Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.com

Document heading doi:10.12980/APJTB.4.2014C537

Production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 and its broad antibacterial spectrum

Sivaramasamy Elayaraja[1*], Neelamegam Annamalai[2], Packiyam Mayavu[2], Thangavel Balasubramanian[2]

[1]*Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China*

[2]*Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai-608502, Tamilnadu, India*

PEER REVIEW

Peer reviewer

Dr. Jiquan Zhang, Ph.D., Key Lab of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China.
Tel: 86-532-82898571
Fax: 86-532-82898578
E-mail: zhangjiquan@qdio.ac.cn

Comments

This is a valuable research work in which authors have made attempt to study production, purification and characterization of bacteriocin from marine bacterium *L. murinus* AU06. In addition to this, purified bacteriocin was tested for its antimicrobial activity against fish pathogens.
Details on Page S310

ABSTRACT

Objective: To study the production, purification and characterization of bacteriocin from *Lactobacillus murinus* AU06 isolated from marine sediments and its broad spectrum of inhibition against fish pathogens.
Methods: The selected strain was used in production, purification and characterized of bacteriocin. In addition, purified bacteriocin was tested for its antimicrobial activity against fish pathogens.
Results: In the present study, the bacteriocin production was found to be higher at 35 °C, pH 6.0 and was purified to 4.74 fold with 55. 38 U/mg of specific activity with the yield of 28.92%. The molecular weight of the purified bacteriocin was estimated as 21 kDa. The purified bacteriocin exhibited complete inactivation of antimicrobial activity when treated with proteinase K, pronase, chymotrypsin, trypsin, pepsin and papain. The purified bacteriocin exhibited broad inhibitory spectrum against both Gram positive and negative bacteria.
Conclusions: It is concluded that the ability of bacteriocin in inhibiting a wide-range of pathogenic bacteria is of potential interest for food safety and may have future applications in food preservative.

KEYWORDS

Lactobacillus murinus, Bacteriocin, Purification, Antibacterial, Pathogen

1. Introduction

Bacteriocins are natural peptides secreted by many varieties of bacteria for the purpose of killing other bacteria. This provides them with a competitive advantage in their environment, eliminating competitors to gain resources. These peptides are ribosomally synthesized, although some are extensively post-translationally modified.

Lactic acid bacteria (LAB) are a diverse group of microorganisms that produce lactic acid as the primary end-product of the fermentation of carbohydrates[1-3]. LAB plays a critical role in food processing and spontaneous fermentation and is used in a wide range of fermented food. LAB exerts a strong antagonistic activity against many food contaminating microorganisms as a result of the production of organic acids, hydrogen peroxide, diacetyl, inhibitory enzymes and bacteriocins[4-6]. LAB species belonging to the genera *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Pediococcus*, *Oenococcus*, *Enterococcus*, *Leuconostoc* and *Carnobacterium* produce a variety of bacteriocins, most

*Corresponding author: Sivaramasamy Elayaraja, Key Laboratory of Experimental Marine Biology, Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China.
Tel.: +86-13573827556
Fax: + 86-532-82898578
E-mail: elayabact@gmail.com
Foundation Project: Supported by Ministry of Earth Sciences (MoES)-COMAPS and OASTC (G4/4073/2007).

Article history:
Received 3 Feb 2014
Received in revised form 10 Feb, 2nd revised form 13 Feb, 3rd revised form 20 Feb 2014
Accepted 13 Mar 2014
Available online 5 Apr 2014

of which can be grouped into several classes[7]. These bacteriocin producing bacteria are probably amongst the most promising natural food biopreservatives[8-10].

Based on structural, physicochemical and molecular properties, bacteriocins from LAB can be subdivided into three major classes[9,11]. Class 1 bacteriocins are lantibiotics, *i.e.* small, cationic, hydro-phobic, and heat-stable peptides that contain unusual amino acids (*e.g.* the thioether amino acids lanthionine and/or 3-methyl-lanthionine) that are post-translationally formed. Class 2 bacteriocins are small, cationic, hydrophobic, heat-stable peptides that are not post-translationally modified, except for cleavage of a leader peptide from the pre-bacteriocin peptide. Within this class, three subclasses can be distinguished: Subclass 2a or pediocin-like bacteriocins with a strong antilisterial effect, possessing the consensus sequence Tyr-Gly-Asn-Gly-Val in their N-terminus; Subclass 2b or bacteriocins that require two polypeptide chains for full activity; and Subclass 2c or bacteriocins that do not belong to the other subgroups. Class 3 bacteriocins are a group of large, hydrophilic, heat-labile proteins.

Bacteriocins are ribosomally synthesized antimicrobial peptides that are considered to be safe natural biopreservatives as they are sensitive to proteases in the gastrointestinal tract and are effective in controlling food-borne pathogens[12]. Nisin is the only FDA-approved bacteriocin, which is widely used for preservation of pasteurized processed cheese[13]. However, the limited activity spectrum of nisin with respect to pH and its inherent insolubility has underscored the need for additional bacteriocins that demonstrate superior stability over a wide range of pH and are suitable for food fermentation and preservation processes. Hence, an attempt was made in this study on production, purification and characterization of bacteriocin from marine bacterium *Lactobacillus murinus* AU06 (*L. murinus*). In addition to this, purified bacteriocin was tested for its antimicrobial activity against fish pathogens.

2. Materials and methods

2.1. Isolation and biochemical characterization of bacteriocin producing LAB

The bacteriocin producing LAB *L. murinus* AU06 was isolated from marine sediments of Parangipettai coast. The samples were aseptically weighted and 1:10 dilution was subsequently made by dissolving the sample in 100 mL of sterile 50% aged sea water and was serially diluted, spreaded on respective plates water followed by making a 10 fold serial dilution. A volume of 0.1 mL from each dilution was then subcultured in duplicate into the MRS agar (Merck, Germany) for isolating lactobacilli. The strain was cultured in MRS agar plate and incubated in an anaerobic jar at 37 °C for 48 h[14]. Isolated colonies with typical characteristics of LAB were picked from each plate and transferred to MRS slants.

The selected strain was identified by employing the standard schemes of morphological, physiological, biochemical characteristics up to genetic level[15]. The molecular identification of the isolate was achieved by 16S rRNA gene sequencing. In brief, DNA extractions were carried out by phenol chloroform method[16,17]. The primer sequences were chosen from the conserved regions as reported earlier for the bacterial 16S rRNA gene[18]. Sequencing was done using the forward primer (5′-CAGGCCTAACACATGCAAGTC-3′) and reverse primer (5′-GGGCGGTGTGTACAAGGC-3′). PCR reactions were performed with the following conditions: denaturation-30 cycles consisting of 95 °C for 1 min, annealing-55 °C for 1 min and extension-72 °C for 1.5 min, followed by a final extension step of 5 min at 72 °C. After cycling, the PCR products were detected by electrophoresis on a 1% agarose gel, stained with ethidium bromide and visualized under UV light. The 16S rRNA gene sequence was analyzed by an automated DNA Sequencer (Megabace, GE) and homology with those sequences in the GenBank database was analyzed with Clustal-X software. A phylogenetic tree was constructed by neighbor-joining method using Clustal-X version 1.81 and MEGA version 4.1[19-21].

2.2. Influence of growth conditions on the production of bacteriocin

Effect of temperature, pH and incubation time on bacteriocin production was performed with 100 mL of MRS broth in 500 mL of Erlenmeyer flasks. The mixture of bacteriocin and MRS broth (1%, v/v) was inoculated with an overnight culture and incubated at different temperatures (25, 30, 35, 40 and 45 °C), pH (4.5, 5.0, 5.5, 6.0 and 6.5) and incubation time at every 6 h interval. Samples collected after 48 h (expect for incubation time effect) were examined for bacteriocin production (AU/mL).

2.3. Production and purification of bacteriocin from L. murinus AU06

For bacteriocin production, *L. murinus* AU06 was grown in MRS broth (Hi Media Laboratory, Pvt Ltd. India) (pH 6.5) seeded with 1 % inoculum, maintained at optimized culture condition for 48 h. After incubation, cells were removed from the growth medium by centrifugation at 15000 r/min for 15 min, 4 °C. The cell-free supernatant was adjusted to pH 6.5 using 1 mol/L NaOH and it was used as crude bacteriocin. The bacteriocin activity was measured by standard well diffusion assay described by Kang and Lee[22].

The bacteriocin from the culture supernatant was purified by simple purification method described by Lozano *et al*[23]. Briefly, 400 g of ammonium sulfate per liter of culture supernatant was added and allowed to settle for 24 h at 4 °C. The protein precipitates were collected by centrifugation at 6000 r/min for 20 min and dissolved in 50 mL of 20 mmol/L sodium phosphate buffer (pH 6.0). Further, it was applied on diethylaminoethyl-cellulose column (1.5×40.0 cm) equilibrated with 0.1 mol/L Tris-HCl buffer (pH 9) and eluted with linear salt gradient of NaCl (0-1 mol/L). The active

fractions were pooled together, concentrated by ammonium sulphate, loaded on Sephadex G-75 column (1.594 0 cm) equilibrated with 0.1 mol/L Tris-HCl buffer (pH 9) and eluted with same buffer at a flow rate of 0.5 mL/min and then eluted fractions were assayed for bacteriocin activity.

2.4. Effect of enzymes, pH, temperature, detergents and amylase inhibitors on bacteriocin activity

The proteinaceous nature of the inhibitory compounds was confirmed by testing their sensitivity to proteolytic enzymes. Aliquots of the purified bacteriocin were treated with the following enzymes (1 mg/mL) and incubated for 2 h at 30 °C: proteinase K (HiMedia, Mumbai), chymotrypsin (HiMedia, Mumbai), trypsin (HiMedia, Mumbai), pepsin (HiMedia, Mumbai), α-amylase (Sigma, USA) and catalase (HiMedia, Mumbai). Antimicrobial activity was monitored by using the agar spot test method as described elsewhere.

The effect of temperature on activity of the purified bacteriocin was tested by incubating at various temperature between 30 and 90 °C and the residual activity was tested after 20, 60, 90 and 120 min. To find out the effect of pH on bacteriocin activity it was tested by incubating at various pH through adjusting between 4.0 to 10.0 with sterile 1 mol/L NaOH or 1 mol/L HCl.

The effect of surfactants on bacteriocin activity was tested by adding 1% sodium dodecyl sulfate (SDS), Tween 20, Tween 80, urea, Triton X-114 and Triton X-100 with the purified bacteriocin separately. Untreated bacteriocin was considered as control and its activity was taken as 100%. All treated and untreated bacteriocin (control) were incubated at 37 °C and then tested for antimicrobial activity by using the agar spot test method.

2.5. Antibacterial activity assay

The antimicrobial activity of the purified bacteriocin was detected against different indicator strains by agar well diffusion method[24]. The purified bacteriocin (100 μL) was added in 10 mm wells on nutrient agar plates previously spreaded with 100 μL suspension of each indicator strain containing 2×10^8 CFU/mL and the plates were incubated for 48 h[25].

3. Results

3.1. Isolation and identification of bacteriocin producing strain L. murinus AU06

In the present study, bacteriocin producing lactic acid bacteria AU06 was isolated from the marine sediments of Parangipettai coast (Latitude 11°29′ N and Longitude 79°46′ E). The strain AU06 is a Gram-positive and non endospore forming bacillus, with catalase but without oxidase, which grows in both aerobic and anaerobic environments (Table 1). On the basis of 16S rRNA gene sequence and phylogenetic analysis, the strain AU06 is closest to *L. murinus* (NR 042231). Concluding from the above results, the strain was identified as *L. murinus* and designated as *L. murinus* AU06 (GenBank Accession No. JN987182) (Figure 1).

Table 1
Physiological and biochemical characteristics of bacteriocin producing strain *L. murinus* AU06.

Name of the test	Observation
Gram′s reaction	+
Shape	Rod
Spore formation	−
Motility	+
Catalase	+
Glucose	+
Arabinose	+
Galactose	+
Lactose	+
Indole	−
Sorbitol	+
Maltose	+
Gas formation	+
Ribose	+
Manitol	−

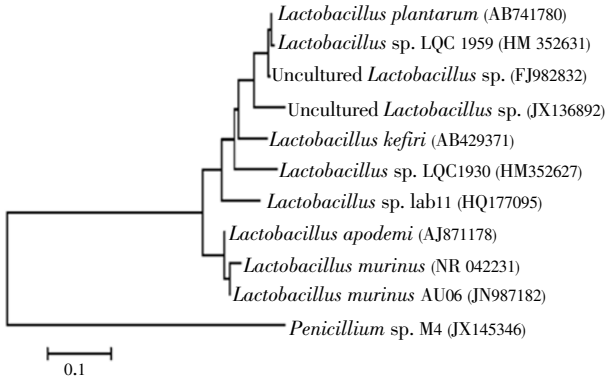

Figure 1. Phylogenetic tree showing the taxonomic position of *L. murinus* AU06.

3.2. Growth and bacteriocin production by L. murinus AU06

The influence of growth conditions on bacteriocin studies revealed that, incubation temperature and pH played an important role in cell growth as well as bacteriocin production. Furthermore, bacteriocin production (2 323 AU/mL) was found to be higher at 35 °C, pH 6.0 and lower at 45 °C, pH 6.5 (383 AU/mL) respectively (Figure 2 and 3). The decrease in antimicrobial activity observed after a longer incubation time could be due to the degradation of the bacteriocin by proteolytic enzymes present in the medium, or else by the low pH.

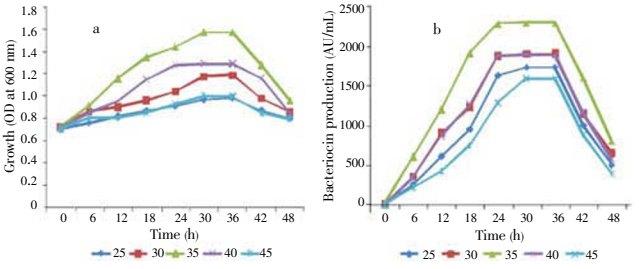

Figure 2. Cell growth and bacteriocin production at various temperatures.
a: Cell growth; b: Bacteriocin production.

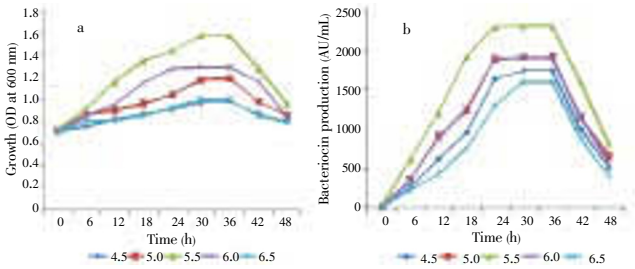

Figure 3. Cell growth and bacteriocin production at different pH.
a: Cell growth; b: Bacteriocin production.

3.3. Production and purification of bacteriocin from L. murinus AU06

Regarding bacteriocin production, cell growth was started from late log phase itself and maximum was obtained in early stationary growth phase at 30th h of the culture. Growth beyond stationary phase resulted decrease in bacteriocin production (Figure 4). In purification, bacteriocin from culture supernatant was concentrated by ammonium sulfate precipitation followed by cation exchange chromatography and hydrophobic interaction chromatography. The bacteriocin from culture broth of *L. murinus* AU06 was purified to 4.74 fold with 55.38 U/mg of specific activity with the yield of 28.92% (Table 2).

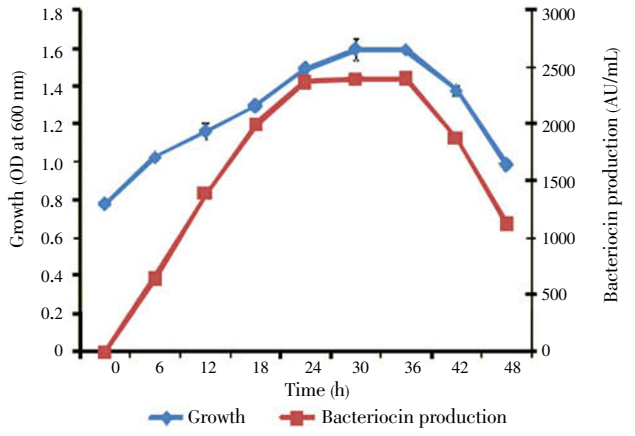

Figure 4. Growth kinetics vs. bacteriocin production under optimized culture conditions.

Table 2
Summary of the purification profile of bacteriocin from culture supernatant of *L. murinus* AU06.

Purification step	Total activity (U)	Total protein (mg)	Specific activity (U/mg)	Purification (Fold)	Yield (%)
Culture filtrate	2240	192.1	11.66	1	100
$(NH_4)_2SO_4$ precipitate	1920	98.7	19.45	1.66	85.71
DEAE-cellulose	1250	42.5	29.41	2.52	55.80
Sephadex G-75	648	11.7	55.38	4.74	28.92

3.4. Molecular weight determination

The molecular weight of the purified bacteriocin of *L. murinus* AU06 was estimated as 21 kDa (Figure 5) by SDS-PAGE gel electrophoresis. Single protein band was observed when stained with Comassie brilliant blue and it clearly indicated the purity of the protein.

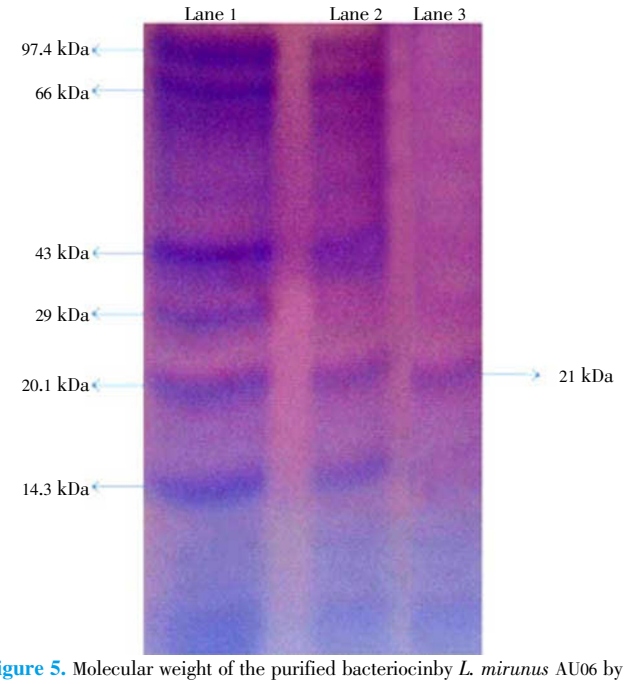

Figure 5. Molecular weight of the purified bacteriocinby *L. mirunus* AU06 by SDS-PAGE.
Lane 1: Molecular weight markers; Lane 2: Crude; Lane 3: Purified bacteriocin.

3.5. Effect of enzymes, temperature, pH, detergents and proteinaceous inhibitors on bacteriocin

The proteinaceous nature of the bacteriocin was confirmed by testing their sensitivity with proteolytic enzymes. The bacteriocin of *L. murinus* AU06 exhibited complete inactivation of antimicrobial activity after the treatment of bacteriocin with proteinase K, chymotrypsin, trypsin and pepsin which confirming its proteinaceous nature (Table 3). Treatment of the bacteriocin with catalase and α-amylase did not result in any changes of antimicrobial activity indicating that the inhibition recorded was not hydrogen peroxide and that carbohydrate moieties were not required for antimicrobial activity.

Table 3
Effect of enzymes on activity of purified bacteriocin from *L. murinus* AU06.

Enzyme	Bacteriocin activity
Proteinase K	–
Chymotrypsin	–
Trypsin	–
Pepsin	–
α-Amylase	+
Catalase	+

Effect of temperature studies revealed that the bacteriocin was stable over a broad range of temperature between 30-80 °C. It retained more than 60% of its original activity even at 60 °C for 30 min and declined afterwards (Figure 6). Effect of pH on bacteriocin activity revealed that, 100% active stability was observed between the pH 6-7 and more than 70% of activity was retained at pH 4 (Figure 7). The

bacteriocin activity was moderately resistant to detergents such as SDS, Triton X-100, Tween 80, Tween 20 and ethylene diamine tetra acetic acid whereas highly sensitive to Triton X-114 and urea (Table 4).

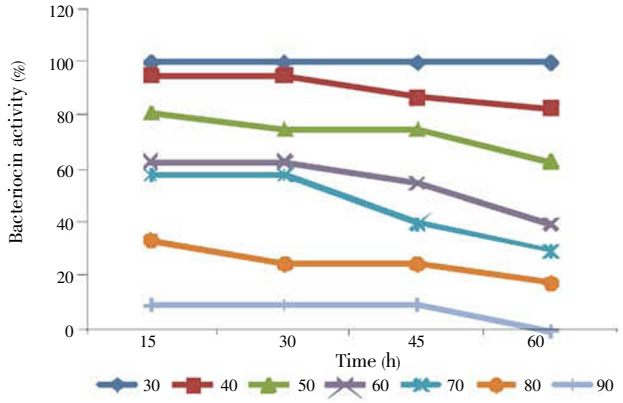

Figure 6. Effect of temperature on activity of purified bacteriocin from *L. murinus* AU06.

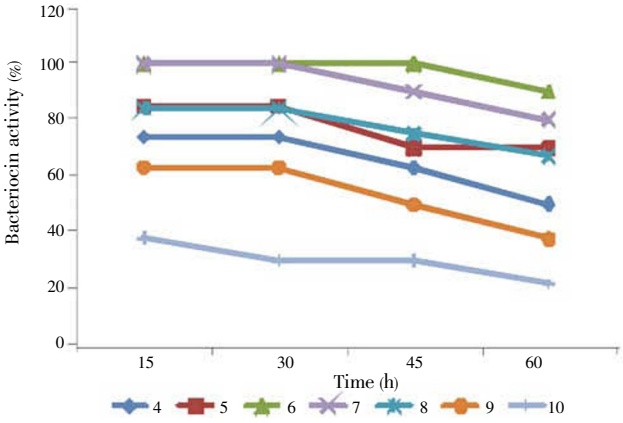

Figure 7. Effect of pH on activity of purified bacteriocin from *L. murinus* AU06.

Table 4

Effect of detergents on activity of purified bacteriocin from *L. murinus* AU06.

Chemicals	Concentration	Residual activity (%)
Triton X-100	1%	25
Triton X-114	1%	-
Tween 80	1%	60
Tween 20	1%	42
SDS	1%	80
Urea	1%	-
EDTA	5 mmol/L	75

3.6. Determination of inhibitory spectrum

The purified bacteriocin of *L. murinus* AU06 was tested against Gram positive and Gram negative bacteria (Table 5). Among the 9 indicator strains tested, 7 were found to be sensitive. The significant inhibition was observed against *Micrococcus* sp. and *Staphylococcus aureus* (*S. aureus*) and *Pseudomonas aeruginosa* and *Escherichia coli* whereas lower inhibition found with *Enterococcus faecalis*, *Bacillus licheniformis* and *Listeria monocytogenes*.

Table 5

Antimicrobial activity of purified bacteriocin from *L. murinus* AU06 against various bacterial pathogens.

Bacterial strain	Zone of inhibition (mm)	Antagonistic activity
S. aureus	24.0	++++
Vibrio sp.	0.0	-
Enterococcus faecalis	10.0	+
Listeria monocytogenes	15.0	++
Escherichia coli	18.0	+++
Micrococcus sp.	28.0	++++
Klebsilla pneumonia	0.0	-
Bacillus licheniformis	13.5	++
Pseudomonas aeruginosa	22.0	+++

-: No activity; +: Inhibitory zone diameter within 5-10; ++: Inhibitory zone diameter within 10-15; +++: Inhibitory zone diameter within 15-20; ++++: Inhibitory zone diameter within 20-25.

4. Discussion

Bacteriocins are small peptides contains 30-60 amino acid with antimicrobial properties against bacteria usually of the same or closely related species (narrow spectrum), and sometimes against a wide spectrum of species[26,27]. Microorganisms that produce bacteriocins also have immunity mechanisms to present self-protection from committing "suicide"[28,29].

In general, the production of bacteriocin can be significantly influenced by pH, temperature, incubation time, and other environmental factors[30,31]. The results obtained in this study revealed that optimal production of bacteriocin from *L. murinus* AU06 occurs usually at pH above 6, whereas many studies have reported that maximum bacteriocin production was found between pH 4 and 5[32-34]. Annamalai *et al.*[32] found that the optimum temperature for bacteriocin production from *E. faecium* was 35 °C. Todorov and Dicks[35] have reported optimal production (12 800 AU/mL) of bacteriocin ST712BZ was recorded when incubated at 30 °C but only 6 400 AU/mL was recorded when the cells were incubated at 37 °C. Similarly Sankar *et al.*[8] reported the bacteriocin producing *Lactobacillus plantarum* (*L. plantarum*) strain isolated from raw cow's milk samples, showed broad range of antibacterial activity against food borne pathogens.

An increased amount of biological activity has also been reported during purification of other bacteriocin in the pediocin family and may be due to the presence of some inhibitory compound at an earlier stage of the purification[36].

The molecular weight of the purified bacteriocin found in the present study as 21 kDa was analogous to the result obtained by *L. murinus* AU06. Many investigators reported that different molecular mass were higher than the bacteriocin from ST16Pa (6.5 kDa)[37], *L. plantarum* (10 kDa)[38], bacST8KF (3.5 kDa)[39], *L. plantarum* ST194BZ (14.0 kDa) and *E. faecium* (5 kDa)[40,41].

The proteinaceous nature of the bacteriocin of *L. murinus* AU06 exhibited complete inactivation of antimicrobial

activity after the treatment of bacteriocin with proteinase K, chymotrypsin, trypsin and pepsin, which confirms its proteinaceous nature. Similar results have been reported for other proteinaceous inhibitors on bacteriocin of *L. plantarum* bacST202Ch and bacST216Ch[42]. In general, Gram negative bacteria are usually considered to be resistant to the many of bacteriocins from *Lactobacillus* strains[35,42]. Similarly Maurya and Thakur have reported antibacterial spectrum of the isolate, partial purification, characterization and effect of some physical and chemical factors on the activity of bacteriocin from *Leuconostoc* NT-1[4]. *Leuconostoc* NT-1 inhibits several pathogenic and spoilage-causing bacteria. The inhibitory activity against some potent pathogens such as *S. aureus*, including a methicillin-resistant *S. aureus*, *Salmonella typhi* and *Klebsiella pneumoniae* is an interesting feature of the bacteriocin.

The bacteriocin had broad spectrum of inhibition against fish pathogens. However, some reports have been supported to our findings that certain lactic acid bacteriocins, especially the Class 2 bacteriocin pediocin, can inhibit a limited number of Gram negative bacteria including *Shigella* sp., *Salmonella* sp., *Pseudomonas* sp. and *Shigella flexneri*[43,44].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

The authors are greatly thankful to Ministry of Earth Sciences (MoES)-COMAPS and OASTC (G4/4073/2007) for providing financial support and also thankful to the authorities of Annamalai University for providing facilities.

Comments

Background

Bacteriocin is one of the antagonistic compounds found to possess major applications in food and pharmaceutical industries, as food preservative and drug. Many of these lactic acid bacteria are known to produce antibacterial substances including bacteriocins which can inhibit the growth of several pathogenic bacteria.

Research frontiers

The present research depicts the production, purification and characterization of bacteriocin from *L. murinus* AU06 isolated from marine sediments. The produced bacteriocin had broad spectrum of inhibition against fish pathogen.

Related reports

Lactic acid bacteria are a group of microorganisms widely used in the industrial food fermentation. They are the cell factors for the production of high-value metabolites involved in flavor, texture.

Innovations and breakthroughs

The *Lactobacillus* bacteria that play a crucial role in the production of these fermented foods are called lactic acid bacteria. These lactic acid bacteria could produce bacteriocins which exhibit a narrow antimicrobial spectrum and they are modified or unmodified ribosomally synthesized peptides. In the present study, purified bacteriocin was tested for its anti bacterial activity against fish pathogens.

Applications

From the literature survey, it has been found that *L. murinus* AU06 plays an important role in human and animals. This scientific study supports and suggests the use of this bacterium in food preservation and medical application.

Peer review

This is a valuable research work in which attempt was made on production, purification and characterization of bacteriocin from marine bacterium *L. murinus* AU06. In addition to this, purified bacteriocin was tested for its antimicrobial activity against fish pathogens.

References

[1] Kumari A, Akkoç N, Akçelik M. Purification and partial characterization of bacteriocin produced by *Lactococcus lactiss* sp. lactis LL171. *World J Microbiol Biotechnol* 2012; **28**: 1647-1655.

[2] Carr FJ, Chill D, Maida N. The lactic acid bacteria: a literature survey. *Crit Rev Microbiol* 2002; **28**(4): 281-370.

[3] Pringsulaka O, Thongngam N, Suwannasai N, Atthakor W, Pothivejkul K, Rangsiruji A. Partial characterization of bacteriocins produced by lactic acid bacteria isolated from Thai fermented meat and fish products. *Food Control* 2012; **23**: 547-551.

[4] Maurya AP, Thakur RL. Inhibition spectrum, purification and characterization of bacteriocin from *Leuconostoc*NT-1. *Curr Sci* 2012; **103**(12): 1405-1407.

[5] Piard JC, Desmazeaud M. Inhibiting factors produced by lactic acid bacteria: 1. Oxygen metabolites and catabolism end products. *Lait* 1991; **71**: 525-541.

[6] Wilson BA, Thomas SM, Ho Me. The human vaginal microbiome. In: Nelson KE, editor. *Metagenomics of the human body*. Germany: Springer; 2011, p. 91-115.

[7] Klaenhammer TR. Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* 1993; **12**: 39-86.

[8] Sankar NR, Priyanka VD, Reddy PS, Rajanikanth P, Kumar VK, Indira M, et al. Purification and characterization of bacteriocin produced by *Lactobacillus plantarum* Isolated from cow milk. *Int J Microbiol Res* 2012; **3**(2): 133-137.

[9] Atanassova V, Meindl A, Ring C. Prevalence of *Staphylococcus aureus* and staphylococcal enterotoxins in raw pork and uncooked

smoked ham: a comparison of classical culturing detection and RFLP-PCR. *Int J Food Microbiol* 2001; **68**: 105-113.

[10] Leroy F, De Vuyst L. A combined model to predict the functionality of the bacteriocin producing *Lactobacillus* sakeistrain CTC 494. *Appl Environ Microbiol* 2003; **69**(2): 1093-1099.

[11] Nes IF, Diep DB, Havarstein LS, Brurberg MB, Eijsink V, Holo H. Biosynthesis of bacteriocins in lactic acid bacteria. *Antonie Van Leeuwenhoek* 1996; **70**: 113-128.

[12] Cleveland J, Montville TJ, Nes IF, Chikindas ML. Bacteriocins: safe, natural antimicrobials for food preservation. *Int J Food Microbiol* 2001; **71**: 1-20.

[13] Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. *Nat Rev Microbiol* 2005; **3**: 777-788.

[14] De Man JC, Rogosa M, Sharpe ME. A medium for the cultivation of lactobacilli. *J Appl Bacteriil* 1960; **23**: 130-135.

[15] Garrity GM, Boone DR, Castenholz RW. *Bergey's manual of systematic bacteriology*. 2nd ed. Germany: Springer Verlag; 2001.

[16] Marmur J. A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J Mol Biol* 1961; **3**: 208-218.

[17] Kocabiyik S, Caba E. Prevalence of monochloro acetate degrading genotypes among soil isolates of *Pseudomonas* sp. *Biodegradation* 1998; **8**: 371-377.

[18] Marchesi JR, Sato T, Weightman AJ, Martin TA, Fry JC, Hiom SJ, et al. Design and evaluation of useful bacterium specific PCR primers that amplify genes coding for bacterial 16S rRNA. *Appl Environ Microbiol* 1998; **64**: 795-799.

[19] Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol Biol Evol* 1987; **4**: 406-425.

[20] Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; **25**: 4876-4882.

[21] Tamura K, Dudley J, Nei M, Kumar S. MEGA 4: molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; **24**: 1596-1599.

[22] Kang JH, Lee MS. Characterization of a bacteriocin produced by *Enterococcus faecium* GM-1 isolated from an infant. *J Appl Microbiol* 2005; **98**: 1169-1176.

[23] Nieto Lozano JC, Meyer JN, Sletten K, Peláz C, Nes IF. Purification and amino acid sequence of a bacteriocin produced by *Pediococcus acidilactici*. *J Gen Microbiol* 1992; **138**: 1985-1990.

[24] Tagg JR, Mcgiven AR. Assay system for bacteriocins. *Appl Microbiol* 1971; **21**(5): 943.

[25] Iqbal A. Production, purification and characterization of bacteriocins from indigenous clinical staphylococci [dissertation]. Karachi: University of Karachi, Pakistan Research Repository, Higher Education Commission; 1998.

[26] Klaenhammer TR. Bacteriocins of lactic acid bacteria. *Biochimie* 1988; **70**(3): 337-349.

[27] Rantsiou K, Drosinos EH, Gialitaki M, Metaxopoulos I, Comi G, Cocolin L. Use of molecular tools to characterize *Lactobacillus* spp. isolated from Greek traditional fermented sausages. *Int J Food Microbiol* 2006; **112**(3): 215-222.

[28] Draper SJ, Moore AC, Goodman AL, Long CA, Holder AA, Gilbert SC, et al. Effective induction of high titer antibodies by viral vector vaccines. *Nat Med* 2008; **14**(8): 819-821.

[29] Waleed KMAT, Ibrahem AAA, Mohammed QAA, Al alusi Nadia T, Salmah I. Isolation of *Lactobacillus* salivarius from children and purification of bacteriocin to inhibition cancer cell *in vitro*. In: Proceeding of the International Conference on Advanced Science, Engineering and Information Technology; 14-15 January 2011; Bangi, Malaysia; 2011, p. 14-15.

[30] Mortvedt-Abildgaa CI, Nissen-Meyer J, Jelle B, Grenov B, Skaugen M, Nes IF. Production and pH-dependent bactericidal activity of lactocin S, a lantibiotic from *Lactobacillus* sakeiL45. *Appl Environ Microbiol* 1995; **61**: 175-179.

[31] Annamalai N, Manivasagan P, Balasubramanian T, Vijayalakshmi S. Enterocin from *Enterococcus* faecium isolated from mangrove environment. *Afr J Biotechnol* 2009; **8**(22): 6311-6316.

[32] Abo-Amer AE. Molecular characterization of antimicrobial compound produced by *Lactobacillus* acidophilusAA11. *Acta Microbiol Immunol Hung* 2007; **54**: 107-119.

[33] Usmiati S, Marwati T. Selection and optimization process of bacteriocin production from *Lactobacillus* sp.1. *Indonesian J Agric* 2009; **2**(2): 82-92.

[34] Tarelli GT, Carminati D, Giraffa G. Production of bacteriocins active against *Listeria monocytogenes* and *Listeria innocua* from dairy enterococci. *Food Microbiol* 1994; **11**: 243-252.

[35] Todorov SD, Dicks MTL. Bacteriocin production by *lactobacillus* pentosusst712bz isolated from Boza. *Brazilian J Microbiol* 2007; **38**: 166-172.

[36] Xiraphi N, Georgalaki M, Driessche GV, Devreese B, Beeumen JV, Tsakalidou E, et al. Purification and characterization of curvaticin L442, a bacteriocin produced by *Lactobacillus curvatus* L442. *Antonie van Leeuwenhoek* 2006; **89**: 19-26.

[37] Todorov SD, Furtado DN, Saad SMI, Tome E, Franco BD. Potential beneficial properties of bacteriocin producing lactic acid bacteria isolated from smoked salmon. *J Appl Microbiol* 2011; **110**: 971-986.

[38] Eijsink VG, Axelsson L, Diep DB, Håvarstein LS, Holo H, Nes IF. Production of class II bacteriocins by lactic acid bacteria, an example of biological warfare and communication. *Antonie Van Leeuwenhoek* 2002; **81**: 639-654.

[39] Powell JE, Todorov SD, Van Reenen CAV, Dicks LMT, Witthuhn RC. Growth inhibition of *Enterococcus mundtii* in Kefir by in situ production of bacteriocin ST8KF. *Lait* 2006; **86**: 401-405.

[40] Todorov SD, Dicks LMT. Effect of growth medium on bacteriocin production by *Lactobacillus plantarum* ST194BZ, a strain isolated from Boza. *Food Technol Biotechnol* 2005; **43**: 165-173.

[41] Ennahar S, Sashihara T, Sonomoto K, Ishizaki A. Class IIa bacteriocins: biosynthesis, structure and activity. *FEMS Microbiol Rev* 2000; **24**: 85-106.

[42] Todorov SD, Ho P, Vaz-Velho M, Dicks LM. Characterization of bacteriocins produced by two strains of *Lactobacillus plantarum* isolated from Beloura and Chourico, traditional pork products from Portugal. *Meat Sci* 2010; **84**: 334-343.

[43] Lauková A1, Czikková S, Vasilková Z, Juris P, Mareková M. Occurrence of bacteriocin production among environment enterococci. *Lett Aappl Microbial* 1998; **27**: 178-182.

[44] Kwon DY, Koo MS, Ryoo CR, Kang CH, Min KH, Kim WJ. Bacteriocin produced by *Pediococcus* sp. in kimchi and its characteristics. *J Microbiol Biotechnol* 2002; **12**: 96-105.