Design, Expression and Characterization of Lactiscin—a Novel Broad-Spectrum Peptidic Bacteriocin

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Abstract

Bacteria-derived antimicrobial peptides known as peptidic bacteriocins offer a promising alternative to traditional antibiotics in the face of the emergence of multidrug-resistant bacteria. Here, a nucleotide sequence of the gene encoding *Lactococcus lactis*-derived peptidic bacteriocin designated as lactiscin selectively identified from the GenBank® database was synthesized with an added 6·His sequence and cloned into *Escherichia coli*. Upon low-temperature expression at 16°C, the His-tagged peptide could be produced in both soluble form and insoluble inclusions. Efficient purification of the soluble His-tagged peptide was achieved *via* immobilized-Ni^{2+} affinity chromatography (IMAC) and its estimated molecular mass of ~ 13.4 kDa was determined by tricine-sodium dodecyl sulfate polyacrylamide gel electrophoresis. The purified peptide was highly active against both Gram-positive and Gram-negative bacteria as it exhibited a minimal inhibitory concentration of 0.45 mg/mL, 0.15 mg/mL, 0.35 mg/mL and 0.45 mg/mL against. *Escherichia coli, Vibrio parachemolyticus, Staphylococcus aureus and Micrococcus luteus*, respectively. In addition, the lactiscin peptide still retained antimicrobial activity over a pH range of 3.0–12.0 and heat stability of 100°C for 30 minutes. A membrane integrity study revealed that this peptidic bacteriocin was able to induce *E. coli* membrane permeabilization in a concentration-dependent manner, albeit it showed a negligible toxic effect on erythrocytic cells. Gel retardation assay demonstrated that the lactiscin bacteriocin could suppress the migration of genomic DNA extracted from pathogenic bacteria, suggesting the presence of bacteriocin-responsive binding genomic. Our findings of lactiscin—a novel broad-spectrum bacteriocin would be a valuable additive for the application of food industry as a potential bio-preservative.

Key Points

- A novel recombinant peptidic bacteriocin—lactiscin showed high activity against both Gram-positive and Gram-negative pathogenic bacteria.
- Lactiscin showed good stability against pH and heat, but no hemolytic toxicity.
- This broad-spectrum peptidic bacteriocin would contribute to the future development of a natural food preservative for the application of food industry.

Introduction

The pathogenic microorganisms found in food are primarily responsible for foodborne diseases (Yi et al. 2018). They are a global concern in the industrial sector and a threat to human healthcare (Le Loir et al. 2003). As a result, controlling the proliferation of these organisms is critical. Various physical and chemical preservatives are the principal technique for controlling food quality (Yu et al. 2020). However, this approach can potentially have multiple adverse clinical effects on human health and impair food quality (Vally et al. 2009). Effective, natural, safe preservative or antibacterial agents as alternatives are desperately needed in the food industry.

Antimicrobial peptides (AMPs) have gained popularity as a food preservation alternative. AMPs are naturally occurring antibiotics produced by all organisms ranging from bacteria to plants, vertebrates, and invertebrates (Fjell et al. 2012; Goh and Philip 2015). AMPs are a critical component of the animal's innate immune response against the other category of pathogens (Cotter et al. 2005; Xu et al. 2017). Of particular interest, AMPs from bacteria known as bacteriocins were first used in food preservation about 20 years ago. One of the benefits of bacteriocins is that they can be used as alternative antibiotics (Olejnik-Schmidt et al. 2014) or as food biopreservatives. Bacteriocins, like conventional antibiotics, exhibit antibacterial activity against harmful microorganisms found in food (Bharti et al. 2015). Bacteriocins also minimize chemical preservatives because of their capacity to preserve food without affecting the product or threatening human health. Peptidic bacteriocins in particular can exhibit a wide range of activities by which they either kill (bactericidal) (Nes et al. 1996) or inhibit bacterial growth (bacteriostatic) (Zacharof et al. 2001). The diversity of natural AMPs is classified into four categories: biological origins, function activity, structural properties, and species rich in amino acids (Klaenhammer 1993; Nes et al. 1996). Bacteriocins are also the initial line of defense secreted by food-grade lactic acid bacteria (LAB) (Cotter et al. 2005; Mesa-Pereira et al. 2018). This property enables food scientists to control the growth of specific bacterial species in food. Additionally, these bacteriocins are promising antibiotic options for treating illnesses in people and food-producing animals.

The use of bacteriocins in food preservation has reduced the number of traditional antibiotics applied to improve food safety and shelf life. The most well-known bacteriocin is nisin, which is synthesized by *Lactococcus lactis* and *Streptococcus* species (Delves-Broughton 1996; Shin et al. 2016). Nisin is extensively used as a preservative and food additive. It is active against many Gram-positive pathogens (Laridi et al. 2003), but it has little or no effect on Gram-negative bacteria (Müller-Auffermann et al. 2015). Gram-negative cell walls are far less permeable than Gram-positive cell walls, preventing nisin from penetrating the target molecules. The bacteriocin use in industries necessitates a simple purification system that is controlled and capable of producing large quantities in a short period. As a result, the restrictions of bacteriocin derived from nature are alleviated. Therefore, measures to increase production are necessary to maximize the benefits of bacteriocin use. Additionally, the industry's need for alternate food preservation methods and the limitations of nisin is cause for concern. Numerous reports have been made of unstudied bacteriocin-related data in databases.

Thus, this research aims to examine the bacteriocins in the database. A modified search strategy was used to identify putative bacteriocin-coding genes. The gene was selected and synthesized using KWT49638.1 DNA as a template. The capacity of the recombinant bacteriocin to inhibit both Gram-positive and Gram-negative bacteria was examined after it was cloned, expressed, and purified.

Materials And Methods

Amino acid sequence alignments and homology-based modeling

Multiple sequence alignments and a distance tree displaying the relationship of the peptides of the lactiscin gene (Accession: OP925095) with the corresponding segment of eight related peptides members in the Bacteriocin classIIa family i.e., Lactococin972, E50-52, Pediocin PA-1, Sakacin-P, Avicin A, Enterocin CRL35, Mesentericin Y10 and Enterocin-P were analyzed with Protein BLAST server (https://blast.ncbi.nlm.nih.gov/Blast.cgi?). Sequence alignments was done using ClustalW program through Clustal server (http://www.ebi.ac.uk/Tools/msa/clustalw2/). The crystal structure was analyzed by using I-TASSER server (https://zhanggroup.org/I-TASSER/) and PyMOL program version 2.5.

Construction of recombinant plasmid with His-tagged fusion.

The lactiscin bacteriocin template gene was obtained from the National Center for Biotechnology Information (NCBI) public database. Codon optimization was performed on the *E. coli* expression system using the pET25b + expression vector (KMUTT, Thailand). The gene was amplified *via* polymerase chain reaction (PCR) using Taq DNA polymerase (New England, Biolac). The PCR product was purified using QIAquick Gel Extraction Kit (QIAGEN, USA) and then digested with CutSmart® *Nde*1 and CutSmart® *Bam*H1. After that, the gene was inserted into pET25b + by ligation with T4 DNA ligase (New England Biolabs, USA). The resulting plasmid was transformed into *E. coli* DH5α by heat shock method at 42°C. The positive colonies were confirmed by PCR amplification using a T7 primer pair and subsequently verified by dideoxy-chain-termination nucleotide sequencing using an automated analyzer (Bioneer, Thailand).

Protein expression and purification

The plasmid was extracted from *E. coli* DH5α and transformed into the protease-deficient expression host of *E. coli* BL21(DE3)pLysS. The recombinant vector bacteria were cultured in a 2·Yeast Extract Tryptone (2·YT) medium supplemented with 100 µg/mL ampicillin and 34 µg/mL chloramphenicol. Cell growth was measured using a UV-visible spectrophotometer at 600 nm until it reached ~ 0.6 at 37°C with shaking at 200 pm. The recombinant peptide expression was then induced at a final concentration of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG). The culture was incubated overnight at 16°C with shaking (150 rpm). Cells were harvested using centrifugation at 5000 rpm for 20 minutes at 4°C using JS-5.3 Swinging-Bucket Rotor, Beckman. The cells were disrupted with a Q2000 ultra-sonication before centrifuging for 20 minutes at 9000 rpm, 4°C. The supernatant containing the recombinant peptide was purified using IMA C with an affinity-based Ni^{2+}-NTA column. The pre-equilibrated buffer contained 20 mM Tris-HCl (pH 8.0), 5 mM sodium chloride, and 20 mM imidazole. While the equilibrium buffer consists of 20 mM Tris-HCl (pH 8.0), 5 mM sodium chloride, and 500 mM imidazole. The eluted component was collated, concentrated, and dialyzed using an MW 3000-kDa dialysis tube. The pure lactiscin bacteriocin protein concentration was determined using the Bradford-based micro assay (Bio-RAD, Hercules, CA, USA). (Emami Bistgani et al. 2017).

Tricine-SDS-PAGE and Western blot analysis

The protein purity was determined by analyzing fractions on (16% gel w/v) tricine-SDS-PAGE (Schägger 2006; Ma et al. 2016). Gel staining was done with Coomassie Brilliant Blue G-250 and verified by Western

blot analysis using anti-6·His Tag monoclonal antibodies conjugated to HRP (horseradish peroxidase). The blotted gel was treated with the chemiluminescent substrate tetramethylbenzidine (TMB) for a few minutes. The outcome was measured using a gel doc instrument. The Western blot signal confirmed the presence of protein of 13 kDa apparent molecular mass.

Determination of minimal inhibitory concentration value

The MIC of lactiscin bacteriocin against *Escherichia coli* (ATCC 8739), *Micrococcus luteus* (TISTR 745),

Vibrio parahaemolyticus (ATCC 17802) and *Staphylococcus aureus* (ATCC 6538) was obtained using the microdilution method. The organisms were cultured in nutrient agar at 37°C overnight. A few isolated colonies were transferred into sterilized Mueller Hinton Broth (MBH) and cultured for 4 hours at 37°C, 200 rpm. The bacteria cells were collected in an exponential phase and diluted to $1 \cdot 10^5$ CFU/mL in fresh MBH. Then, 50 µL of each sample was added to a 96-well plate containing 50 µl of the varying concentrations of lactiscin bacteriocin solution, then incubated at 37°C for 16 hours. Absorbance reading was measured at 600 nm using a microplate reader and compared to positive and negative controls of 50 µg/mL ampicillin and 50 mM Tris-HCl pH 8.0 buffer. The MIC was the lowest peptide concentration that inhibited bacteria growth with an OD of less than 0.2 (Masadeh et al. 2019).

Stability assays

The pH stability of purified lactiscin bacteriocin was tested by adjusting the pH with HCl and NaOH. The concentrations were 3.0–4.0 of 50 mM glycine-HCl, 4.0–7.0 of 50 mM sodium acetate, 7.0–9.0 of 50 mM Tris-HCl, and 9.0–12.0 of 50 mM glycine-NaOH. The lactiscin bacteriocin was evaluated for residual antibacterial activity at each specified pH using *V. parahaemolyticus* (ATCC 17802) and *S. aureus* (ATCC 6538). At room temperature for 2 hours, the cell suspension was incubated with 50 µL of lactiscin bacteriocin at twice the MIC. The incubation period and temperature were changed to 16 hours at 37°C. We assessed the remaining activity against the indicators and used untreated samples as a negative control (Sadeghi et al. 2018).

The thermal stability of the purified lactiscin bacteriocin was investigated by immersing the samples in a thermostatic water bath. For 30 minutes, temperatures 50, 60, 70, 80, 90, and 100°C were used. The residual activity was assessed against the indicators. A sample at 4°C was utilized as a positive control, while a buffer of 50 mM Tris-HCl (pH 8.0) was used as a negative control.

Bacterial membrane permeabilization via **β-galactosidase test**

The β-galactosidase assay was performed to assess how the lactiscin bacteriocin disrupted the membrane. An *E. coli* cell was cultured in a lactose broth at 37°C for 18 hours to induce the production of the enzyme. The cell was centrifuged three times, and the pellet was washed three times in a 1·PBS buffer. The suspension was then adjusted to a concentration of $1 \cdot 10^6$ CFU/100 µL in 1·PBS. A reaction mixture containing 100 µL of this suspension was combined with 100 µL of purified lactiscin bacteriocin at 0.05, 0.15, 0.25, and 0.35 mg/mL concentrations. 30 µL of o-nitrophenyl- β-D-galactoside (ONPG) was

added to each reaction mixture. The 96-well plates containing the solution were incubated at 37°C, over varying intervals. The activity was measured by spectrophotometric absorbance at 405 nm (Luca et al. 2013; Almaaytah et al. 2017).

Mobility shift DNA-binding assay

The genomic DNA from *E. coli* was extracted according to the manufacturer's protocol using the QIAprep Spin Miniprep Kit (QIAGEN, USA). Purity was OD 260/280 ≥ 1.8 using the Thermo Scientific Nanodrop™ 1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA). A varied concentration of lactiscin bacteriocin in PBS was combined with a different volume of extracted DNA. 2 µL of a loading dye was added to the mixture and incubated for 10 minutes at room temperature. The migration of the DNA was assessed by 0.8% agarose gel electrophoresis and monitored by UV illumination using a Gel Doc_IT 310 imaging system. A buffer of 50 mM Tris-HCl (pH 8.0) was used as a negative control (Almaaytah et al. 2017).

Evaluation of hemolytic toxicity

With modifications, the erythrocyte toxicity test was carried out as previously described by Mazzarino et al. (2015). The sheep erythrocytes were washed in PBS pH 5.7 and centrifuged for 10 minutes at 5000·*g* at 4°C to remove the supernatant (Washing repeated trice). After the last wash, the pellet was resuspended in PBS to a final concentration of 4% blood. 50 µL of blood was mixed with 100 µL of various peptide solution concentrations (0.1, 0.25, 0.45, 0.9 mg/ml) and incubated in Eppendorf tubes at 37°C for 1 hour. The erythrocytes were then separated at 10,000·*g* for 5 minutes in a tabletop centrifuge. 75 µL of supernatant was carefully collected from each tube and placed in a 96 well plate. 10 mM PBS was utilized as a negative control and 10 mM TritonX-100 as a positive control. Spectrophotometric absorbance at 541 nm was used to determine activity.

Statistical analysis

All experiments were done in triplicate, and results were expressed as the mean ± standard deviation. Data analysis was performed using Duncan test with SPSS 19.0 (IBM Corp., Armonk, NY). In all analyses, statistical significance was set at a confidence level of 95%.

Results

The 3D structure analysis

To identify the structure of peptide, we initially performed amino acid-sequence alignments of the bacteriocin locus KWT49638 gene with those of representative known structures of the bacteriocin classIIa family. The resulting of conservative sequence was conserved YGN motif as illustrated in Fig. 1A. The 3D-crystal structure of the bacteriocin locus KWT49638 gene contained uncharged-polar and positive charged residues, e.g. His^{7}, Arg^{10}, Arg^{23}, His^{28} and Lys^{36}. These residues we hypothesize that they might be responsible for specific interactions with the outer membrane leading to permeability with positive charged region of phospholipids and surface in the bacterial cell membrane (Fig. 1B).

Comparison of induction profiles, expression of recombinant lactiscin and purification

The lactiscin gene was successfully amplified, yielding around 250bp amplification products. SDS-PAGE analysis of the lactiscin bacteriocin showed its presence in the soluble fraction of the cell lysate, and the peptide band matched the predicted molecular mass of ~ 13.14 kDa. The band size in the lane corresponds to the gene of KWT49638.1 bacteriocin. After induction with IPTG, varied temperatures were used to obtain the soluble lactiscin peptide. The incubation temperatures are presented in Fig. 2A at 30°C (lanes 4–5), 37°C (lanes 6–7), and 16°C (lanes 8–9) and compared to gene-free host cells (lanes 2–3). Western blot analysis corroborated the results, as shown in Fig. 2B. After 3 and 16 hours of induction, the protein band was seen in the supernatant. However, it was more intensely observed at 16°C overnight than in gene-free host cells at 30°C and 37°C. This result indicates that the optimal conditions for expressing lactiscin bacteriocin in soluble fraction were 0.1 mM IPTG at 16°C overnight and low-speed shaking. The lactiscin bacteriocin has a 6·His tag at the C-terminus, facilitating easy purification. Thus, following sonication-mediated cell lysis, the peptide was purified by IMAC using a high imidazole concentration. A sharp peak with more than 95% purity was achieved and SDS-PAGE was used to separate the final pure peptide (Fig. 2C). The final determined concentration was 0.8 mg/mL using Bradford's reagent (Bio-Rad).

Antibacterial activity of the purified lactiscin peptide

The purified lactiscin bacteriocin was diluted to evaluate and quantify the antibacterial activity, and MIC experiments were performed. The recombinant peptide had substantial antibacterial action against Gram-positive and Gram-negative bacteria (Fig. 3). The peptide was active against *V. parahaemolyticus* (ATCC 17802) and *S. aureus* (ATCC 6538), with 0.45 mg/mL MIC values. The lactiscin bacteriocin activity against these two types of foodborne pathogens was concentration dependent.

Effects of pH and temperature on lactiscin antibacterial activity

The influence of pH on lactiscin bacteriocin activity was studied. When tested at pH levels ranging from 3.0 to 12.0, the recombinant peptide was confirmed to be stable (Fig. 4A). Temperature experiments demonstrated that the lactiscin bacteriocin was heat stable. It retained a broad spectrum of inhibitory activity at high temperatures from 70–100°C. However, increasing the temperature to 100°C for more than 30 minutes had a negligible effect on the lactiscin bacteriocin's antibacterial activity. Compared to an untreated sample, the action against *V. parahaemolyticus* (ATCC 17802) was reduced by 50% and against *S. aureus* (ATCC 6538) by 58.28% (Fig. 4B). This excellent thermal stability of lactiscin bacteriocin makes it ideal for application in the food industry.

Effects of lactiscin on intracellular β-galactosidase and DNA retardation

A membrane integrity assay was done to determine whether the lactiscin bacteriocin peptide inhibited bacterial growth by membrane permeabilization. Using *E. coli* as indicator strain, the enzymatic activity of an intracellular β-galactosidase in converting ONPG to galactose and onitrophenol was measured using a microplate reader at

405 nm. As the concentration and duration of exposure to lactiscin bacteriocin increase after 3 hours of incubation, the cytoplasmic membrane permeability reached its maximum (Fig. 5A). The result revealed that the recombinant peptide could significantly cause membrane damage, leading to bacterial cell lysis. After 550 min of treatment. The intensity of different of peptide were substantially different from the PBS control.

DNA retardation test was conducted to determine if the lactiscin bacteriocin works by affecting intracellular targets. The study demonstrated that lactiscin bacteriocin has the potential to bind DNA. The lactiscin bacteriocin inhibited the DNA mobility and the gel compared to the positive and negative controls (Fig. 5B). This implies that the peptide may be capable of triggering microbial death *via* membrane disruption and binding to intracellular targets.

No hemolytic toxicity toward erythrocytes

A hemolytic assay was performed on erythrocytic cells to evaluate the security of lactiscin bacteriocin. The hemolytic toxicity data obtained for lactiscin bacteriocin at various doses after 1 hour of incubation compared to the negative control is shown in (Fig. 6). Incubating lactiscin bacteriocin with erythrocytes at the measured quantities did not result in a substantial decrease in viable cells. This data demonstrates that the lactiscin bacteriocin is not toxic to tested red blood cells.

Discussion

This study successfully produced a new peptidic bacteriocin—lactiscin in *E. coli*, as it is frequently utilized as a fast-growing host cell. Previously, divercin V41 (class IIa) and entrocin P bactofencin (class IId) are two examples of peptidic bacteriocins successfully developed in an *E. coli* system (Cui et al. 2021). Here, the lactiscin peptide production in the soluble lysate of the cell fraction was achieved following induction with 0.1 mM IPTG at 16°C overnight. The cold shock approach aids in slowing down the protein translation process. Cell growth and protein synthesis are reduced at lower temperatures to improve protein solubility and increase soluble protein expression (Ingham and Moore 2007). Our results clearly demonstrated a one-vector system's efficiency in bacteriocin production at controlled conditions. It is worth mentioning that bacteriocin gene synthesis is typically connected with toxicity and protease to the host cells. In this study we decided to use BL21 (DE3) pLysS *E. coli* cells designed for applications that require high-level expression of non-toxic recombinant proteins, low background expression in uninduced cells. The ion protease is absent in (DE3) pLysS cells, as is the outer membrane protease, OmpT. The lack of these proteases decreases heterologous protein degradation. As a result of this condition, the cell density received with certain medium is low, affecting the yield (Mesa-Pereira et al. 2018). Therefore, we herein used the 2·YT culture formula to boost the lactiscin peptide production and hence increase cell

densities. Previous studies have also exploited the 2·YT medium to culture and produce bacteriocins such as nukacin ISK-1 (Nagao et al. 2005) and epidermicin NI01 (Sandiford and Upton 2012). It should be also noted that incorporating the 6·His tag sequence in the optimized gene for IMAC purification has significantly improved the production yield. The food industry could thus apply this technique to produce the bacteriocin rapidly, efficiently, and with high yield and purity.

The purified lactiscin bacteriocin was shown to have broad-spectrum antibacterial properties against various pathogens at low concentrations. Although most bacteriocins published thus far have been demonstrated to be more effective or confined to Gram-positive bacteria our cloned peptidic bacteriocin was able to suppress a Gram-negative bacterial species. This outcome implies that it would be expected to halt the rapid proliferation of foodborne pathogens. One of the parameters used to assess a newly obtained bacteriocin is its tolerance to low-high pH and thermal treatment as such features would be required to determine the usefulness of their application in the food processing industry (Silva et al. 2018). As demonstrated in our study, after 2-hour incubation, the lactiscin bacteriocin displayed significant stability at pH 3 to 12 against the indicator organisms used (*V. parahaemolyticus* and *S. aureus*). However, when the incubation time increased, a decline in the peptide activity was observed. Possibly, protein aggregation or proteolytic hydrolysis could explain the observed decrease in such a activity. (Aasen et al. 2000). Interference from NaOH and HCl during pH adjustment may also reduce bacteriocin activity. Furthermore, it has been discovered that optimal bacteriocin activity is frequently associated with optimal pH of the indicator organisms. (Md Sidek et al. 2018).

The temperature would be another important factor for disturbing the stability of the bacteriocins. Herein, lactiscin bacteriocin displayed heat stability toward bioactivity when subjected to temperatures ranging from 4-100°C for 30 minutes. However, prolonged exposure at 100°C against the indicator strains leads to a decline in the lactiscin bacteriocin activity. At 4°C, it maintained intense activity on the indicator microorganisms despite the relatively low temperature. Previously, bacteriocin derived from *L. lactis* was found to be active for only 15 minutes at temperatures ranging from 4 to 100°C (Mehwish Aslam, 2012). Another bacteriocin's heat stability was reported to be at 121°C for only 15 minutes (Joshi et al. 2006). Thus, the lactiscin bacteriocin's capacity to suppress foodborne bacteria while staying active at high pH and temperature is intriguing for future food preservation uses.

It is necessary to study the potential mechanisms of bacterial cell death *via* peptide membrane interaction (Hancock and Chapple 1999) since the membrane is the primary barrier to antibiotic penetration (Li et al. 2013). The degree of intracellular β-galactosidase enzyme release from perforated *E. coli* cells was used to assess the lactiscin bacteriocin's potential to rupture bacterial membranes. The peptide was able to permeate and perforate *E. coli* plasma membranes, resulting in the release of the β-galactosidase enzyme. The rapid increase in optical density readings with time corresponds to the pace at which o-nitrophenol is produced. Our findings inferred that the lactiscin peptide is responsible for considerable membrane damage, which results in fast cell death. In line with this result, a rise in optical density was observed following the hydrolysis of ONPG by sonorensin on *S. aureus* membrane (Chopra et

al. 2015), AGAAN, and pepcon—a novel consensus antimicrobial peptide on *E. coli* cell membrane (Almaaytah et al. 2017; Ajingi et al. 2021).

Peptides do not just affect the cytoplasmic membrane of bacteria; they also affect other cell structures like intracellular biopolymers, nucleic acids, and proteins (Li et al. 2013). Thus, multiple peptide inhibitory action culminates in microbial cell death. A mobility shift DNA-binding test was done to determine whether the lactiscin peptide would target additional biological components. The data indicated that the peptide could bind to the target bacterial genomic DNA. As similar to previous studies (Gottschalk et al. 2013; Miao et al. 2016), the tested genomic DNAs were wholly trapped and unable to travel along with the gel. Hence, lactiscin bacteriocin may perhaps exert dual modes of action *via* membrane damage and DNA inhibition. Additionally, hematological and biochemical measures are frequently used to determine the threat of toxicity in animals and are regarded as reliable indicators of physiological circumstances (Mazzarino et al. 2015). Under our experimental conditions, the treatment with the lactiscin bacteriocin clearly revealed its harmless effect on erythrocytes. Taken together, our results suggests that the lactiscin bacteriocin may possess fast-killing kinetics, making it suitable for application against pathogens.

In conclusion, lactiscin—a novel peptidic bacteriocin has been shown to possess a broad spectrum of action, pH stability, and heat resistance. This characteristic permits the Lactiscin to display its capability in controlling microbes. However, at the high pHs, the activity was dropped. We hypothesize by altering the charge state of the recombinant peptide, the extreme basic pH influences how successful it is against bacteria. The heat tolerant test revealed that the Lactiscin was heat stable. It apparently displayed dual inhibitory activity against bacterial cells. The approach for cloning, expression, and characterization used in this study would be paving the way for future research. Furthermore, the properties of lactiscin bacteriocin can be viewed as a benefit when used to control foodborne pathogens. It is worthwhile to study future industrial uses of this essential bioactive peptide.

Declarations

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Author contribution:

N. R. Performed the investigation, Writing-original draft, Data curation and Formal analysis. **Y. S. A.** Assisted in proofreading the manuscript, Formal analysis, and Methodology. **S. N.** Performed the investigation, and Formal analysis. **J. N. U.** Helps in Data curation and Formal analysis. **S. R.**Assist in Methodology and Validation. **T. R.** Helped in Supervision and Formal analysis. **P. P.** Assists in Supervision, conceptualization, and Formal analysis. **C. A.** Supervision, Formal analysis, and Validation. **N. J.** Performed the main Supervision, Funding acquisition, Conceptualization, Resources, and Validation. The findings were discussed by all authors, and they all contributed to the final manuscript.

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Data availability: The paper contains all the data related to this studyincluding the accession number (OP925095) of our codon-optimized gene, additional information can be provided on request.

Ethics approval: This study did not include any human or animal participants and was carried out in accordance with safety standards.

Declaration of competing interest

The authors declared that there is not any competing interest.

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Figures

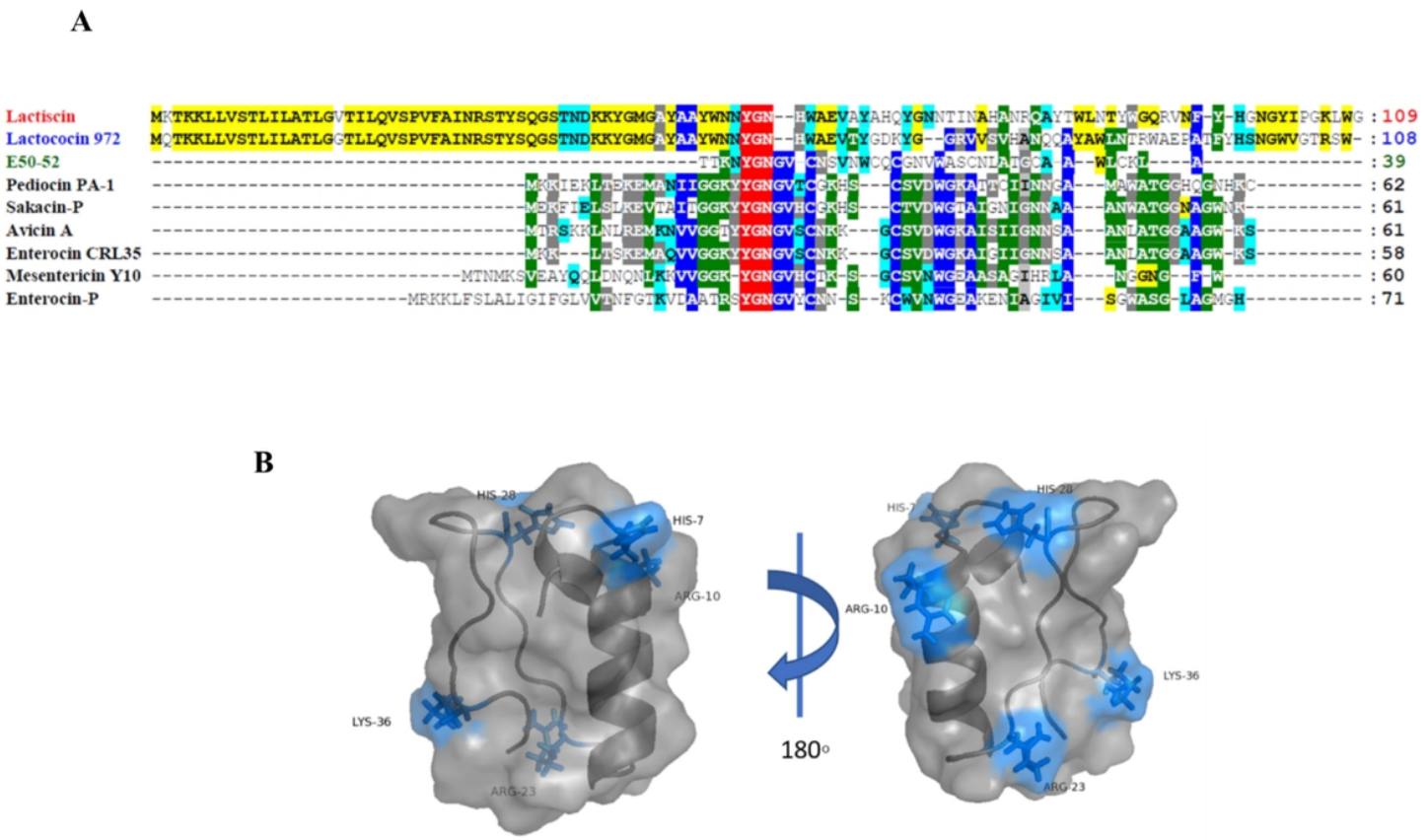

Figure 1

Sequence alignment of peptidic bacteriocin designated as lactiscin with related peptides members in the bacteriocin classIIa **(A)**. Homology-based 3D modeled structure of Lactiscin and positive residues are shown in blue **(B)**.

Fig. 2

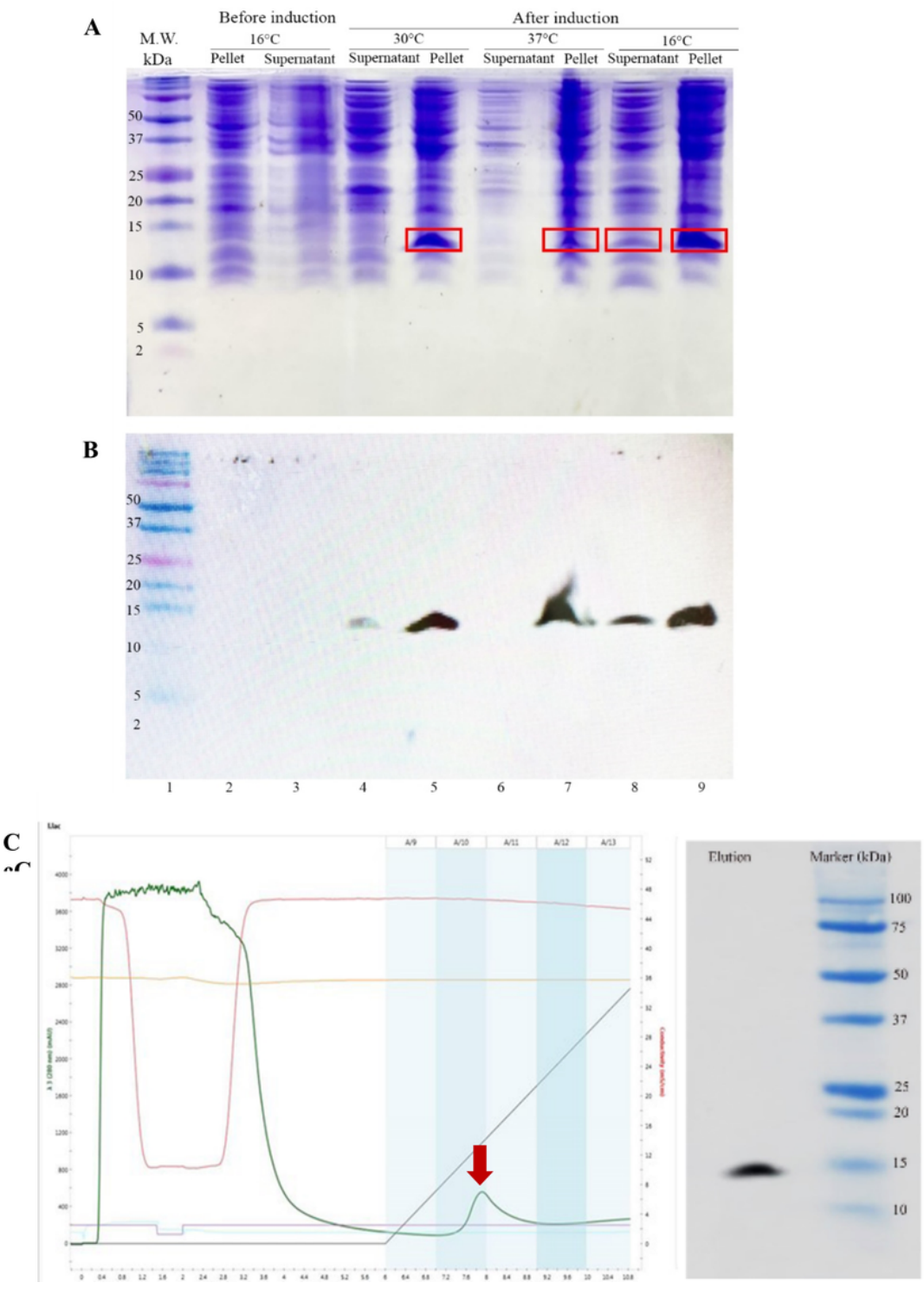

Figure 2

Expression of recombinant Lactiscin bacteriocin. SDS–PAGE gel stained with Coomassie Brilliant Blue R-250. Pellet protein fraction (lane 2) and soluble cell lysate (lane 3) without gene after induction with IPTG. Pellet protein fraction (lane 4) and soluble cell lysate (lane 5) with Lactiscin bacteriocin gene after induction with IPTG at 37°C. Pellet protein fraction and soluble cell lysate (lane 6, 7) with Lac bacteriocin gene after induction with IPTG at 30°C. Pellet protein fraction and soluble cell lysate (lane 8, 9) with

Lactiscin bacteriocin gene after induction with IPTG at 16°C (**A**). The protein has a molecular weight of approximately 13 kDa (**B**). A Western blot of His-tagged protein probed with Mouse anti Histidine tag antibody, clone AD1.1.10 (MCA1396) confirmed the results of Lactiscin bacteriocin purification. The Lactiscin Bacteriocin purification through a Ni^{2+}-NTA column with Fast protein liquid chromatography (FPLC) Western blot on nitrocellulose membrane analysis of protein fraction corresponding to the indicated peak by using 25% of 20mM Tris HCl pH 8.0, 5mM Sodium chloride, and 500 mM imidazole for elution buffer (**C**).

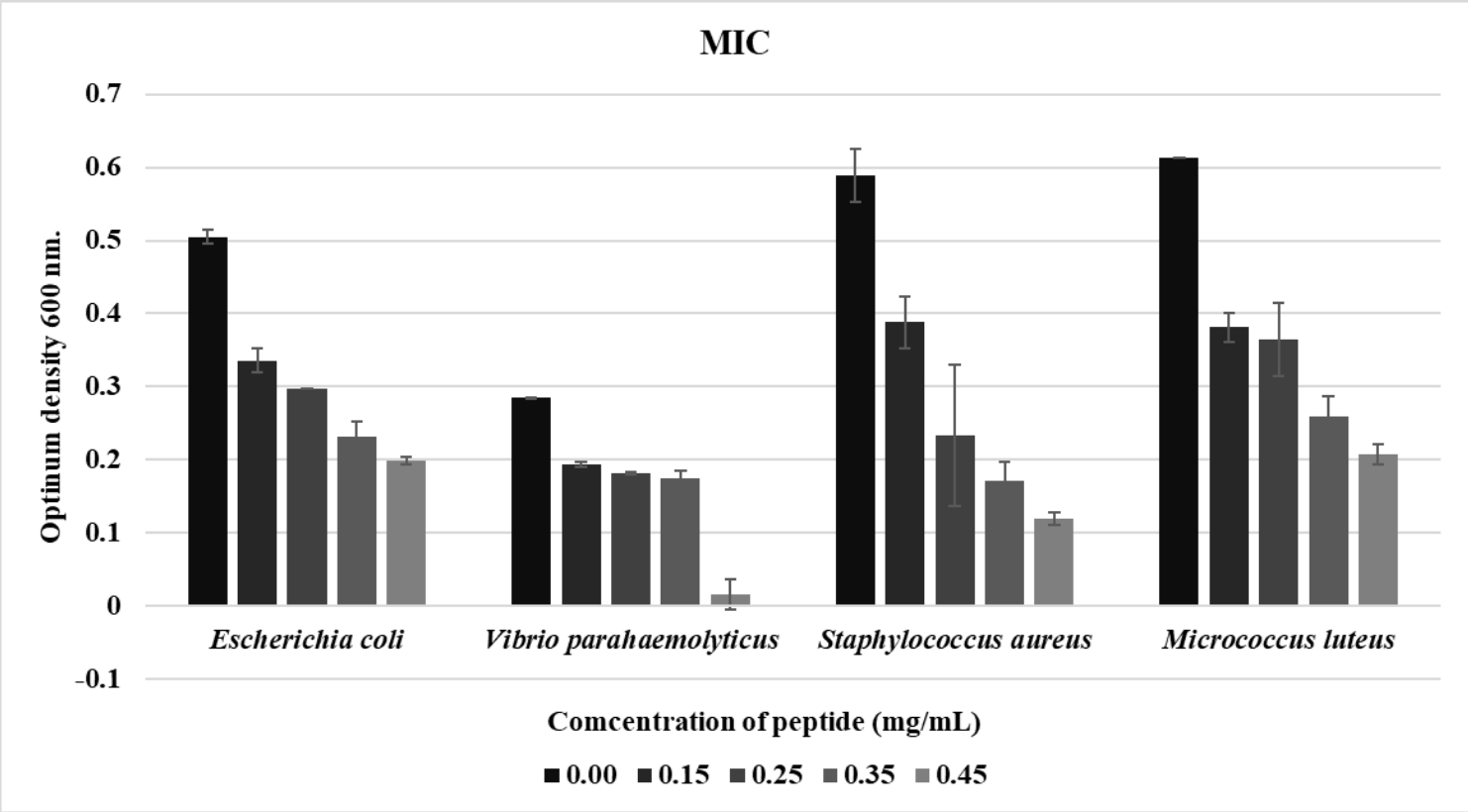

Figure 3

OD600 value of the bacteriocin suspensions treated with different concentration of Lactiscinbacteriocin. The data indicate the OD600nm of *Escherichia coli*, *V. parahaemolyticus*, *S. aureus* and *Micrococcus luteus*. Mean data for each sample (n = 3). Error bars illustrated the standard deviation

Fig. 4

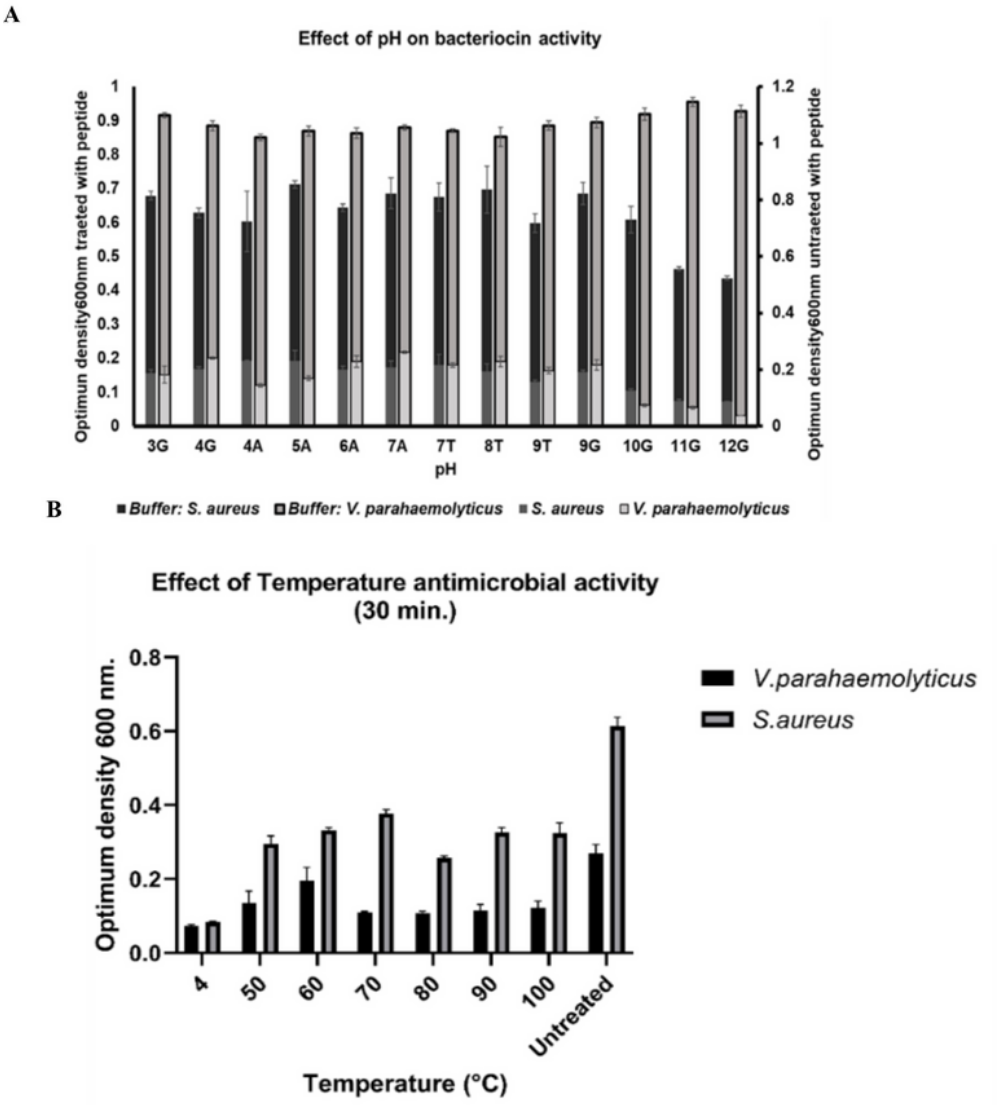

Fig. 4 The pH stability of the purified Lacticin bacteriocin activity against *S. aureus* and *V. parahaemolyticus*. Each pH concentration was incubated with the followings. ■ 50mM buffer with *S. aureus*, ■ 0.45 mg/ml Lacticin with *S. aureus*, ▨ 50mM buffer with *V. parahaemolyticus*, ▨ 0.45 mg/ml Lacticin with *V. parahaemolyticus*, (pH 3 of 50mM glycine-HCl(3G), pH 4 of 50mM glycine-HCl(4G), pH 4 of 50mM sodium acetate(4A), pH 5 of 50mM sodium acetate(5A), pH 6 of 50mM sodium acetate(6A), pH 7 of 50mM sodium acetate(7A), pH 7 of 50mM Tris-HCl(7T), pH 8 of 50mM Tris-HCl(8T), pH 9 of 50mM Tris-HCl(9T), pH 9 of 50mM glycine-NaOH(9G), pH 10 of 50mM glycine-NaOH(10G), pH 11 of 50mM glycine-NaOH(11G),and pH 12 of 50mM glycine-NaOH(12G), respectively. Error bars indicate standard errors of the mean from at least three independent experiments (**A**). Effect of temperature on Lacticin activity and stability. For enzyme activity reaction mixture was incubated at different temperatures (4, 50-100 °C) and for stability, the enzyme was pre-incubated at respective temperatures for 30min, and reaction was conducted as standard assay method (**B**). Mean data for each sample (n = 3) and Error bars illustrated the standard deviation.

Figure 4

See image above for figure legend

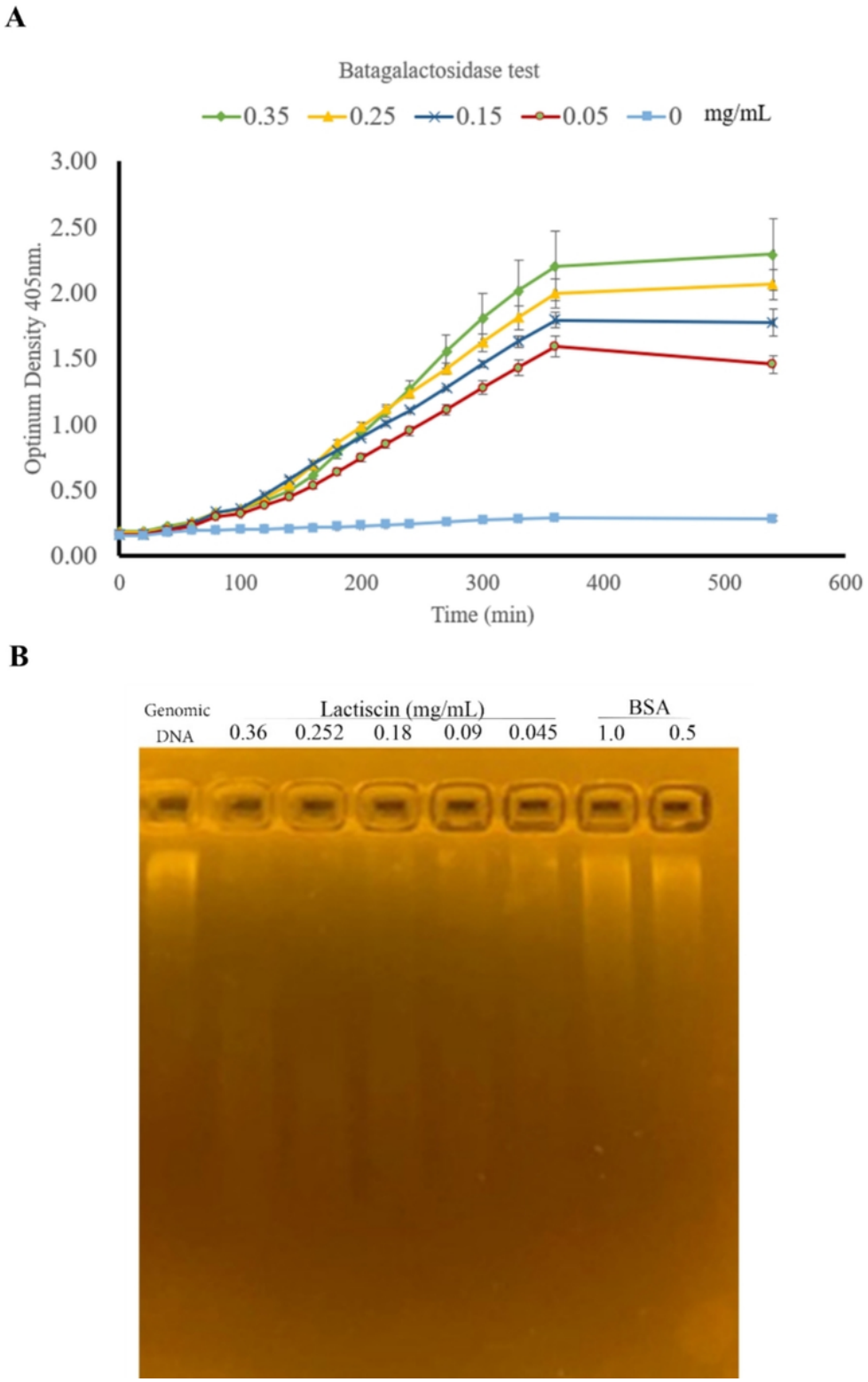

Figure 5

Time dependence of cytoplasmic membrane permeabilization of *E. coli* bacterial cells after treatment with four concentrations of Lactiscin bacteriocin (0.35, 0.25, 0.15, and 0.05mg/mL) compare with 50mM Tris-HCl pH8.0 as a negative control (**A**). Gel retardation assay. Lane1 (+) *E. coli* DNA devoid of peptide, Lane 2-6; Lactiscin bacteriocin (0.36, 0.252, 0.18, 0.09 and 0.045mg/mL) and Lane 7-8; BSA (1 and 0.5 mg/mL) (**B**).

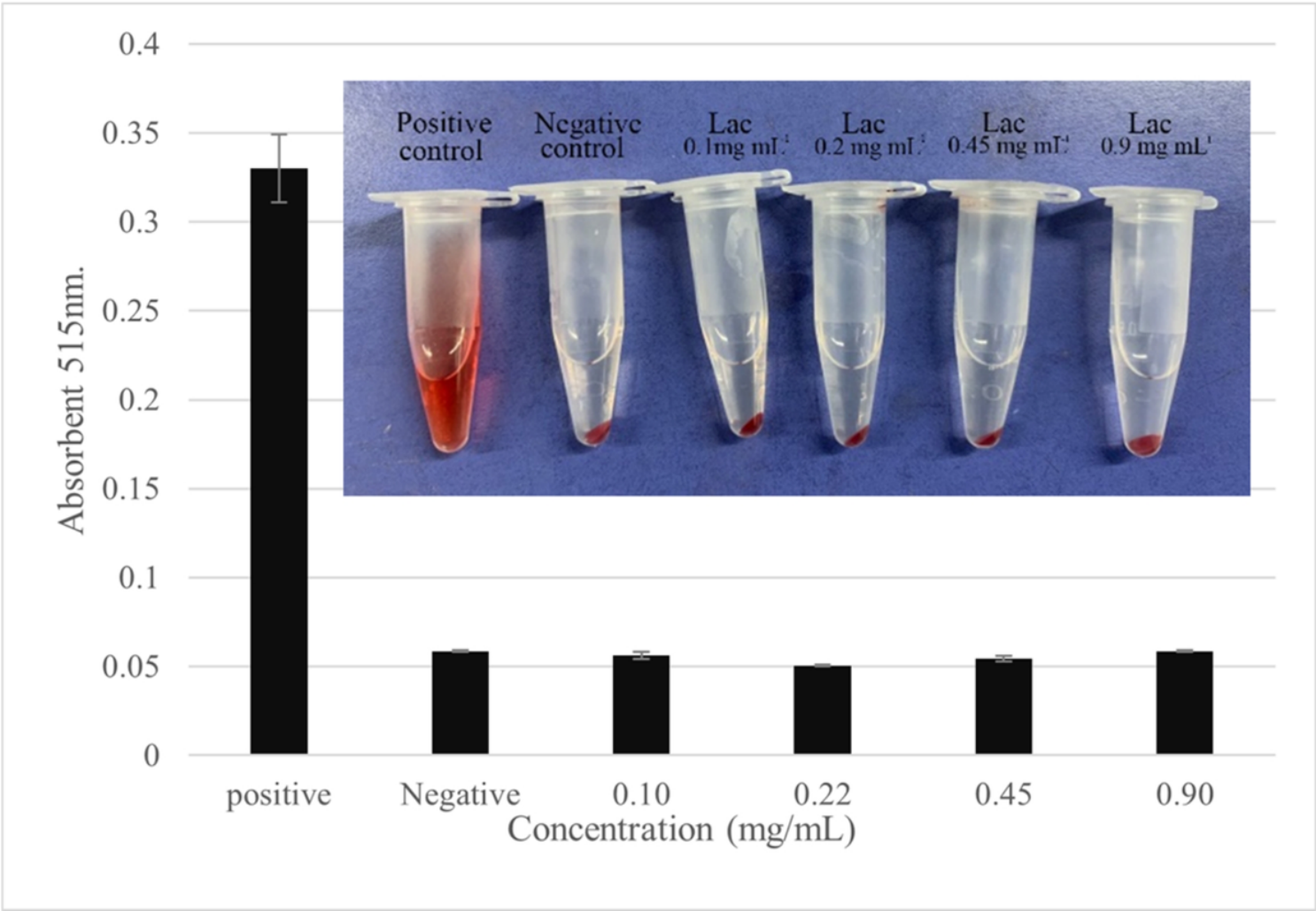

Figure 6

Hemolysis assay with Lactiscin bacteriocin. Relative rate of hemolysis in human erythrocytes following 1 h incubation at 37°C with different concentrations of Lactiscin bacteriocin. An image of samples after centrifugation at 10,000 rpm for 5 min is inscribed. Negative control (10mM PBS pH8.0), Positive control (10mM TritonX-100), and Lactiscin bacteriocin (0.1, 0.2, 0.45, and 0.9 mg mL^{-1}). Mean data for each sample (n = 3). Only in the positive control tube is there a noticeable concentration of hemoglobin in the supernatant.