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Characterization of Antimicrobial Activities of *Pediococcus pentosaceus* Vtcc-B-601

Dat Nghe and Tu Nguyen*

[1]School of Biotechnology, Hochiminh International University, Hochiminh city, Vietnam.

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ABSTRACT

In this study, *Pediococcus pentosaceus* VTCC-B-601 was investigated and characterized for bacteriocin production. The antimicrobial activities were produced strongly at the late exponential phase ($5x10^8$ CFU/ml), corresponding to the activity of cephalosporin (13.3µg/ml) against *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and *Micrococcus luteus* ATCC 10240. The bacteriocin activity was reduced after proteinase K treatment while the activity was still stable in high temperature. This work supplied a *Pediococcus pentosaceus* bacteriocin identification that was useful in food preservation, clinical use, and agriculture.

INTRODUCTION

Pediococcus pentosaceus, a gram positive bacteria, belongs to a group of homofermentative lactic acid bacteria (LAB), is the best-known major member of probiotic bacteria (Gibson and Fuller, 2000; Rolfe, 2000), which can prevent cardiovascular diseases, prevent harmful pathogens from accessing the gastrointestinal mucosa (Rinkinen *et al*., 2003; Duggan *et al*., 2002) and provoke immune reaction (Ouwehand *et al*., 2003; Vinderola *et al*., 2005). Bacteriocins of *Pediococcus* are small, heat stable and non-lanthionine containing peptides belonging to the class II which are biologically active proteins demonstrating a bactericidal mode of action in foodborne pathogens, including *Bacillus cereus, Clostridium perfringens, Listeria species and S. aureus* (Jiménez *et al*., 1993; Klaenhammer, 1988; Jack and Tagg, 1995; Tagg *et al*., 1976; Carminati *et al*., 1989; Spellberg, 2008). Antibiotic-resistant pathogens have been becoming more and more severe, resulting in the potential epidemic threatening human life. The ratio of pathogens resisted to available antibiotics was extremely high and has been tending to increase rapidly.

In Vietnam, *Streptococcus pneumonia*, a very common cause of respiratory infection had the highest prevalence of penicillin-resistant (71.4%) and erythromycin-resistant (92.1%) of the 11 countries in the Asian network for surveillance of resistant pathogens, 75% of pneumococci was resistant to three or more classes of antibiotic (Martys, 1982).

Obviously, pathogens have been resisting to available antibiotics by mutations or genetic material changes with a very high rate, which progressed faster than the development of new antibiotic invention. In addition, some available antibiotics have side-effects on consumer (Martys, 1982). In fact, chloramphenicol has effects on the performance of bone marrow, resulting in negative effects on blood cell production. Besides, macrolide can cause vomit, diarrhea, and ototoxicity.

In food industry, food formulated with chemical preservatives or antibiotics for longer shelf-life influences on human health. Besides, using antibiotic in aquaculture for long time will cause many risks of potential epidemics. Therefore, development of the natural antimicrobial agents is a great interest for the application in food preservation, agriculture, clinical use, and environmental science (Cleveland et al., 2001). For the above reasons, detection and characterization of bacteriocin in *Pedio pentosaceus* (VTCC-B-601) are necessary.

* *Corresponding Author*
E-mail: nhktu@hcmiu.edu.vn
Tel: 084-8-37244270; Fax: 084-8-37242195

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MATERIAL AND METHODS

Bacteria strains, growth condition

Pediococcus pentosaceus (VTCC-B-601) was purchased from Vietnam Type Culture Collection (VTCC). *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and *Micrococcus luteus* ATCC 10240, were purchased from American Type Culture Collection (ATCC). *Pediococcus pentosaceus* was grown in Deman, Rogosa and Sharpe (MRS) at 37°C. All other strains were grown on LB medium at 37°C.

Sample preparation

Pediococcus pentosaceus (VTCC-B-601) was cultured in MRS medium (pH 6.5) (De Man *et al.*, 1960). The cultures were studied for the growth condition necessitating for bacteriocin production.

Bacteriocin assay

Antimicrobial effects were tested on pathogens by the agar - well - diffusion assay as described by Toba (Toba *et al*., 1991). The tested microorganisms were grown for 18 to 24h in 10 ml LB medium, then dilute to 7.10^{10} CFU/ml. The 150 µl of cell-free filtrates was added into each well in the 50 µl pathogen-spread plate. The plates were incubated for 12 h at 37 °C. The clear zone around each well showing antimicrobial activity was measured and analyzed.

Thermostability test

Cell-free filtrate was treated with temperature. Two ml of cell-free filtrate were poured into test tubes and capped tightly, then treated at 70 °C, 80 °C, 90 °C, 100 °C for 30 minutes and 121°C for 15 minutes. These tubes were cooled and tested for antimicrobial activity (Maja et al., 2010).

Test for proteinase K sensitivity

Five ml of cell-free filtrate were added with proteinase K to get final concentration at 100µg/ml, 500µg/ml and 1000µg/ml (Rajaram *et al*., 2009). The reaction was incubated at 37°C for 2h before antimicrobial test.

RESULTS AND DISCUSSION

Detection of antimicrobial activities of *Pediococcus pentosaceus*

Cell-free-supernatant was harvested at early exponential phase (OD = 0.5, 10h), late exponential phase (OD = 1.39, 20h), stationary phase (OD = 1.35, 36h), and dead phase (OD = 0.5, 49h) (Figure 1). Supernatant of *Pediococcus pentosaceus* was collected after centrifugation at 12000 g for 15 min, then the clear supernatant was sterilized by filtration (0.45µm). The cell-free filtrates were used for antimicrobial assay and further characterization. The activities of antimicrobial compounds considerably varied along with the changes of growth phases of *Pediococcus pentosaceus* VTCC-B-601 on *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and *Micrococcus luteus* ATCC 10240. Remarkably, the average inhibition zone value is 13.08 mm in early exponential phase, 19.58 mm in late exponential phase, 18.41 mm in stationary phase, and 16.66 mm in dead phase (Figure 2, Table 1).

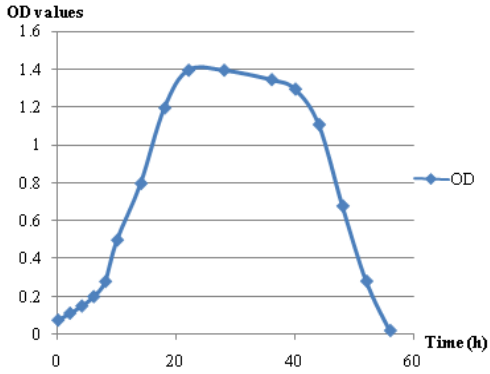

Fig. 1: The growth curve of *Pedio pentosaceus* (VTCC-B-601) in MRS medium at 37°C.

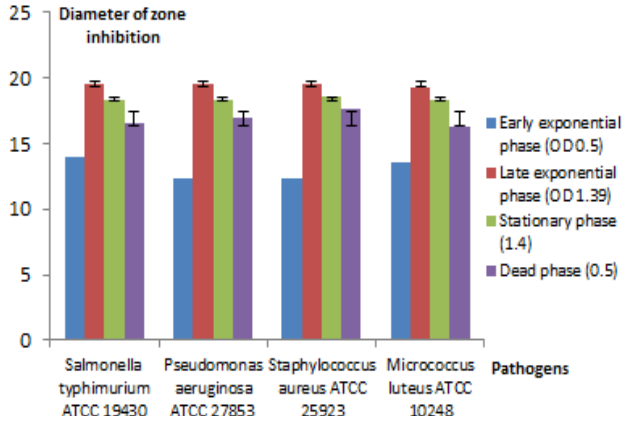

Fig. 2: The antimicrobial activities of *Pediococcus pentosaceus* (VTCC-B-601) cell-free-supernatant on pathogens.

Table. 1: The statistic analysis of antimicrobial activity of *Pedio pentosaceus* (VTCC-B-601) cell free supernatant at different growth phases on indicator strains.

Indicator strains	Early exponential phase (mm)	Lately exponential phase (mm)	Stationary phase (mm)	Dead phase (mm)
Salmonella typhimurium ATCC 19430	14.00 ± 0.00 ^{a}	**19.67 ± 0.58** ^{c}	18.33 ± 0.58 ^{bc}	16.67 ± 0.58 ^{b}
Pseudomonas aeruginosa ATCC 27853	12.33 ± 0.58 ^{a}	**19.67 ± 0.58** ^{c}	18.33 ± 0.58 ^{bc}	16.33 ± 0.58 ^{b}
Pseudomonas aeruginosa ATCC 27853	12.33 ± 0.58 ^{a}	**19.67 ± 0.58** ^{c}	18.33 ± 0.58 ^{bc}	16.33 ± 0.58 ^{b}
Staphylococus aureus ATCC 25923	12.33 ± 0.58 ^{a}	**19.67 ± 0.58** ^{c}	18.67 ± 0.58 ^{bc}	17.33 ± 0.58 ^{b}
Micrococcus luteus ATCC 10240	13.67 ± 0.58 ^{a}	**19.33 ± 0.58** ^{c}	18.33 ± 0.58 ^{bc}	16.33 ± 0.58 ^{b}

In this study, bacteriocin produced by *Pediococus pentosaceus* (VTCC-B-601) inhibited effectively the growth of *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and

Micrococcus luteus ATCC 10240. These pathogens were chosen as indicator strains because of their prevalence in environment and the high resistance to many kinds of available antibiotics. Particularly, *Salmonella typhimurium*, a gram negative bacteria cause gastroenteritis and diarrhea in human and animal, resisted to streptomycin, ampicillin, rifampicin, nalidixic acid, chloramphenicol, and trimethoprin. *Pseudomonas aeruginosa* resisted to β-lactam, carbapenems, aminoglycoside, and fluoroquinolones.

Therefore, the success of this study may be useful in the contribution to the resolving of antibiotic-resistant microorganism as well as applying this bacteriocin extensively in aquaculture, clinical treatment, food preservation. The study exploited the antimicrobial activity of *Pediococus pentosaceus* bacteriocin originated in Vietnam in order to extend the antimicrobial agent sources in Vietnam and worldwide on commonly highly resistant pathogens.

Clearly, bacteriocin production was different in the different growth phases. At the early exponential phase, bacteria growth was not enough to produce high amount of bacteriocin. At the late exponential phase, the antimicrobial activity was highest because of the highest growth.

At stationary and dead phase, the antimicrobial activity trended to decrease moderately. The decreasing of antimicrobial activity in stationary and dead phases may be due to the degradation by proteinase in culture. Therefore, it was appropriate to harvest *Pediococus pentosaceus* (VTCC-B-601) cell-free-supernatant at the late exponential phase for strong antimicrobial activities.

Bacteriocin characterization

The cell-free-filtrate was treated with proteinase K at 100µg/ml, 500µg/ml and 1000µg/ml. The results in figure 4 showed the antimicrobial activity test on Micrococcus luteus ATCC 10240. The antimicrobial activities strongly reduced in cases of proteinase K used at 100µg/ml, 500µg/ml and 1000µg/ml were (figure 4). The inhibition zone nearly disappeared around the sampled wells. Results from proteinase K treatment proved that antimicrobial activities in cell free-supernatant of this bacteria was due to proteinaceous bacteriocin, not H_2O_2 or other antimicrobial agents (Mohankumar , 2011).

Table. 2: The statistic analysis of antimicrobial activity of *Pedio pentosaceus* (VTCC-B-601) cell-free-supernatant after treatment with different temperature levels.

Temperature treatments	*Salmonella typhimurium* ATCC 19430	*Pseudomonas aeruginosa* ATCC 27853	*Staphylococus aureus* ATCC 25923	*Micrococcus luteus* ATCC 10240
Room	19.67 ± 0.58 [a]	17.67± 0.58 [a]	18.33 ± 0.58 [a]	20.0 ± 1 [a]
70°C	19.67 ± 0.58 [a]	17.67± 0.58 [a]	18.33 ± 0.58 [a]	20.0 ± 1 [a]
80°C	19.67 ± 0.58 [a]	17.67± 0.58 [a]	18.33 ± 0.58 [a]	20.0 ± 1 [a]
90°C	19.67 ± 0.58 [a]	17.67± 0.58 [a]	18.33 ± 0.58 [a]	20.0 ± 1 [a]
100°C	19.67 ± 0.58 [a]	17.67± 0.58 [a]	18.33 ± 0.58 [a]	20.0 ± 1 [a]
Autoclave	18.67± 0.58 [a]	17.00 ± 0.00 [a]	16.67± 0.58 [a]	18.33±0.58[a]

As seeing in table 2 and figure 3, there was no reduction in antimicrobial activities of bacteriocin in all temperature treatments from 70°C to 100°C, even autoclaved at 121°C for 15 minutes. *Micrococcus luteus* was used as an illustration for the antimicrobial activities of heated bacteriocin. Consequently, the bacteriocin produced by *Pediococus pentosaceus* (VTCC-B-601) was stable as even heating at 100°C for 30 minutes or autoclaving at 121°C for 15 minutes. With this properties, *Pediococus pentosaceus* bacteriocin could be used as the improved food preservative.

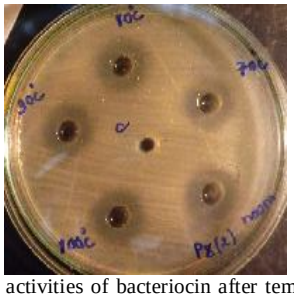

Fig. 3: Antimicrobial activities of bacteriocin after temperature-treatment on *Micrococcus luteus* at 70°C, 80°C, 90°C, 100°C. The MRS medium was used as control (C).

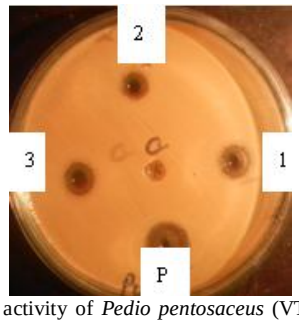

Fig. 4: Antimicrobial activity of *Pedio pentosaceus* (VTCC-B-601) cell-free-filtrate after proteinase K treatment on *Micrococcus luteus* ATCC 10240. (1): 100µg/ml; (2): 500µg/ml; (3): 1000µg/ml. *Pedio pentosaceus* (VTCC-B-601) cell-free-supernatant was used as a positive control (P). The MRS medium was used as negative control (C).

CONCLUSION

The study identified the bacteriocin activities of *Pedio pentosaceus* (VTCC-B-601) on *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and *Micrococcus luteus* ATCC 10240.. This temperature stable bacteriocin showed the antimicrobial activities on *Salmonella typhimurium* ATCC 19430, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococus aureus* ATCC 25923, and *Micrococcus luteus* ATCC 10240. Therefore, this work supplied a *Pediococcus pentosaceus* bacteriocin identification which might be useful in food preservation, clinical use, and agriculture.

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