Journal of Agricultural Science; Vol. 8, No. 10; 2016
ISSN 1916-9752 E-ISSN 1916-9760
Published by Canadian Center of Science and Education

Characterization and Antibacterial Mode of a Novel Bacteriocin with Seven Amino Acids from *Lactobacillus plantarum* in Guizhou Salted Radish

Meizhong Hu[1,2], Lijuan Dang[1], Haizhen Zhao[1], Chong Zhang[1], Yingjian Lu[3], Jiansheng Yu[2] & Zhaoxin Lu[1]

[1] College of Food Science and Technology, Nanjing Agricultural University, Nanjing, Jiangsu, P.R. China

[2] Tongren Polytechnic College, Tongren Guizhou, P.R. China

[3] Department of Nutrition and Food Science, University of Maryland, College Park, Maryland, USA

Correspondence: Zhaoxin Lu, College of Food Science and Technology, Nanjing Agricultural University, No.1 Weigang Nanjing, Jiangsu 210095, P.R. China. Tel: 86-25-8439-6583. Fax: 86-25-8439-6431. E-mail: fmb@njau.edu.cn

Received: July 6, 2016 Accepted: August 11, 2016 Online Published: September 15, 2016

doi:10.5539/jas.v8n10p120 URL: http://dx.doi.org/10.5539/jas.v8n10p120

Abstract

Traditional Chinese fermented vegetables are excellent probiotic food with probiotic lactic acid bacteria that are benefical to the health. A novel bacteriocin with molecular weight, 825 Da was found successfully from Lactobacillus plantarum 163, which was isolated from Guizhou salted radish. The complete amino acid sequence was speculated as YVCASPW based on the mass spectrometry, and was named as bacteriocin 163-1. The bacteriocin 163-1 was highly thermostable and stability over a broad pH range (pH 3-6), sensitive to protease K and pepsin, and exhibited a wide range of antimicrobial activity not only against lactic acid bacteria (LAB) but also against other foodborne pathogens including Gram-positive and Gram-negative bacteria. Bacteriocin 163-1 could disrupt the cell membrane of bacteria. The observations of the transmission electron microscopy and laser confocal microscopy on the cell membrane of Escherichia coli and Staphylococcus aureus showed that bacteriocin 163-1 could result in forming pores on the cell membrane and then cytolysis of the bacteria. The new bacteriocin with broad-spectrum antibacterial activity will be useful in preservation of vegetable, fruit and food as well agricultural bio-controlling.

Keywords: *Lactobacillus plantarum*, bacteriocin, action mode of antibacteria, bio-preservative

1. Introduction

Traditional Chinese fermented vegetables are excellent probiotic food with probiotic lactic acid bacteria (LAB), which has various health benefits including anticonstipation, anticancer, antioxidative and immune-boosting. The functional ingredients from fermentation by LAB such as bacteriocins could be used as bio-preservative. LAB strains are generally recognized as safe (GRAS) microorganisms (Burdock & Carabin, 2004), and bacteriocins have also achieved GRAS status. Some bacteriocins with remarkable thermostability and pH stability show a significant inhibitory activity against spoilage and pathogenic bacteria, indicating that bacteriocin could be potentially used as an effective bio-preservative in the food industry.

Some bacteriocins are already commercially available, such as nisin (produced by *Lactococcus lactis*), used to keep and extend the shelf life of food products in many countries in the world. However, nisin has some drawbacks when applied in the food industry. For instance, nisin does not perform the good antibacterial activity against Gram-negative bacteria, and it is only effective under an acidic environment. These limits are not positive for nisin to be applied in process and conservation for foods. Thus, an alternative novel bacteriocin with good antibacterial activities is essential for the food industry. Recently, the researcher is focusing on the mining of novel bacteriocin from LAB for such as *Lb. plantarum* from salted vegetable, yoghurt, fermented meat (Biscola et al., 2013; Ahmad et al., 2014; Alvarez-Sieiro et al., 2016).

Generally, most bacteriocins generated from LAB appear to share a common mode of action which form a pore in the sensitive bacterial membrane, and therefore lead to sensitive cell death (Castellano et al., 2003; Oppegård et al., 2007). In addition, other type of bacteriocins such as lantibiotic has exhibited different mode of action by

binding themselves to lipid II, and therefore lead to cell death through false cell wall synthesis (Cotter et al., 2005). Furthermore, some of bacteriocins such as Pep 5 cause lysis of treated cells, which is another mode of action (Bierbaum & Sahl, 1987). The effective use of bacteriocins in food preservation requires a better understanding of their mode of action and their inhibitory action under different biochemical conditions naturally occurring in food.

In this study, the purification, identification, antimicrobial spectrum, biochemical and genetic characteristics of new bacteriocin from *Lb*. *plantarum* 163 in Guizhou salted radish were comprehensively investigated, and the action mode of the bacteriocin against to *S. aureus* and *E. coli* was discussed.

2. Materials and Methods

2.1 Strains, Media and Growth Conditions

Lb. *plantarum* 163 was screened from Chinese fermented salted radish (Hu et al., 2013) and was stored in China General Microbiological Culture Collection Center (No. 8224). The lactic acid bacteria and fungi used in this study and their respective growth conditions are listed in Table 1.

Table 1. Antimicrobial activity profile of bacteriocin 163-1 produced from the isolated *Lactobacillus plantarum* 163

Strains	Source [a]	Growth conditions	Antimicrobial activity [b]
Staphylococcus ureus	ATCC 25923	nutrient broth/37 °C	19.38±0.68
Listeria monocytogenes	ATCC 19114	nutrient broth/37 °C	16.25±0.58
Bacillus pumilus	CMCC 63202	nutrient broth/37 °C	16.28±0.47
Bacillus cereus	AS 1.1846	nutrient broth/37 °C	13.07±0.27
Micrococcus luteus	CMCC 28001	nutrient broth/37 °C	13.07±0.27
Lactobacillus thermophilus	Our Lab	MRS medium/37 °C	9.55±0.52
Lactobacillus rhamnosus	Our Lab	MRS medium/37 °C	9.60±0.54
E. coli	ATCC 25922	nutrient broth/37 °C	15.35±0.67
Pseudomonas aeruginosa	AS 1.2620	nutrient broth/37 °C	9.91±1.30
Pseudomonas fluorescens	AS 3.6452	nutrient broth/37 °C	12.92±1.46
Penicillium notatum	AS 3.4356	Potato Dextrose/30 °C	0
Aspergillus niger	AS 3.6459	Potato Dextrose/30 °C	0
Rhizopus stolonifer	AS 3.822	Potato Dextrose/30 °C	0

Note. [a] ATCC, American type culture collection; CMCC, China center of medicine culture collection; AS, China General Microbiological Culture Collection Center. [b] Well: 5 mm, Means of three replicate values.

2.2 Purification of Bacteriocin 163-1

Lb. *plantarum* 163 was activated in MRS broth at 37 °C for 14 h without agitation. 10 mL pre-culture was inoculated into 1,000 mL MRS broth, and incubated at 37 °C for 24 h without agitation. The bacterial cells then were harvested by centrifugation (6,000 g, 15 min) at 4 °C, and the cell-free supernatant collected was filtrated by 0.45 μm membrane. Then, the cell-free supernatant was separated by preparative chromatography (Waters, 600) with Waters SunFire OBD-C18 columns (19×150 mm) in a 40 min isocratic elution of 95% water-acetonitrile (5%) and containing 0.1% trifluoroacetic acid (TFA). The elution was monitored continuously at 267 nm and all individual peaks were collected. Then, all individual peaks were further purified by reversed-phase high-performance liquid chromatography (RP-HPLC, UltiMate 3000) using the following conditions: Agilent Eclipse XDB-C18 columns, (4.6 × 250 mm), followed by an isocratic elution using 95% water-acetonitrile (5%) and containing 0.1% TFA for 40 min. Each individual peak was collected and further used for antimicrobial test using *Bacillus pumilus* CMCC 63202 as indicator.

2.3 Determination of the Primary Structure of Bacteriocin 163-1

The molecular mass and amino acid sequence of purified compound (bacteriocin 163-1) were analyzed by a matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltonics, Bremen, Germany) using α-cyano-4-hydroxycinnamic acid as a reference (Hu et al., 2013; Chen et al., 2014).

2.4 Antimicrobial Spectrum of Bacteriocin 163-1

The bacteriocin 163-1 from preparative chromatography were used to evaluate the antimicrobial activity against indicator organisms (seven of positive bacteria, three of gram-negative bacteria and three of fungi). Antibacterial activity of bacteriocin 163-1 was measured using agar well diffusion assay method (Ruixiang et al., 2015).

2.5 Effects of Enzyme, Temperature and pH on the Activity of Bacteriocin 163-1

The effect of Protease K (30 U/mg), Trypsin (2.5 KU/mg), α-Chymotrypsin (1 KU/mg) and Pepsin (3 KU/mg) on the activity of bacteriocin 163-1 was determined. The purified bacteriocin 163-1 from preparative chromatography was incubated at the optimum pH at 37 °C for 3 h) with the enzymes of the final concentration of 5 mg/mL. The influence of temperature on the activity of bacteriocin 163-1 was examined by treating at 60 °C, 80 °C and 100 °C for 10, 20 and 30 min, respectively in a thermostatic water bath and at 121 °C for 20 min in an autoclave. Lastly, the pH value of the purified bacteriocin 163-1 was adjusted to 2-10 and then kept at 37 °C for 3 h in a thermostatic water bath. The residual antimicrobial activities were measured after enzymatic, temperature and pH treatments using agar diffusion assay method using *Bacillus pumilus* CMCC 63202 as the indicator (Gao et al., 2010; Zhang et al., 2013).

2.6 Effect of Bacteriocin 163-1 on the Sensitive Cell Growth and Time-Kill Kinetics

The activity unit of bacteriocin 163-1 was defined as the reciprocal of the highest dilution with antimicrobial activity and was expressed in activity units (AU) per milliliter (Barefoot & Klaenhammer, 1983; Pucci et al. 1988).

In order to determine the effect of bacteriocin 163-1 on the sensitive cell growth, *S. aureus* (ATCC 25923) or *E. coli* (ATCC 25922) were activated in LB medium, then bacteriocin 163-1 were added at a concentration of 6.4 Au/mL and 12.8 Au/mL. The sterile distilled water treatment was used as a control while the sterile LB medium was used as a baseline. The OD_{600} of all treatments were measured at 2, 3, 4, 5, 6, 7, 8, 10, 12 and 16 h. In order to determine the Time-kill kinetics, *S. aureus* (ATCC 25923) or *E. coli* (ATCC 25922) was activated in LB medium and incubated at 37 °C for 6 hours with agitation. Then bacteriocin 163-1 was added at a concentration of 12.8 AU/mL and the bacteria were counted at 0.5, 1, 1.5, 2, 2.5 and 3 h.

2.7 Effect of Bacteriocin 163-1 on Release of Proteins and Nuclear Acid in Cells

To determine the effect of bacteriocin 163-1 on release of proteins and nuclear acid in bacteria cells, *S. aureus* (ATCC 25923) or *E. coli* (ATCC 25922) were activated in LB medium, and incubated at 37 °C for 6 hours with agitation, then bacteriocin 163-1 were added at a concentration of 6.4 AU/mL. The bacterial cells then were harvested by centrifugation (6,000 g, 15 min) at 4 °C. The OD_{280} and OD_{260} of the cell-free supernatants were measured by 0.5, 1, 1.5, 2, 2.5 and 3 h. The sterile distilled water was used as a control while the sterile LB medium was used as a baseline.

S. aureus (ATCC 25923) or *E. coli* (ATCC 25922) were activated and incubated at 37 °C for 5 hours with agitation, then bacteriocin 163-1 were added at a concentration of 6.4 Au/mL for the determination of the lactic dehydrogenase (LDH) release. After bacterial cells were harvested by centrifugation at 6000 g for 15 min at 4 °C, the LDH contents of the cell-free supernatants were measured at 0.5, 1, 1.5, 2, 2.5 and 3 h using LDH kit (Nanjing Jiancheng Bioengineering Institute, China). The sterile distilled water treatment was used as a control while the sterile LB medium was used as a baseline.

2.8 Transmission Electron Microscopy (TEM) Observations on the Sensitive Cell Membrane Treated with Bacteriocin 163-1

S. aureus (ATCC 25923) or *E. coli* (ATCC 25922) were activated in LB medium, and incubated at 37 °C for 8 hours (*E. coli* 4 h) with agitation, then bacteriocin 163-1 were added at a concentration of 6.4 AU/mL, incubated at 37 °C for 2 hours, bacterial cells were harvested by centrifugation at 6,000 g for 15 min at 4 °C. The bacterial cells were washed by 2.5% Gluteraldehyde twice, finally bacterial cells were fixed by 2.5% Gluteraldehyde and the cell membrane observation was done by using Transmission Electron Microscopy (HITACHI, H-600, Japan). The sterile distilled water treatment was used as a control.

2.9 Bacteriocin 163-1 Localization on Celluar

S. aureus (ATCC 25923) or *E. coli* (ATCC 25922) were activated in LB medium, and incubated at 37 °C for 8 hours (*E. coli* 6 h) with agitation, then fluorescein isothiocyanate (FITC)- Bacteriocin 163-1 were added at a concentration of 1 mg/L, and incubated at 37 °C for 0.5 hours with agitation (in dark), bacterial cells were harvested by centrifugation at 6,000 g for 15 min at 4 °C, then the bacterial cells were washed by 2.5% Gluteraldehyde twice. Then, the bacterial cells were suspended using 2.5% Gluteraldehyde (in dark) and finally

observed by Laser confocal microscopy (LSM-710, Zeiss, Germany) using Fluorescence wavelength at 488 nm and 40X object lens.

3. Results and Discussion

3.1 Purification and Identification of the Bacteriocin 163-1

After the purification by preparative chromatography and RP-HPLC, one substance with antibactreia activity, where the retention time was 10.6 min (Figure 1a) was obtained. Then, the molecular mass and amino acid sequence of the bacteriocin 163-1 was identified by means of MALDI-TOF-MS, It was found that molecular mass of bacteriocin 163-1 was 825 Da (Figure 1b). MALDI-TOF-MS/MS fragment of bacteriocin 163-1 are shown in Figure 1b. Based on the previous report about peptide fragmentation nomenclature (Roepstorff & Fohlman, 1984), the primary sequence of bacteriocin 163-1 was determined to be: Y-V-C-A-S-P-W.

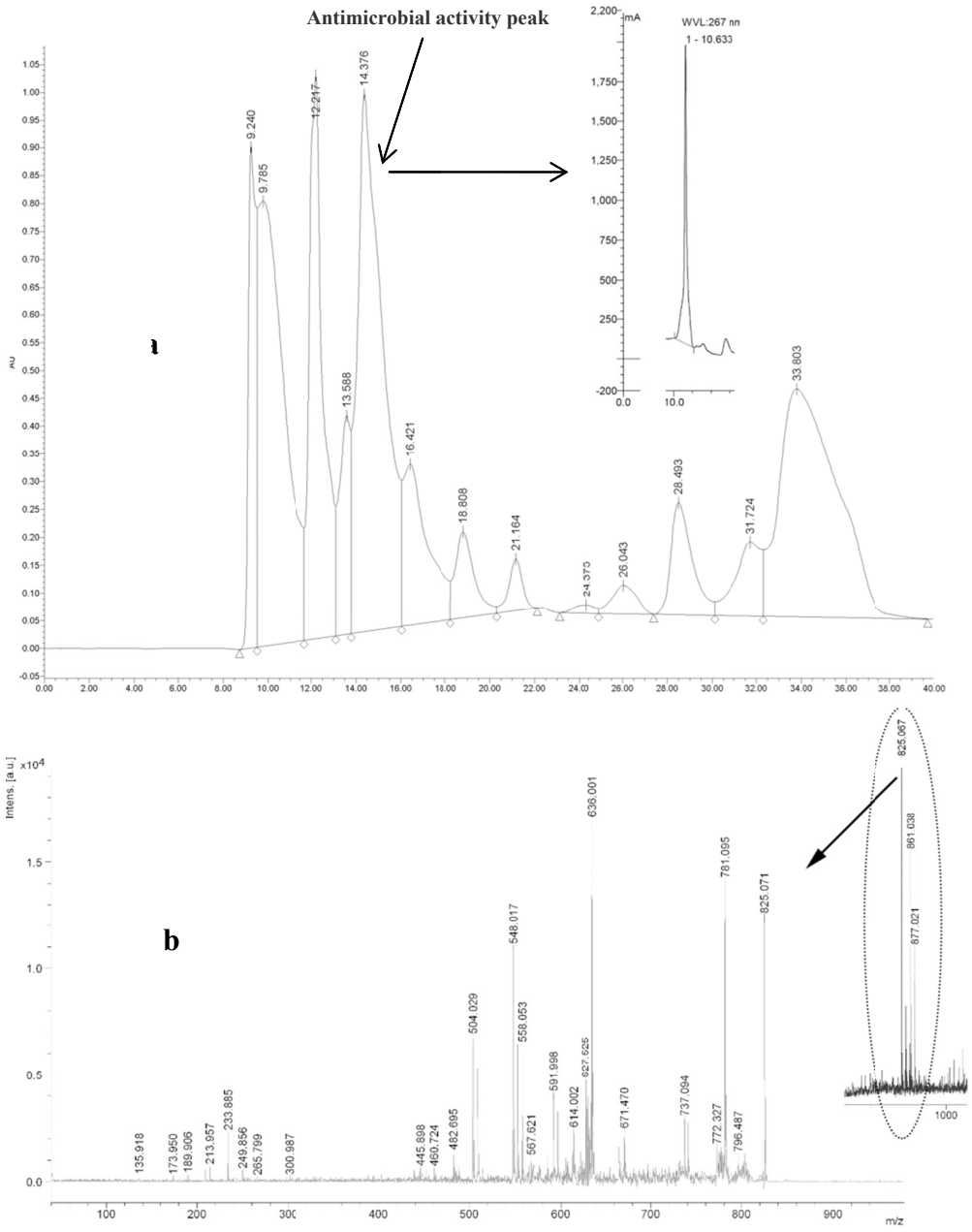

Figure 1. a) The purified chromatograms of bacteriocin 163-1 with retention time of 10.633 s as identified; b) MALDI-TOF-MS of bacteriocin 163-1, the molecular ion peak is represented by a major peak at 825 Da, 861: [4*CHCA-3H+1K+3Na]+, 877: [4*CHCA-3H+2K+2Na]+, CHCA: α-cyano-4-hydroxycinnamic acid

Bacteriocin is an active peptide produced by bacteria. From previous studies, many bacteriocins produced by *Lb. plantarum* have been found and identified, such as plantaricin Y produced by *Lb. plantarum* 510 (Chen et al., 2014), plantaricin LD1 Produced by *Lb. plantarum* LD1 (Gupta & Tiwari, 2014). Some of *Lb. plantarum* could produce more than one type of plantaricin, for instance *Lb. plantarum* C11 could produce plantaricin A, plantaricin F, plantaricin E (Hauge et al., 1999). In our previous work, *Lb. plantarum* 163 could produce plantaricin 163 which consist of 32 amino acid with the molcecular weight of 3553 Da (Hu et al., 2013).

Bacteriocin 163-1 was another antibacterial substance from *Lb. plantarum* 163. The molecular weight of bacteriocin produced by *Lb. plantarum* are between 2 kDa-5 kDa, for example, plantaricin C19 (3.8 KDa) (Atrih et al., 2001), plantaricin UG1 (3-10 KDa) (Enan et al., 1996), plantaricin MG (2180 Da) (Gong et al., 2010), plantaricin-149 (2.2 KDa) (Kato et al., 1994), plantaricin ASM1 (5045.7 Da) (Hata et al., 2010), and plantaricin 163 (3553 Da) (Hu et al., 2013). In this study, we discovered a new bacteriocin 163-1with 825 KDa of molecular weight, which was similar to those found in molecular level, such as Acidocin NX2 (824 Da) (Zhang et al., 2014). And it was confirmed that the sequence of bacteriocin 163-1 was no homology by search of protein BLAST (BLAST) against the GenBank database (http://www.ncbi.nlm.nih.gov/BLAST) and Bactibase (http://bactibase.pfba-lab-tun.org), thus suggesting that bacteriocin 163-1 may be a novel bacteriocin.

3.2 Antimicrobial Spectrum of Bacteriocin 163-1

The antimicrobial activities of bacteriocin 163-1 was shown in Table 1. The bacteriocin 163-1 significantly inhibited the growth of Gram-positive bacteria (*S. aureus*, *Listeria monocytogenes*, *Bacillus pumilus*, *Bacillus cereus*, *Micrococcus luteus*, *Lactobacillus thermophilus*, *Lactobacillus rhamnosus*) and the Gram-negative bacteria (*E. coli*, *Pseudomonas aeruginosa*, and *Pseudomonas fluorescens*), but had no antimicrobial activity against fungi such as *Penicillium notatum*, *Aspergillus niger*, and *Rhizopus nigricans*, indicated that bacteriocin 163-1 had a broad antimicrobial spectrum. As shown in previous literature, most of bacteriocin, such as plantaricin F, plantaricin E, plantaricin J could only showed antibacterial activity against homologous species (Anderssen et al., 1998), whereas only a few plantaricin appeared to be a broad spectrum of antimicrobial activity. A bacteriocin with broad antibacterial activity may be more useful and valuable in agro-food industry.

3.3 Effects of Enzyme, Temperature and pH on the Activity Bacteriocin 163-1

Effects of enzyme, temperature and pH on the activity bacteriocin 163-1 were investigated and the results were shown in Table 2. The antimicrobial activity of bacteriocin 163-1 was found to be fully lost after enzymatic treatments with protease K, pepsin, and partially lost after enzymatic treatments with trypsin, α-Chymotrypsin, thus highlighting the typical property of a peptide.

Further, the stability of bacteriocin 163-1 was tested at 60 °C, 80 °C, 100 °C and 121 °C and under a different pH (2-10). Surprisingly, it was observed that the activity recovery of bacteriocin 163-1was more than 90% by treatments of 60-121 °C in pH 4, appeared to be good thermostability. On other hand, its recovery was more than 70% when tested at pH 3-10, of which showed remarkable pH stability in pH 3-8. The result was consistent with previous findings about bacteriocin peptides such as plantaricin MG (Gong et al., 2010), plantaricin 163 (Hu et al., 2013). The good physicochemical properties of bacteriocin 163-1 could offer an essential promise for its application in the processing and preservation of foods as well bio-controlling of plant diseases.

3.4 Effect of Bacteriocin 163-1 on Cell Growth and Time-Kill Kinetics S. aureus and E. coli

The effect of bacteriocin 163-1 on cells growth and Time-kill kinetics of *S. aureus* and *E. coli* was shown in Figure 2. The growths of *S. aureus* and *E. coli* were partly inhibited when they were treated with bacteriocin 163-1 at a concentration of 6.4 AU/mL (Figures 2a and 2b). However, *S. aureus* growth was fully inhibited, but *E. coli* growth was done before 8 h of culture when bacteriocin 163-1 was added at a concentration of 12.8 AU/mL (Figures 2a and 2b), suggested *S. aureus* was more sensitive to bacteriocin 163-1 than *E. coli* and it needed higher concentration of the bacteriocin to fully inhibit growth of *E. coli*. The growth of *S. aureus* was highly inhibited after 1 h with the treatment of 12.8 AU/mL (Figure 2c). The growth of *E. coli* was significantly reduced from 0.5 h to 3 h, and a 5-log reduction was observed after 3 h when treated with bacteriocin 163-1 at a concentration of 12.8 AU/mL (Figure 2d).

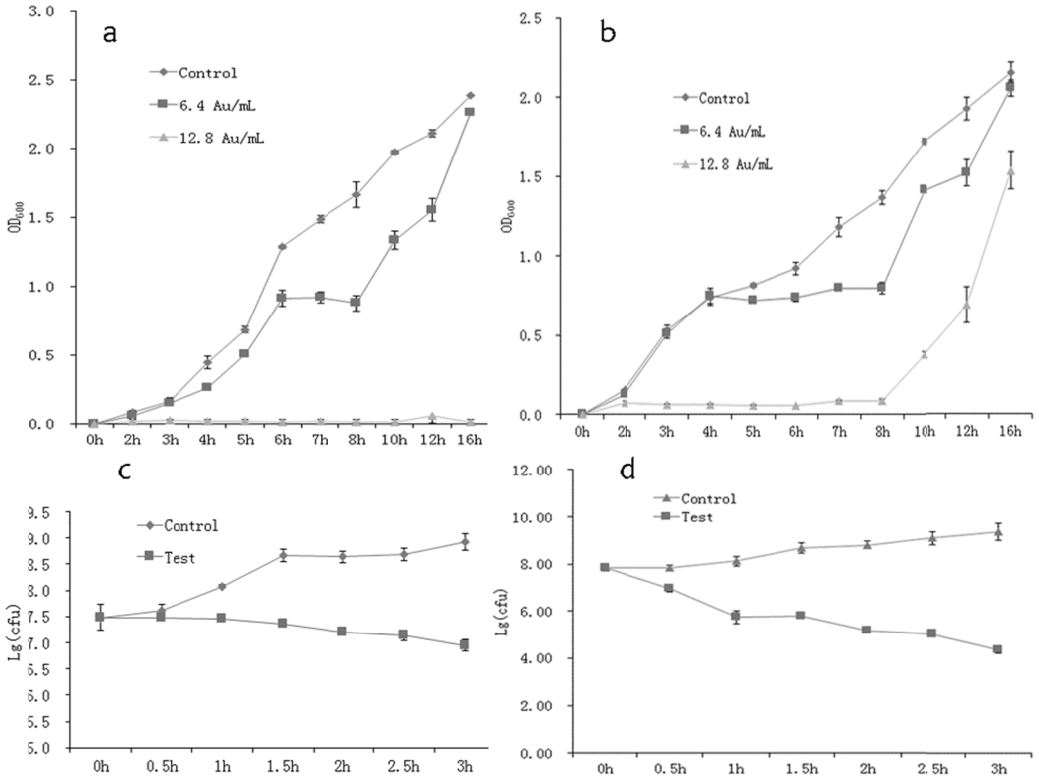

Figure 2. a) The effect of *S. aureus* biological growth by Bacteriocin 163-1; b) The effect of E. coli biological growth by Bacteriocin 163-1; c) Time-kill kinetics of Bacteriocin 163-1 against *S. aureus*; d) Time-kill kinetics of Bacteriocin 163-1 against *E. coli*

3.5 Effect of Bacteriocin 163-1 on Cell Conponents

To determine whether the bacteriocin 163-1 had an impact on the proteins, nuclear acid and LDH of *S. aureus* and *E. coli*, the bacteriocin 163-1 were added to the medium at a concentration of 6.4 Au/mL. It was found OD_{280} (extracellular proteins), OD_{260} (extracellular nuclear acid) and extracellular LDH activity were significantly increased after adding the bacteriocin (Figure 3), suggested that the the addition of bacteriocin 163-1 resulted in massive leakage of proteins, nuclear acid and LDH from the cells. The result implied that the bacteriocin may be disrupted the cell structure of the bacteria as cell membrane (Castellano et al., 2003; Oppegård et al., 2007).

In order to confirm above speculation, the cell structure was observed by Transmission Electron Microscopy (TEM). The non-treated *E. coli* cell grow normally its cell wall was smooth and not broken (Figure 4a), whereas the structures of cell membrane with the bacteriocin-treated were damaged (Figure 4b), and the holes were observed in the cell membrane (Figure 4b, arrow tip) and even the cells were deformed and distorted. In the case of *S. aureus* cells, similar effects were observed (Figure 4c), moreover, a larger number of cavity cells due to leakage out of cell component were found by treatment of the bacteriocin (Figure 4d). The results of TEM suggested that bacteriocin 163-1 could damage the cell wall and cross the cell membrane of the bacteria. Then, cytoplasms and components inside the cells were leaked to form empty hole, finally resulted in cell death.

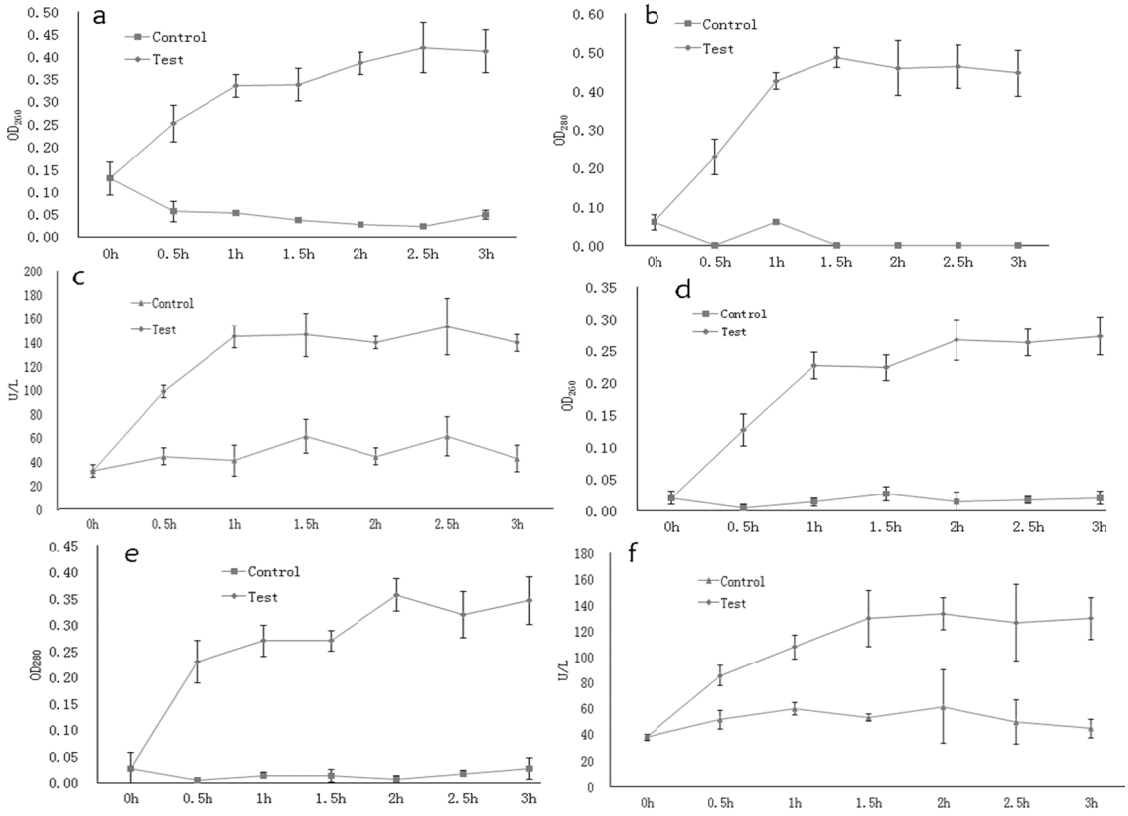

Figure 3. a) Change of the cell-free supernatant OD_{260} of *S. aureus* by Bacteriocin 163-1-treated ; b) T Change of the cell-free supernatant OD_{280} of *S. aureus* by Bacteriocin 163-1-treated; c) Change of the cell-free supernatant LDH of *S. aureus* by Bacteriocin 163-1-treated; d) Change of the cell-free supernatant OD_{260} of *E. coli* by Bacteriocin 163-1-treated; e) Change of the cell-free supernatant OD_{280} of *E. coli* by Bacteriocin 163-1-treated; f) Change of the cell-free supernatant LDH of *E. coli* by Bacteriocin 163-1-treated

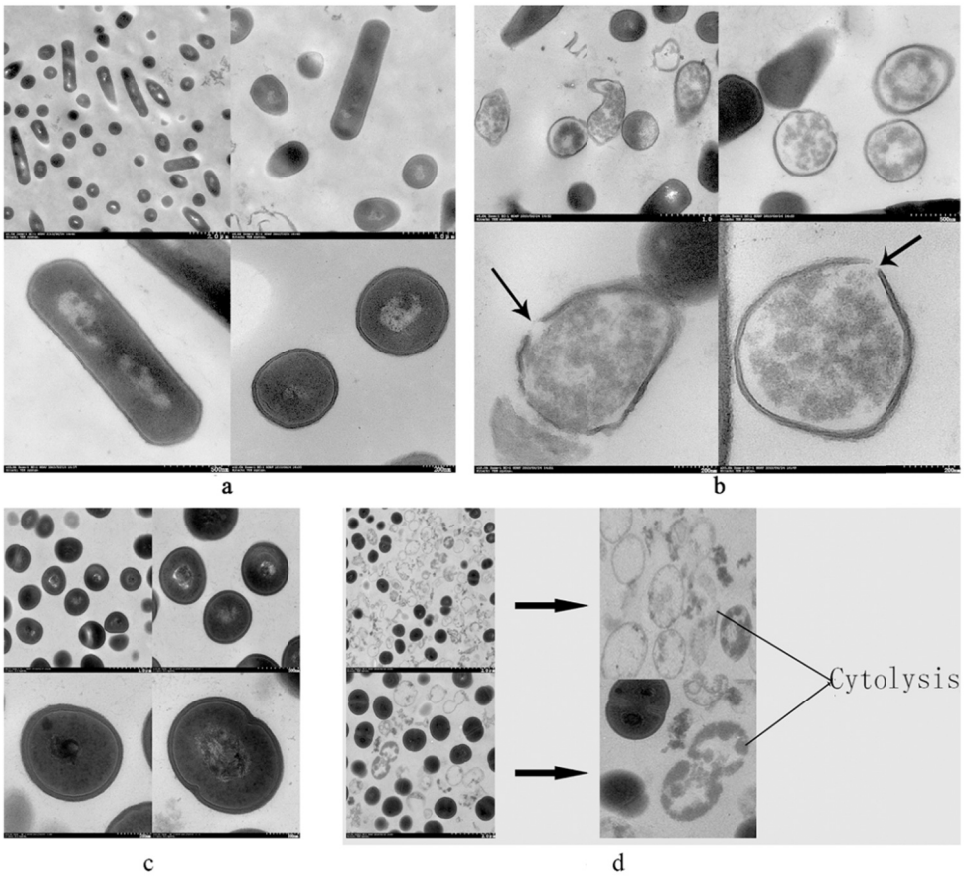

Figure 4. a) E. Coli treated without bacteriocin 163-1; b) E. Coli treated with bacteriocin 163-1(Circular for slitting, rod for crosscutting); c) S. aureus treated without bacteriocin 163-1; d) S. aureus treated with bacteriocin 163-1

3.6 Bacteriocin 163-1 Localization in Cell

FITC-Bacteriocin 163-1was subjected to observe action part of bacteriocin 163-1 in the bacteria cell by laser confocal microscopy (LCM). The observation results were shown in Figure 5. The fluorescence with FITC-Bacteriocin 163-1 was observed in whole cells of *E. coli* (Figures 5a and 6b) it was only done in cell wall or membrane of *S. aureus* due to leakage up of cell component (Figures 5c and 5d), indicating that the bacteriocin entered into *E. coli* cells and *S. aureus* cells by across the membrane. Some bacteriocins could form a pore in the sensitive bacterial membrane that leads to sensitive cell death, such as nisin, subtilin, lacticin 3147 (Bierbaum & Sahl, 2009; Chatterjee, Paul, Xie, & van der Donk, 2005; Wiedemann, Benz, & Sahl, 2004). Therfore, the results also suggested that the action mode of bacteriocin 163-1 for *E. coli* and *S. aureus* might be pore mode, then cytolysis.

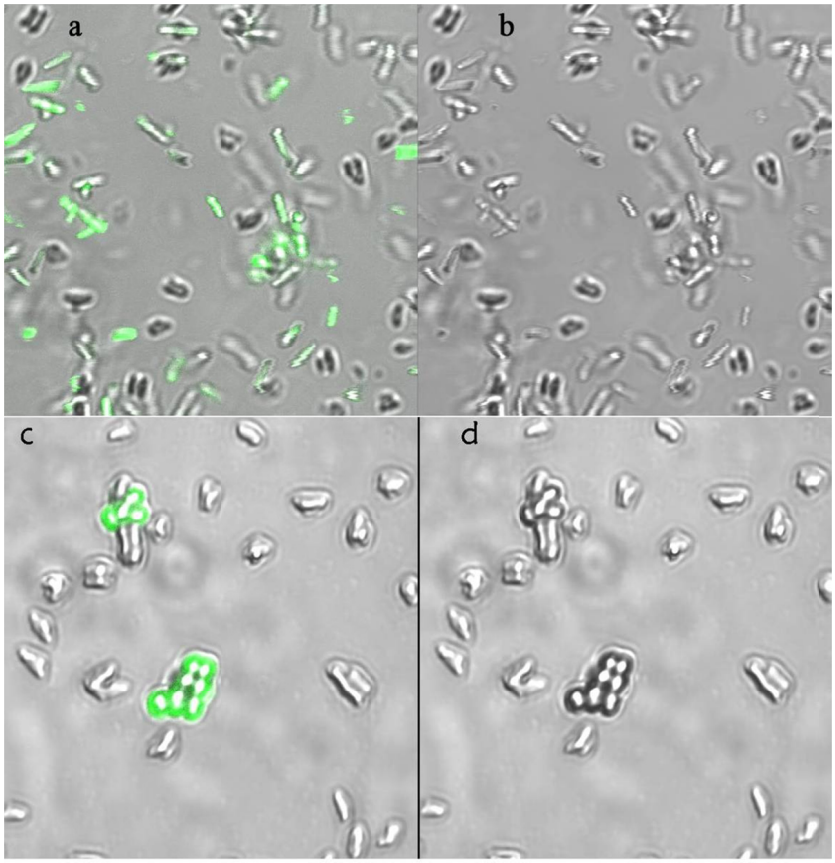

Figure 5. Fluorescence observations of bacteriocin 163-1-treated E.coli and S.aureus cells. a) *E. coli* treated with bacteriocin 163-1 were observed with fluorescence mode by Laser confocal microscopy; b) *E. coli* treated with bacteriocin 163-1 were observed with no fluorescence mode; c) *S. aureus* treated with bacteriocin 163-1 were observed with fluorescence mode by Laser confocal microscopy; d) *S. aureus* treated with bacteriocin 163-1 were observed with no fluorescence mode

4. Conclusion

In this study, bacteriocin 163-1 was purified through the preparative chromatography and reversed-phase chromatography, and its molecular mass (825 Da) was determined by MALDI-TOF-MS. The primary sequence of bacteriocin 163-1 was deduced as Y-V-C-A-S-P-W. Bacteriocin 163-1 was sensitive to the proteases and exhibited a broad-spectrum antimicrobial activity not only against lactic acid bacteria, but also against some foodborne pathogens. In addition, bacteriocin 163-1 appeared as a remarkable thermostability and pH stability. The fluorescence test with FITC-Bacteriocin 163-1 showed bacteriocin 163-1 acted on the cell membrane of bacteria and resulted in pore formation on the cell membrane and then cytolysis. The results indicated that the novel bacteriocin might be a potential candidate as a bio-preservative in agro-food industry in the future.

Acknowledgements

The authors would like to thank the Priority Academic Program Development of the Jiangsu Higher Education Institutions (PAPD) and Jiangsu Collaborative Innovation Center of Meat Production and Processing, Quality and Safety Control for financial support.

References

Ahmad, V., Muhammad Zafar Iqbal, A. N., Haseeb, M., & Khan, M. S. (2014). Antimicrobial potential of bacteriocin producing Lysinibacillus jx416856 against foodborne bacterial and fungal pathogens, isolated from fruits and vegetable waste. *Anaerobe, 27*, 87-95. http://dx.doi.org/10.1016/j.anaerobe.2014.04.001

Alvarez-Sieiro, P., Montalbán-López, M., Mu, D., & Kuipers, O. (2016). Bacteriocins of lactic acid bacteria: Extending the family. *Applied Microbiology & Biotechnology, 100*(7), 2939-2951. http://dx.doi.org/10.1007/s00253-016-7343-9

Anderssen, E. L., Diep, D. B., Nes, I. F., Eijsink, V. G., & Nissen-Meyer, J. (1998). Antagonistic activity of *Lactobacillus plantarum* C11: two new two-peptide bacteriocins, plantaricins EF and JK, and the induction factor plantaricin A. *Applied and Environmental microbiology, 64*(6), 2269-2272.

Atrih, A., Rekhif, N., Moir, A., Lebrihi, A., & Lefebvre, G. (2001). Mode of action, purification and amino acid sequence of plantaricin C19, an anti- Listeria bacteriocin produced by *Lactobacillus plantarum* C19. *International Journal of Food Microbiology, 68*(1), 93-104. http://dx.doi.org/10.1016/S0168-1605(01)00482-2

Barefoot, S. F., & Klaenhammer, T. R. (1983). Detection and activity of lactacin B, a bacteriocin produced by Lactobacillus acidophilus. *Applied and Environmental microbiology, 45*(6), 1808-1815.

Bierbaum, G., & Sahl, H. (1987). Autolytic system of Staphylococcus simulans 22: Influence of cationic peptides on activity of N-acetylmuramoyl-L-alanine amidase. *Journal of Bacteriology, 169*(12), 5452-5458.

Bierbaum, G., & Sahl, H.-G. (2009). Lantibiotics: Mode of action, biosynthesis and bioengineering. *Current Pharmaceutical Biotechnology, 10*(1), 2-18. http://dx.doi.org/10.2174/138920109787048616

Biscola, V., Todorov, S. D., Capuano, V. S. C., Abriouel, H., Gálvez, A., & Franco, B. D. G. M. (2013). Isolation and characterization of a nisin-like bacteriocin produced by a Lactococcus lactis strain isolated from charqui, a Brazilian fermented, salted and dried meat product. *Meat Science, 93*(3), 607-613. http://dx.doi.org/10.1016/j.meatsci.2012.11.021

Burdock, G. A., & Carabin, I. G. (2004). Generally recognized as safe (GRAS): History and description. *Toxicology Letters, 150*(1), 3. http://dx.doi.org/10.1016/j.toxlet.2003.07.004

Castellano, P., Raya, R., & Vignolo, G. (2003). Mode of action of lactocin 705, a two-component bacteriocin from Lactobacillus casei CRL705. *International Journal of Food Microbiology, 85*(1), 35-43. http://dx.doi.org/10.1016/S0168-1605(02)00479-8

Chatterjee, C., Paul, M., Xie, L., & van der Donk, W. A. (2005). Biosynthesis and mode of action of lantibiotics. *Chemical Reviews, 105*(2), 633-684. http://dx.doi.org/10.1021/cr030105v

Chen, Y.-S., Wang, Y.-C., Chow, Y.-S., Yanagida, F., Liao, C.-C., & Chiu, C.-M. (2014). Purification and characterization of plantaricin Y, a novel bacteriocin produced by *Lactobacillus plantarum* 510. *Archives of Microbiology, 196*(3), 193-199. http://dx.doi.org/10.1007/s00203-014-0958-2

Cotter, P. D., Hill, C., & Ross, R. P. (2005). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology, 3*(10), 777-788. http://dx.doi.org/10.1038/nrmicro1273

Enan, G., El-Essawy, A. A., Uyttendaele, M., & Debevere, J. (1996). Antibacterial activity of *Lactobacillus plantarum* UG1 isolated from dry sausage: Characterization, production and bactericidal action of plantaricin UG1. *International Journal of Food Microbiology, 30*(3), 189-215.

Gao, Y., Jia, S., Gao, Q., & Tan, Z. (2010). A novel bacteriocin with a broad inhibitory spectrum produced by Lactobacillus sake C2, isolated from traditional Chinese fermented cabbage. *Food Control, 21*(1), 76-81. http://dx.doi.org/10.1016/j.foodcont.2009.04.003

Gong, H. S., Meng, X. C., & Wang, H. (2010). Plantaricin MG active against Gram-negative bacteria produced by *Lactobacillus plantarum* KLDS1.0391 isolated from "Jiaoke", a traditional fermented cream from China. *Food Control, 21*(1), 89-96. http://dx.doi.org/10.1016/j.foodcont.2009.04.005

Gupta, A., & Tiwari, S. K. (2014). Plantaricin LD1: A Bacteriocin Produced by Food Isolate of *Lactobacillus plantarum* LD1. *Applied Biochemistry and Biotechnology, 172*(7), 3354-3362. http://dx.doi.org/1007/s12010-014-0775-8

Hata, T., Tanaka, R., & Ohmomo, S. (2010). Isolation and characterization of plantaricin ASM1: A new bacteriocin produced by *Lactobacillus plantarum* A-1. *International Journal Of Food Microbiology, 137*(1), 94-99. http://dx.doi.org/10.1016/j.ijfoodmicro.2009.10.021

Hauge, H. H., Mantzilas, D., Eijsink, V. G., & Nissen-Meyer, J. (1999). Membrane-mimicking entities induce structuring of the two-peptide bacteriocins plantaricin E/F and plantaricin J/K. *Journal of Bacteriology, 181*(3), 740-747.

Hu, M., Zhao, H., Zhang, C., Yu, J., & Lu, Z. (2013). Purification and Characterization of Plantaricin 163, a Novel Bacteriocin Produced by *Lactobacillus plantarum* 163 Isolated from Traditional Chinese Fermented Vegetables. *Journal of Agricultural and Food Chemistry, 61*(47), 11676-11682. http://dx.doi.org/10.1021/jf403370y

Kato, T., Matsuda, T., Ogawa, E., Ogawa, H., Kato, H., Doi, U., & Nakamura, R. (1994). Plantaricin-149, a bacteriocin produced by *Lactobacillus plantarum* NRIC 149. *Journal of Fermentation and Bioengineering, 77*(3), 277-282.

Oppegård, C., Rogne, P., Emanuelsen, L., Kristiansen, P. E., Fimland, G., & Nissen-Meyer, J. (2007). The two-peptide class II bacteriocins: Structure, production, and mode of action. *Journal of Molecular Microbiology and Biotechnology, 13*(4), 210-219. http://dx.doi.org/10.1159/000104750

Pucci, M. J., Vedamuthu, E. R., Kunka, B. S., & Vandenbergh, P. A. (1988). Inhibition of Listeria monocytogenes by using bacteriocin PA-1 produced by Pediococcus acidilactici PAC 1.0. *Applied and Environmental Microbiology, 54*(10), 2349-2353.

Roepstorff, P., & Fohlman, J. (1984). *Biomed. Mass Spectrometry* (Vol. 11, p. 601).

Ruixiang, Z., Gaili, D., Tianyou, Y., Shengyang, N., & Ying, W. (2015). Purification, Characterization and Antibacterial Mechanism of Bacteriocin from Lactobacillus Acidophilus XH1. *Tropical Journal of Pharmaceutical Research, 14*(6), 989-995. http://dx.doi.org/10.4314/tjpr.v14i6.8

Wiedemann, I., Benz, R., & Sahl, H.-G. (2004). Lipid II-mediated pore formation by the peptide antibiotic nisin: A black lipid membrane study. *Journal of Bacteriology, 186*(10), 3259-3261. http://dx.doi.org/10.1128/JB.186.10.3259-3261.2004

Zhang, H., Liu, L., Hao, Y., Zhong, S., Liu, H., Han, T., & Xie, Y. (2013). Isolation and partial characterization of a bacteriocin produced by *Lactobacillus plantarum* BM-1 isolated from a traditionally fermented Chinese meat product. *Microbiology and Immunology, 57*(11), 746-755. http://dx.doi.org/10.1111/1348-0421.12091

Zhang, Q., Lu, Y., Liu, X., Bie, X., Lv, F., & Lu, Z. (2014). Preservative Effect of Food-Based Fermentate from Lactobacillus acidophilus NX2-6 on Chilled Pork Patties. *Journal of Food Protection, 77*(3), 459-465. http://dx.doi.org/10.4315/0362-028X.JFP-13-359