have been classified in four distinct classes on the basis of biochemical and genetic characterization: (I) lantibiotics, (II) small heat-stable, non-lanthionine peptides including (IIa) *Listeria*-active peptides, (IIb) incorporation complexes consisting of two peptides for activity and (IIc) thiol-activated peptides, (III) large heat-labile proteins, and (IV) complex bacteriocins, carrying lipid or carbohydrate moieties (Riley, 2009). Bacteria strains, which produce bacteriocins classes I and II, have been suggested as promising probiotics. Bacteriocins produced by lactic acid bacteria (LAB) have studied the most, showing their remarkable potential as food conservatives (Abee *et al.*, 1995), or as therapeutics for veterinary or medical uses (Riley, 2009). Nevertheless, only a few studies have focused on marine bacterium isolation from marine animals and the search for their ability to produce bacteriocins (Desriac *et al.*, 2010).

In this study, we assessed bacteriocinogenic potential and antimicrobial spectrum of 12 marine bacteria strains isolated from cobia (*Rachycentron canadum*), snubnose pompano (*Trachinotus blochii*), black tiger shrimp (*Penaeus monodon*) and ornate spiny lobster (*Panulirus ornatus*) in Nha Trang Bay and Cam Ranh Bay against together themselves and against food-borne and animal pathogens. In addition, the sensitivity to heat, pH and enzymes of crude bacteriocins were also investigated and discussed. These knowledges are basis to apply bacteriocins as food preservatives or new drugs in medicine and aquaculture in Vietnam.

## MATERIALS AND METHODS

### Strains and growth conditions

Total 12 bacteriocin producing strains were screened from hundreds of marine bacteria strains isolated from the gut of cobia (*Rachycentron canadum*), snubnose pompano (*Trachinotus blochii*), black tiger shrimp (*Penaeus monodon*) and ornate spiny lobster (*Panulirus ornatus*) in Nha Trang Bay and Cam Ranh Bay (Nguyen Van Duy *et al.*, 2012; Nguyen Van Duy and Pham Thu Thuy, 2012, Nguyen Van Duy and Nguyen Thi Ngoc Thanh, 2012). The indicator strains include food-borne and animal pathogens supported from Nha Trang University (strains Sal1, C1, B1.1), Institute of Veterinary Research and Development of Central Vietnam (strains GA, CP, SA), Nha Trang Pasteur Institute (strain VP2865) and Australian Institute of Marine Science (strain DY05) (Table 1). All bacteria were grown aerobically in trypticase soy broth (TSB) (HiMedia, India) supplemented with 2% NaCl at 30°C for 1 day on a rotary shaker at 180 rpm per min.

**Chemicals and reagents**

Proteinase K 10 IU $mg^{-1}$, trypsin 10 000 BAEE U $mg^{-1}$, α-chymotrypsin 10 000 BAEE U $mg^{-1}$, α-amylase 200 IU $mg^{-1}$ and lipase 20 000 IU $mg^{-1}$ were purchased from Promega (USA). Other chemicals and reagents were from Merck (Germany).

**Assay of antimicrobial activity**

Antimicrobial activity was determined by agar-well diffusion method as described by Todorov and Dicks (2009) with some modifications. Selected marine bacteria were inoculated into TSB medium and incubated at 30°C with agitation until mid logarithmic phage of growth ($OD_{600}$ = 0.8 - 1.5). Then cell-free supernatants were harvested by centrifugation at 6000 rpm/min for 30 min at 4°C. Plates were overlaid with 3 ml soft agar containing $10^8$ cells per ml of indicator bacteria. Wells were cut and 100 µl of the supernatant of $10^8$ cells per ml of growing isolates was loaded into each well. Plates were incubated at 37°C for 24 - 36 h and then the diameter of inhibitory zone around the well was measured.

**Assay of bacteriocin activity**

Bacteriocin activity was determined by agar-well diffusion method as described above with some modifications. The pH of the supernatant fluid was adjusted to 7.0 with 1N NaOH or 1N HCl to remove the effect of organic acid and then treated with catalase (Promega, USA) at the final concentration of 0.5 mg $ml^{-1}$ at 37°C for 30 min to remove the effect of hydrogen peroxide. To check the protein property of bacteriocin, proteinase K or trypsin (Promega, USA) at the final concentration of 1 mg $ml^{-1}$ were treated with supernatant at 50°C for 3 hours.

**Effect of physiochemical factors on the activity of crude bacteriocin**

***The effect of enzymes***

The supernatant was first adjusted pH to 7.0 and treated with catalase as described above. To check the chemical property of bacteriocin, proteinase K, trypsin, α-chymotrypsin and lipase or α-amylase (Promega, USA) at a final concentration of 1 mg $ml^{-1}$ were added. Afterward, the supernatant were incubated at their optimal temperatures as recommended by the manufacture. In particular, trypsin and proteinase K were incubated at 50°C for 3 hours, and lipase and α-amylase at 37°C and 20°C for 2 hours, respectively. The residual activity after enzyme treatment was determined as described previously.

***Heat and pH stability***

For the determination of heat stability, the cell-free neutralized supernatant of bacteriocin was preincubated at different temperatures 60-100°C for 15 - 30 min and 121°C for 15 min. The remaining bacteriocin activity was checked by agar-well diffusion method as described above. Similarly, the effect of pH on the bacteriocin was determined by adjusting the cell-free supernatant to pH 2.0–12.0 with 1 N HCl or 1 N NaOH. After 30 min of incubation at 30°C, the samples were readjusted to pH 7.0 and the bacteriocin activity was determined (Todorov and Dicks, 2009).

## RESULTS AND DISCUSSIONS

**Antimicrobial spectra of 12 marine bacteria strains**

The results from Table 1 have shown that the cell-free supernatants from all 12 isolates exhibited antimicrobial activity against at least one of selected indicators. Among them, the culture extracts of bacteria isolated from snubnose pompano (*T. blochi*) such as D9.1, D10 and D15 inhibited the growth of most of indicators with the strongest activity (20 - 40 mm in inhibitory zone diameter) against *Proteus* spp. (N1.4, T9, T14, B3.7.1), *V. owensii* DY05, *Bacillus pumilus* B3.10.2B, *B. cereus* B1.1 and *Staphylococcus aureus* SA. In addition, the strains L5B and M2, which originated from spiny lobster (*P. ornatus*), showed their strong antimicrobial activity against *Proteus* spp. (CT1.1, T9, T14) and *E. coli* GA with the inhibitory zone diameter more than 30 mm. Nevertheless, the strain N1.4 was indicated as the weakest inhibitory agent that only slightly prevent 6/21 indicator strains from the growth with relatively small inhibitory zone diameter

Antimicrobial activity plays an important role for a bacterium in community to exclude or inhibit competitive bacteria, which enable to be performed through producing non-specific antimicrobial substances, such as organic acids or hydrogen peroxide, and target-specific toxins such as bacteriocins or BLIS, and bacteriophages (Riley, 2009; Desriac *et al.*, 2010). In this research, 12 marine bacteria strains showed their wide antimicrobial spectra against several important food-borne and animal pathogens. For example, *E. coli* strains can cause serious food poisoning in humans and gut diseases in animals, while *S. aureus* is a common cause of skin infections, respiratory disease and also food poisoning in humans. Some strains of *B. cereus* and *Salmonella* are also harmful to humans and cause foodborne illness (Abee *et al.*, 1995).

In addition, the *Vibrio* strains, one of the most important pathogens recognized in larval cultures, provoking a high mortality in marine animals (Desriac *et al.*, 2010) were inhibited significantly by cell-free supernatants of strains D9.1, D10, D15 and L5B. Interestingly, *Vibrio owensii* strain DY05 has just recently been demonstrated as an agent of disease causing rapid and high mortality in phyllosoma larva of ornate spiny lobster (*P. ornatus)* cultured in Australia (Goulden *et al.*, 2012). In our research, *Proteus* spp. strains isolated from snubnose pompano (strains D10 and D15) and lobster (strains T9 and T14) expressed their strong antagonistic activity against the lobster pathogen DY05, indicating the potential of these bacteria for the development as probiotics in lobster culture. However, further study on the pathogenicity of these potential probiotics is required. In addition, some *Proteus* strains (CT1.1, G1, N1.4, T9 and B3 7.1) with antimicrobial activity found in this study could belong to novel species with undetermined toxicity (Nguyen Van Duy and Pham Thu Thuy, 2012; Nguyen Van Duy and Nguyen Thi Ngoc Thanh, 2012).

**Table 1. Antimicrobial activity of cell-free supernatants from 12 marine bacteria strains of Vietnam origin against together themselves and against food-borne & animal pathogens**

| Animal hosts | | Cobia | | | | Snubnose pompano | Ornate spiny lobster | | Black tiger shrimp | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Isolates / Indicators | CT1.1 | D9.1 (CR9.1) | D10 (CR10) | D15 (CR15) | G1 | L5B (R2) | M2 | N1.4 | T9 | T14 | B3.10.2B | B3.7.1 |
| Against together | *Proteus* sp. CT1.1 | 0 (*) | + | + | + | + | + | +++ | + | + | + | + | + |
| | *Bacillus cereus* D9.1 | 0 | 0 | + | + | 0 | + | 0 | + | + | + | + | 0 |
| | *P. mirabilis* D10 | 0 | + | 0 | 0 | +++ | ++ | ++ | + | 0 | + | 0 | + |
| | *P. mirabilis* D15 | + | + | + | 0 | + | + | ++ | + | + | + | ++ | ++ |
| | *Proteus* sp. G1 | 0 | + | + | + | 0 | 0 | + | 0 | + | 0 | + | + |
| | *Enterococcus faecalis* L5B | + | 0 | + | + | 0 | 0 | 0 | 0 | ++ | + | + | 0 |
| | *Klebsiella pneumoniae* M2 | 0 | + | + | + | + | ++ | 0 | + | 0 | + | + | + |
| | *Proteus* sp. N1.4 | 0 | +++ | + | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 |
| | *Proteus* sp. T9 | + | +++ | ++ | ++ | +++ | +++ | +++ | 0 | 0 | 0 | 0 | 0 |
| | *P. mirabilis* T14 | + | +++ | ++ | ++ | ++ | +++ | + | +++ | +++ | 0 | +++ | ++ |
| | *B. pumilus* B3.10.2B | 0 | +++ | ++ | ++ | 0 | 0 | + | 0 | 0 | 0 | 0 | 0 |
| | *Proteus* sp. B3.7.1 | 0 | +++ | +++ | ++ | 0 | 0 | + | 0 | 0 | 0 | ++ | 0 |
| Against food-borne & animal pathogens | *Escherichia coli* GA | + | + | + | + | 0 | +++ | + | 0 | 0 | ++ | 0 | 0 |
| | *Staphylococcus aureus* SA | 0 | 0 | ++ | ++ | 0 | 0 | + | 0 | 0 | + | 0 | 0 |
| | *Clostridium perfringens* CP | 0 | + | + | + | + | 0 | 0 | 0 | 0 | 0 | 0 | + |
| | *Salmonella enterica* Sal1 | 0 | ++ | + | + | + | 0 | 0 | 0 | + | 0 | + | + |
| | *Vibrio parahaemolyticus* C1 | 0 | 0 | + | + | 0 | 0 | 0 | 0 | 0 | + | + | + |
| | *V. parahaemolyticus* VP286S | 0 | + | + | + | 0 | + | 0 | 0 | 0 | 0 | 0 | 0 |
| | *V. owensii* DY05 | + | + | ++ | +++ | 0 | + | + | 0 | ++ | ++ | 0 | 0 |
| | *B. cereus* B1.1 | ++ | + | ++ | + | ++ | 0 | 0 | 0 | + | + | 0 | 0 |

*(*) The diameter of inhibitory zone +, >10 mm, ++, >20 mm; +++, >30 mm; 0, no inhibition zone*

**Bacteriocin activity of 12 marine bacteria strains**

The most sensitive indicators of 12 strains of research interest were selected for screening bacteriocin activity of marine bacteria. After neutralized to pH7 to exclude the effect of organic acids, and then treated with catalase to remove the effect of hydrogen peroxide, cell-free supernatants of 12 marine bacteria strains were spotted onto soft agar plates seeded with selected sensitive indicators. The results were presented in Table 2.

**Table 2. Bacteriocin activity of 12 marine bacteria of Vietnam origin against sensitive indicators**

| No. | Isolates | Inhibitory zone diameter (D-d, mm) | Sensitive indicators |
|---|---|---|---|
| 1 | L5B | 23 | *P. mirabilis* T14 |
| 2 | D15 | 15 | *P. mirabilis* T14 |
| 3 | D10 | 14.5 | *P. mirabilis* T14 |
| 4 | G1 | 14 | *Proteus* sp. T9 |
| 5 | CT1.1 | 13.5 | *P. mirabilis* T14 |
| 6 | D9.1 | 12 | *P. mirabilis* T14 |
| 7 | B3.7.1 | 7 | *S. enterica* Sal1 |
| 8 | B3.10.2B | 5 | *S. enterica* Sal1 |
| 9 | T9 | 5 | *S. enterica* Sal1 |
| 10 | T14 | 4 | *K. pneumoniae* M2 |
| 11 | M2 | 4 | *Proteus* sp. T9 |
| 12 | N1.4 | 4 | *Proteus* sp. CT1.1 |

The results from Table 2 have indicated the cell-free neutralized supernatants of 6 strains (B3.7.1, B3.10.2B, T9, T14, M2 and N1.4) much smaller in inhibitory zone diameter than their natural supernatants, suggesting that their antimicrobial activities could result from organic acid production. In the contrary, the activity of 6 remaining strains were found to be relatively stable after their supernatants were neutralized. Even no inhibitory activity of these strains was observed after proteinase K treatment, indicating the proteinaceous nature of inhibitory compounds, which may be bacteriocins or BLIS. These results are similar to the bacteriocin activity reported in 5 *Pseudoalteromonas* isolates and 3 unidentified isolates from marine animals in Australia (Wilson *et al.*, 2010) and in *Vibrio* strains isolated from seahorses (Balcazar *et al.*, 2010). Thus, 6 these strains were selected for further study on physico-chemical characteristics

**Effect of enzymes, pH and temperature on bacteriocin activity**

Crude bacteriocins from 6 selected strains were tested for their sensitivity to various enzymes (Table 3 and Figure 1). The antimicrobial activity remained or little decreased after catalase treatment, indicating that inhibitory activity was not caused by $H_2O_2$. Although crude bacteriocins of all of 6 strains were completely inactived by proteinase K treatment, inhibitory activity of supernatants produced by 6 strains was different after the exposure to trypsin and chymotrypsin. In particular, a complete inactivation was observed when bacteriocin extracts of two strains CT 1.1 and G1 were treated by these two enzymes, while the activity of bacteriocins produced by four remaining strains seemed to be unchanged.

**Table 3. Effect of enzymes, pH and temperature on antimicrobial activity of crude bacteriocins**

| Treatment | Inhibitory zone diameter (D-d, mm) | | | | | |
|---|---|---|---|---|---|---|
| | CT1.1 | G1 | D9.1 | D10 | D15 | L5B |
| Control (*) | 13.5 | 14 | 12 | 14.5 | 15 | 23 |
| ***Enzyme treatment*** | | | | | | |
| Catalase | 13 | 12 | 12 | 14 | 8 | 21 |
| Proteinase K | - | - | - | - | - | - |
| Trypsin | - | | 12 | 8 | 8 | 23 |
| α-Chymotrypsin | - | | 11 | 9 | 8 | 21 |
| α-Amylase | 13.5 | | 12 | 6 | 10 | 23 |
| Lipase | 13.5 | | 11 | 8 | 9.5 | 23 |
| ***pH treatment*** | | | | | | |
| pH 2 | - | - | - | - | - | - |
| pH 4 | 4 | 4 | 4 | 14 | 12 | 9.5 |
| pH 6 | 11 | 8 | 11 | 14 | 15 | 18 |
| pH 7 | 13.5 | 13 5 | 12 | 14.5 | 15 | 23 |
| pH 8 | 12 | 14 | 8 | 7 | 7 | 13 |
| pH 10 | 8 | 7.5 | 6 | 5 | 6 | 8 |
| pH 12 | - | - | 4 | - | - | 4 |
| ***Heat treatment*** | | | | | | |
| 60°C, 15 min | 13.5 | 13 | 11 | 13 | 6 | 18 |
| 60°C, 30 min | 10 | 11 | - | - | 4 | - |
| 100°C, 15 min | 8 | 9 | | - | - | - |
| 100°C, 30 min | - | 4 | | - | - | - |
| 121°C, 15 min | - | - | - | - | - | - |

*(*) Control: cell-free neutralized supernatants without any treatments, - : no antimicrobial activity*

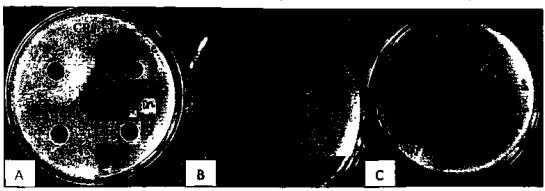


**Figure 1. Effect of enzymes on antimicrobial activity of crude bacteriocins produced by bacteria strains D9.1 (CR9.1) (A), CT1.1 (B) and D15 (CR15) (C).** *BC: control; samples treated with enzymes presented on plates.*

These results are in agreement with results recorded by previous studies, e.g., nisin, plantaricin C and plantaricin D as food biopreservatives applied all over the world (Abee *et al.*, 1995; Gillor *et al.*, 2008). Also, bacteriocin G2 isolated *Lactobacillus plantarum* G2 was inactivated by trypsin treatment but remained inhibitory activity after proteinase K and chymotrypsin treatment (Seatovic *et al.*, 2011). This can be explained by different cleavage mechanisms of types of proteases resulting from the diversity of amino acid sequences in polypeptides. In addition, treatment with α-amylase and lipase did not significantly affect the antimicrobial activity, except for the strain G1, suggesting that the bacteriocins from these strains were not attached to a carbohydrate or lipid moiety (Riley, 2009).

Moreover, inhibitory activity of bacteriocins produced by 6 isolates was found to be stable in the pH range 4.0 to 10.0 (Table 3), even the activity of bacteriocins from D9.1 and L5B still remained 50% at pH 2. These results suggest the potential application of these marine bacteria as probiotics or multi - drugs in aquaculture because bacteriocins produced by them can act in fluctuating pH environments.

Finally, bacteriocins from 6 marine strains were heat-labile bacteriocins (Table 3). Most of them were resistent to the heat treatment at 60°C in 15 min, but lost 75% their activity afer 30 min at 60°C and completely inactivated at 100°C for 30 min or at 121°C for 15 min. Remarkedly, antimicrobal activity of bacteriocins from L5B, D9.1 and D10 was lost completely after incubation at 60°C for 30 min only. These results are similar to data recorded by bacteriocin class III, including large, heat-labile bacteriocins (Riley, 2009), suggesting that the bacteriocins from these isolates in our research could belong to Class III.

Overall, the physico-chemical characteristics of bacteriocins from 6 marine bacteria support important data for further studying on bacteriocin diversity in marine bacteria of Vietnam origin and applying these bacteria as potential probiotics in aquaculture and food technology.

## CONCLUSIONS

The cell-free supernatants from 12 marine bacteria exhibited antimicrobial activity against together themselves and against food-borne and animal pathogens such as *E. coli, Proteus mirabilis, Clostridium perfringens, Salmonella enterica, Vibrio owensii, V. parahaemolyticus, S. aureus* and *B. cereus*. Among them, 6 strains L5B, D15, D10, D9.1, G1

và CT1.1 were found to express their stable and strong antimicrobial activity against sensitive indicators *Proteus* spp.* T14 and T9. Bacteriocins produced by these strains were stable to catalase treatment but completely inactivated when treated with proteinase K. Trypsin or α-chymotrypsin treatment inactived bacteriocins of two strains CT 1.1 and G1 while the activity seemed to be stable in bacteriocins produced by L5B, D15, D10 and D9.1 after the exposure to these enzymes. Most of bacteriocins were resistent to the treatment at 60°C for 15 min, but lost 75% their activity after 30 min at 60°C and completely inactivated at 100°C for 30 min. However, they remained active after incubation at pH 4-10.

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# XÁC ĐỊNH HOẠT TÍNH KHÁNG KHUẨN VÀ TÍNH CHẤT HÓA LÝ CỦA DỊCH BACTERIOCIN THÔ TỪ VI KHUẨN BIỂN VIỆT NAM

**Nguyễn Thị Hải Thanh, Nguyễn Thị Hồng Mai, Nguyễn Văn Duy***

*Viện Công nghệ sinh học và Môi trường, Trường Đại học Nha Trang*

## TÓM TẮT

Bacteriocin là các peptide kháng khuẩn được sản sinh từ vi khuẩn này để ức chế sinh trưởng của các vi khuẩn khác, nhất là các chủng có quan hệ tiến hóa gần gũi. Do đó, chúng là những được phẩm tiềm năng bảo vệ cho sức khỏe con người và động vật. Trong nghiên cứu này, chúng tôi xác định khả năng sinh bacteriocin và phổ kháng khuẩn của 12 chủng vi khuẩn biển được phân lập từ cá giò (*Rachycentron canadum*), cá chim vây vàng (*Trachinotus blochii*), tôm sú (*Penaeus monodon*) và tôm hùm bông (*Panulirus ornatus*) ở Vịnh Cam Ranh và Vịnh Nha Trang. Các thử nghiệm bao gồm xác định hoạt tính kháng lẫn nhau cũng như kháng các tác nhân gây thối thực phẩm và gây bệnh cho động vật. Các vi khuẩn chỉ thị nhạy cảm với ít nhất một bacteriocin thô trong nghiên cứu này bao gồm *E. coli*, *Proteus mirabilis*, *Clostridium perfringens*, *Salmonella enterica*, *Vibrio owensii*, *V. parahaemolyticus*, *Staphylococcus aureus*, và *Bacillus cereus*. Đồng thời ảnh hưởng của nhiệt độ, pH và enzyme tới độ bền và hoạt tính của dịch bacteriocin thô từ các chủng này cũng được xác định. Kết quả chỉ ra rằng hầu hết các bacteriocin đều bị bất hoạt khi xử lý với proteinase K nhưng bền với trypsin và catalase. Các bacteriocin bền ở 60°C trong 15 phút, nhưng hoạt tính giảm 75% sau 30 phút ở 60°C và bất hoạt hoàn toàn ở 100°C trong 30 phút. Tuy nhiên, các bacteriocin này vẫn thể hiện hoạt tính sau khi xử lý ở pH 4-10. Kết quả xác định hoạt tính kháng khuẩn và tính chất hóa lý của các bacteriocin thô giúp nâng cao hiểu biết về đa dạng sinh học của bacteriocin từ vi khuẩn biển Việt Nam, cũng như những ứng dụng xa hơn trong y dược và nông nghiệp.

*Từ khóa*: bacteriocin, hoạt tính kháng khuẩn, tác nhân gây bệnh, tính chất hóa lý, vi khuẩn biển

* Author for correspondence: *E-mail: duynv@ntu.edu.vn*