Purification and Characterization of a Novel Bacteriocin Pediocin Z-1 Produced by Pediococcus Pentosaceus Z-1 Screened From Jinhua Ham

Xiaohong Yu
Yancheng Institute of Technology
Zhicai Yang
Yangzhou University
Keyue Li
Yangzhou University
Rui Liu
Yangzhou University
Huijie Pei
Yangzhou University
Guoyuan Xiong
Anhui Agricultural University
Hai Yu
Yangzhou University
Mangang Wu
Yangzhou University
Qingfeng Ge (qfge@yzu.edu.cn)
bCollege of Food Science and Engineering, Yangzhou University https://orcid.org/0000-0001-7776-3709

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Abstract

Background: Lactic acid bacteria (LAB) can produce bacteriostatic substances, among which bacteriocins attracted wide attention in food preservation for decades. Up to date, nisin (class I bacteriocins) has been considered to be the only bacteriocin produced by *Lactococcus lactis* strains for commercial use. Moreover, there are many other reports concerning the isolated bacteriocins for potential application in food industry while some exhibited a narrow bactericidal spectrum, thermal stability and low acid-base stability. Jinhua ham is a representative of traditional dry-cured meat product in China. The microbial community structure and diversity was speculated to be responsible for flavor and quality formation of Jinhua ham, protecting from the spoilage microorganisms contamination. However, no studies was performed to investigate the bacteriocin from LAB in Jinhua ham. Thus, the objective of this study was to screen out high-efficiency, safe and non-toxic bacteriocin-producing lactic acid bacteria (LAB) from Jinhua Ham and subsequently perform the purification, identification and characterization of its bacteriocin.

Results: The bacteriocin-producing LAB was screened from Jinhua ham and then designated as *Pediococcus pentosaceus* Z-1 by colony morphology and 16S rDNA sequencing. The bacteriocin was then crudely extracted from the bacterial cell-free supernatant by pH adsorption, and further purified by cellulose DEAE-52 ion exchange and Sephadex G-50 chromatography columns. The tricine-SDS-PAGE electrophoresis showed a highly purified protein band with 8227.35 Da with 60 amino acids identified by MALDI-TOF-MS analysis. The bacteriocin was named as pediocin Z-1 and its antibacterial activity exhibited an acid-base stability between pH 2-10 and a thermal stability at a range of 50-110°C. The pediocin Z-1 was sensitive to proteases and showed an inhibitory effect against Gram-positive bacteria and Gram-negative bacteria, including *Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, Salmonella potsdam* and *Escherichia coli*.

Conclusions: Our data suggest that pediocin Z-1 has a comparable effect of antibacterial activity with nisin and it is promising that pediocin Z-1 has great potential for application prospect in the food industry.

Background

Bacteriocins are bacteriostatic proteins or polypeptides produced by ribosomal synthesis during bacterial growth and metabolism and have attracted much attention for food preservation for decades [1]. Most lactic acid bacteria can produce a variety of bacteriostatic substances in the process of growth and metabolism, which antagonize other microorganisms. The bacteriostatic substances produced by lactic acid bacteria are mainly organic acids, hydrogen peroxide and bacteriocins [2]. Bacteriocin is a bacteriostatic protein or polypeptide produced by ribosome synthesis during the bacterial growth and metabolism and has been attracted for attention in food preservation for decades [3]. The bacteriocins are initially discovered from food isolates and can be basically divided into four categories according to their molecular mass, thermo-stability, enzymatic sensitivity and the presence of post-translational

modified amino acid. Notably, class II bacteriocins are mostly found and own the characteristics including un-modified amino acids, heat-stability and generally less than 10 kDa. Class II bacteriocins has sub-groups, namely IIa (pediocin-like bacteriocins), IIb (two-component bacteriocins) IIc (thiol-activated bacteriocins).

Compared to the existing synthetic food preservatives, bacteriocins are considered to be the alternatives for the guarantee of food quality without health concerns [4]. However, up to date, nisin (class I bacteriocins) has been considered to be the only bacteriocin produced by *Lactococcus lactis* strains for commercial use. It has a molecular weight of 3354 Da with 34 amino acids and is evidenced to be nontoxic to animals and humans. As a food preservative, nisin can inhibit Gram-positive bacteria, but has no inhibitory effect on Gram-negative bacteria. Moreover, there are many other reports concerning the isolated bacteriocins for potential application in food preservation [5, 6]. However, some exhibited a narrow bactericidal spectrum. For example, Guerreiro detected the Bacteriocin B231 antibacterial spectrum produced by *Lactobacillus pentosus* B231 and found that it only inhibited *Listeria spp.* [7]. A bacteriocin produced by *Pediococcus pentosaceus* K23-2 isolated from kimchi had good stability under acidic and neutral conditions, but its activity is sharply lost under alkaline conditions, and the bacteriocin can only inhibit Gram-positive bacteria [8]. *Lactobacillus pentosus* DZ35 was isolated from dry-cured meat products showing inhibitory effect on *Staphylococcus aureus* and *Escherichia coli* at the range of pH 2 -11 [9]. The pediocin GS4 is a broad spectrum bacteriocin isolated from the *Pentosaceus* GS4, however, it has antibacterial activity in the limited range of 30℃-50℃ and pH 5-7 [10]. The bacteriocins produced by the three *enterococci* isolated from Egyptian dairy products exhibited narrow-spectrum antibacterial activity, and can only inhibit *Enterococcus Faecalis* and *Staphylococcus aureus* [11]. Thus, the research for the novel bacteriocin has become one of the topical subject in the field of food anti-corrosion.

Jinhua ham is a representative of traditional dry-cured meat product in China and possesses a variety of microorganism species. Our previous study showed that a total of 242 genera of bacteria belonging to 18 phyla was identified in Jinhua Ham factories [12]. The microbial community structure and diversity of Jinhua ham was speculated to be responsible for flavor and quality formation of Jinhua ham, protecting from the spoilage microorganisms contamination. Therefore, the aim of this paper was to screen out high-efficiency, safe and non-toxic bacteriocin-producing LAB from Jinhua Ham. The bacteriocin was then isolated, purified and characterized. The purified bacteriocin was supposed to compare with commercialized nisin for antibacterial spectrum of activity to achieve application prospects in food preservation.

Methods

Bacterial strains

The 162 LAB strains were isolated from Jinhua ham produced by Jinnian Ham Company of Jinhua City, Zhejiang Province, and stored in deMan, Rogosa, and Sharpe (MRS) medium containing 15% glycerol at

-80°C. The indicator bacteria used to determine bacteriostatic spectrum of bacteriocin were *Lactobacillus plantarum, Lactobacillus cerevisiae, Lactobacillus campylobacter, Streptomyces enteri, Saprophytic staphylococci, Lactococcus lactis, Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, Salmonella Potsdam, Escherichia coli*, provided by the key Laboratory of Zoonosis in Jiangsu Province. *S. aureus* was chosen as the indicator strain for antimicrobial assays. The standard strain was *Lactococcus lactis* (Cicc 23609) from China Industrial Culture Collection Center.

Screening of bacteriocin-producing LAB

The activated LAB at a dose of 1% (v/v) isolated from Jinhua ham were inoculated into sterile MRS medium at 30°C. The bacteria were cultured for 24 h and then precipitated by centrifugation at 8,000 g for 30 min at 4°C. The supernatant was filtered through a sterile 0.22 μm filter to obtain the cell-free fermentation solution. The bacteriostatic effect of the supernatant was tested by double-layer agar plate method and turbidity method [13] using *E. coli* and *S. aureus* as indicators. The strains with bacteriostatic effect were re-screened by successively eliminating the effect of organic acid, hydrogen peroxide and suffering the protease hydrolysis test [14, 15]. Briefly, 0.1 M sterile sodium hydroxide were used to adjust the fermentation supernatant to pH of 6.0 to exclude the inhibitory effect of acetic acid and lactic acid. Catalase dissolved in 50 mM phosphate buffer was added to the acellular cell-free supernatant to make the final concentration to 5 mg/mL. The reaction of the mixture was performed at 37°C for 2 h to eliminate the effect of hydrogen peroxide. In the protease hydrolysis test, the final concentration of trypsin, papain and protease K (Shanghai Lanji Technology Development Co., Ltd., Shanghai, China) in bacterial supernatant was 1 mg/mL and incubation was performed at 37°C for 2 h.

Identification of bacteriocin-producing lactic acid bacteria

The morphological and biochemical properties of the bacteriocin-producing strain were identified according to the Bergey's Manual of Determinative Bacteriology [16]. For assaying the 16S rDNA gene sequencing, the target strain was activated and then 1.5 mL culture medium was centrifuged at 10,000 g for 2 min at 4°C. The pellet was taken and the gene of the obtained cells were extracted according to the instructions of the bacterial genomic DNA extraction kit (Tiangen Biotech, DP302-02, Beijing, China). PCR amplification was carried out and procedures were as follows: denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 1 min, 35 cycles, and extension at 72°C for 7 min. Then, the PCR product was sent to Shanghai Sheng gong Bioengineering Technology Service Co., Ltd. (Shanghai, China) for DNA sequencing. The obtained sequencing were compared with the nucleic acid sequences of the model strains with similar homology in the GenBank library (http://www.ncbi.nlm.nih.gov/BLAST), and the phylogenetic tree was constructed by the Neighbor-Joining Method of MEGA 7.0 software (Institute of Molecular Evolutionary Genetics and Department of Biology, Pennsylvania State University, PA, USA).

Determination of bacteriocin activity

The antimicrobial activity of the bacteriocin was determined by using the agar-well diffusion method [17]. The wells were added with 100 μL of cell-free supernatant and active fractions during the purification

process which was subjected to a serial two-fold dilutions. The activity of bacteriocin was defined as the reciprocal of the highest dilution [18] with a clear zone of inhibition in the indicator lawn and expressed as arbitrary unit per milliliter (AU/mL). Protein concentration was measured by using BCA protein kit (Thermo Scientific, 23227, USA). The specific activity of bacteriocin (AU/mg) was calculated by the ratio of the bacteriocin activity (AU/mL) over the protein concentration (mg/mL).

Extraction, purification and identification of bacteriocin

Extraction of crude bacteriocin

The crude bacteriocin was extracted by pH adsorption method according to Daba et al [19] with a minor modification. The activated strain of 1% (v/v) was inoculated into MRS medium and cultured for 24 h at 30°C. The fermentation broth was then incubated at 70°C for 30 min. After the fermentation broth was cooled to room temperature, the pH of the broth was adjusted to 6.0. The bacteriocins were allowed to adsorb onto the cells by gentle stirring for 12 h and then the bacterial cells were collected by centrifugation at 8,000 g for 30 min. The bacterial cells were washed twice with 50 mM sodium phosphate buffer (PBS, pH 6.0). Desorption of bacteriocin from the cell was carried out by adjusting the pH to 2.0 with magnetic stirring in 100 mM NaCl. The supernatant was filtered with 0.22 μm filter and dialyzed with 50 mM PBS (pH 6.0). The crude bacteriocin supernatant was freeze-dried and stored at -80 °C for further purification.

Purification of bacteriocin

The bacteriocin was purified by the cellulose DEAE-52 ion exchange chromatography and Sephadex G-50 chromatography. Briefly, The 5 mL of crude bacteriocin dissolved in 50 mM PBS (pH 7.4) at concentration of 1 mg/ml was loaded onto a 100 mL DEAE-52 ion exchange chromatography column. The bacteriocin was stepwise eluted with PBS buffer (pH=7.4) containing 0, 0.2, 0.4 and 0.6 M NaCl. The effluent was collected with 5 mL per tube, setting the flow rate as 1 mL/min. The eluted peaks were collected and detected at 280 nm. The fractions with antibacterial activity was mixed and dialyzed with PBS buffer for 48 h and then freeze-dried. The bacteriocin was then solubilized in 20 mM PBS (pH 7.4) and loaded onto the Sephadex G-50 column. The active peak with bacteriostatic activity was combined and freeze-dried for further analysis.

Identification of bacteriocin

The bacteriocin was initially detected by Tricine-SDS polyacrylamide gel electrophoresis (Tricine-SDS-PAGE). The gel samples were prepared by protein solution with equal volume of 2×Tricine loading buffer (30% (v/v) glycerol, 8% (w/v) SDS, 3.1% (w/v) DTT, 0.02% (w/v) Coomassie Brilliant Blue G-250, and 250 mM Tris-HCl, pH 6.8). Samples were boiled at 95°C for 5 min. Equal protein amount (10 μg) of each sample was loaded onto a gel system containing 18% polyacrylamide separating gel and 4% polyacrylamide stacking gel. The electrophoresis was run at a constant voltage of 90 V for 120 min. The

gel was stained and destained by Silver staining kit (Sangon Biotech, C500029, Shanghai, China) and the gel was imaged by Gel DOC XR gel imaging system (ChemiDoc XRS+, Bio-Rad Laboratories, Inc., USA).

The molecular mass and sequence of the purified bacteriocin was determined by Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). The purified bacteriocin was mixed with equal volume of the matrix solution containing a-cyano-4-hydroxy cinnamic acid dissolved in 0.1% TFA and 50% acetonitrile. Then 1 μL of the solution was dried on the sample target plate for the MALDI analysis (Test Center of Yangzhou University, Jiangsu, China).

Inhibitory spectrum of purified bacteriocin

The inhibitory spectrum of the purified bacteriocin was assayed by using the method of [13]. The indicator bacteria were *Lactobacillus plantarum, Lactobacillus cerevisiae, Lactobacillus campylobacter, Streptomyces enteri, Saprophytic staphylococci, Lactococcus lactis, Listeria monocytogenes, Staphylococcus aureus, Salmonella typhimurium, Salmonella Potsdam, Escherichia coli*. Nisin (Yuanye Shanghai China) was considered as a control, using the Oxford Cup method to determine the antibacterial activity. The diameter of the inhibition zone was recorded to obtain inhibition spectrum of bacteriocin.

Physico-chemical properties of the purified bacteriocin

The effects of protease, pH, and temperature on activity of bacteriocin and nisin were detected**.** The proteases including proteinase K, pepsin and trypsin at 1 mg/mL was incubated with equal amount of bacteriocin and nisin for 2 h at 37°C. The pH of the purified bacteriocin and nisin solution was adjusted to 2-10, respectively and incubated at 37°C water bath for 30 min. The effect of temperatures at 45, 70, 90, 100, and 110°C on bacteriocin activity was evaluated by incubation at respective water bath for 30 min. The antibacterial activity of bacteriocin against *S. aureus* among different treatments was determined using agar well diffusion method and the diameters of inhibition zone were recorded [20].

Statistical analysis

Each assay was performed in triplicate. The data are presented as means ± standard deviation. Significant differences between groups were evaluated by analysis of variance (FACTORIAL ANOVA) using the software package SAS (Version 9.13) and the plot was made by Sigmaplot 10.0 (Sigmaplot Lab Corporation; USA). $P<0.05$ was considered as statistically significant.

Results And Discussion

Screening of bacteriocin-producing LAB

The 159 strains of LAB in 162 strains isolated from Jinhua ham were observed to inhibit the growth of *S. aureus* and/or *E. coli* (Supplementary Material, Table S1). This was consistent with the view that most of the LAB reported by Birri et al [21] can produce antibacterial substances to inhibit the growth of other

microorganisms. Besides bacteriocins, it was known that the metabolites produced by LAB including organic acids and hydrogen peroxide also exhibited anti-bacterial activity. Thus, it is necessary to exclude the effect of organic acids and hydrogen peroxide on inhibition of bacterial growth. As shown in Table 1, after the elimination of the organic acid, there was 8 strains showed inhibitory activity on indicator bacteria, among which strain L1, L3 and L7 only inhibited *S. aureus*. Strain L2, L4, L5, L6 and L8 had inhibitory effects on both indicator strains. The inhibition zone diameters among the detected strains were significantly different (P<0.05).

In order to screen the bacteriocin-producing LAB that can both inhibit the growth of representative Gram-negative and Gram-positive microbes, strains of L2, L4, L5, L6 and L8 were selected to perform the hydrogen peroxide exclusion test. Catalase was supposed to catalyze the hydrogen peroxide to eliminate its effect. After the elimination of hydrogen peroxide, the antibacterial effect of strain L8 disappeared, suggesting the bacteriostatic substance produced by strain L8 was presumed to be the hydrogen peroxide. The strain L2 still had antibacterial activity against *S. aureus*, and the diameter of the inhibition zone was 10.02±0.53 mm, but showed no antibacterial ability on *E. coli*. Strains L4, L5 and L6 had inhibitory effects on both two indicator bacteria. The bacteriostatic diameter of cell-free supernatant in strain L5 against *E. coli* was 11.36±0.54 mm, which was significantly larger than the inhibition zone diameter of strains of L4 and L6 (P<0.05). The results indicated that in addition to organic acids and hydrogen peroxide, the bacteriostatic component in fermentation supernatant of strain L4, L5 and L6 was possibly to be bacteriocin. Generally, the bacteriocin was small molecular protein and polypeptide, thus the protease detection test was done to evidence the supernatant of strains L4, L5 and L6 with anti-bacterial activity contained proteinaceous substances (Supplementary Material, Table S2). The antibacterial activity of L4 and L6 still has a weak antibacterial activity after trypsin and proteinase K digestion when *E. coli* was used as indicator strain. Notably, the antibacterial activity of the supernatant of strain L5 showed no inhibitory effect on both indicator strains after the enzymes digestion. Therefore, strain L5 is selected for further bacteriocin purification and characterization.

Identification of *Pediococcus pentosaceus*

The target strain L5 was primarily identified by traditional method of colony morphology and Gram staining. The strain L5 showed tetrad and bigeminal forming cocci with a smooth, circular, convex and ivory-white in color of colony (Fig. 1A and Fig 1B). The Gram staining of strain L5 was purple, indicating L5 as a Gram-positive bacteria. In the sugar fermentation test, strain L5 can utilize amygdalin, L-arabinose, D-cellobiose, D-fructose, D-galactose, glucose, sucrose, D-maltose, D-mannose, and melibiose while it cannot be able to metabolize lactose, inulin, D-trehalose, melezitose and D-mannitol, raffinose, D-ribose, D-xylose, D-Sorbitol and L-rhamnose (Supplementary Material, Table S3). Based on the above results, it can be primarily speculated that the assignment of the strain L5 is *Pediococcus pentosaceus*. The 16S rDNA was sequenced and identified for further demonstration of the target strain. A PCR amplification product of approximately 1200 bp was obtained, and a single band were shown to be bright in the agarose gel (Fig. 1C). A comparison based on the alignment results from GenBank using the BLAST software on the NCBI website prove a 98% homology between the target strain and *Pediococcus*

pentosaceus M58834.1. Accordingly, phylogenetic tree was drawn based on the sequence alignment (Fig. 1D). This was consistent with the physiological and biochemical results, confirming that the target strain L5 was a *Pediococcus pentosaceus.*

Recently, bacteriocins produced by *Pediococcus pentosaceus* have been isolated from other resources. Carolina Gutiérrez-Cortés et al. [22] obtained some *Pediococcus pentosaceus* including *P. pentosaceus* 63, 145, 146 and 147 from minas cheese, showing bacteriocin-producing ability. Bacteriocinogenic strains classified as *P. pentosaceus* with anti-Listeria activity were identified from Brazilian artisanal cheese [20], intestine of Mimachlamys nobilis [23], ermented Appam batter [24]. According to Ramanjeet Kaur et al [25], *Pediococcus pentosaceus* LB44 could grow and produce bacteriocin under acidic and alkaline conditions. The strain L5 in current study which was designated as *P. pentosaceus* Z-1 isolated from Jinhua ham expand the bacteriocin production scope from food resources of fermented meat product.

Extraction, purification and identification of bacteriocin

The bacteriocin in cell-free supernatant produced by *P. pentosaceus* Z-1 was stepwise purified by cell adsorption, DEAE-52 exchange chromatography and Sephadex G50 gel filtration (Fig. 2). There were four protein peaks after gradient salt elution from DEAE-52 exchange chromatography. The inhibition zone of fraction c against *S. aureus* was seen to be obviously larger than fraction a while fraction b and c showed no inhibitory zone on *S. aureus* (Fig. 2A). The fraction c were pooled and processed to Sephadex G50 gel filtration. As shown in Fig. 2B, two protein peaks were observed and fraction b with a big inhibition zone were preferred to be the purified bacteriocin. The anti-bacterial activity of the active fraction during the purification process was summarized in Table 2. The bacteriocin extracted from cell-free supernatant by cell adsorption method had led to an improvement of the specific activity by 6.59 fold with a 61.93% recovery. The purified bacteriocin from the active fraction of Sephadex G-50 column showed the specific activity as high as 8712.05 AU/mg, resulting in 40.10 fold increase of specific activity compared to the cell-free supernatant. In addition, the yield of the total activity was gradually decreased during the purification process as the specific activity increased. This was consistent with the basic principle of purification procedures and similar results were also reported, such as the purification of plantaricin ZJ008 and plantaricin K25 [26, 27]. It is suggested that the bacteriocin in cell-free supernatant in present study is effectively purified.

The purity of the bacteriocin was originally identified by Tricine-SDS-PAGE. Abundant protein bands were present in cell-free supernatant with a broad protein molecular range of 1.2-27 kDa (Fig. 2C). As the processing of purification, miscellaneous proteins were excluded and a single protein band at the range of 4.6-10 kDa was emerged in the last active fraction, indicating that the bacteriocin was highly purified. The bacteriocin in the current study was named as pediocin Z-1. The molecular weight of pediocin Z-1 was further detected as 8227.35 Da by MALDI-TOF-MS (Fig. 2D). The purity of the pediocin Z-1 was 96.62% based on the peak area ratio calculation in the total ion chromatogram. As shown in Fig. 2E, the amino acid sequence of pediocin Z-1 was predicted to be MAITLKTELL DQKMTEVFDC SNDQTPLRDA

MCNHVMDDNG HDTMKTIAEA KKWENMNDAE by the MASCOT searching. The sequence of Pediocin Z-1 showed no homology with other known bacteriocins using protein BLAST against the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast) and antimicrobial peptide database (http://aps.unmc.edu/AP/main.php). Thus, pediocin Z-1 was considered as a novel bacteriocin produced by *P. pentosaceus*.

The pediocin Z-1 was preliminarily categorized into class II bacteriocin according to its molecular weight [28], but possessing no conserved motif (YGMGVxC). Similarly, plantaricin GZ1-27 reported by Du et al. [29] belonged to the class II of bacteriocins. However, there was also no the characteristic sequence of Class IIa bacteriocins. Among the 60 amino acid residues of pediocin Z-1, it was composed of 35.8% hydrophobic residues (Ala, Ile, Leu, Met, Phe, Pro, Val) and 64.2% polar residues with 20.0% (Glu, Asp) acidic and 15.7% basic (Arg, Lys, His) amino acids, indicating the pediocin Z-1 with a amphiphilic structure of class II peptide. The N-terminus of the pediocin Z-1 was consisted of the hydrophobic residues and the C-terminus contained a high percentage of hydropholic residues. The structure of the bacteriocin was regularly linked with the mode of action and basic anti-bacterial principle of the bacteriocin [30]. The most-elucidated mechanism of bacteriostatic effect was pore formation, referring to that membrane channel of cells was destroyed by binding of the bacteriocin peptides with membrane components, such as phosphatidylglycerol. Likewise, the pediocin Z-1 is putative to interrupt the integrity membrane of sensitive microorganism by using its amphiphilic structure to cause cell wall depolarization while additional investigations are needed to elucidate the underlying mechanism.

Antibacterial spectrum

The antibacterial spectrum of Pediocin Z-1 was shown in Table 3. Result showed that besides Gram-positive bacterias, the growth of Gram-negative bacteria including *S. typhimurium, S. Potsdam* and *E. coli* were suppressed by pediocin Z-1, indicating that pediocin Z-1 was supposed to exhibit a broad antibacterial activity. However, nisin can only inhibit the Gram-positive bacteria which was agreement with earlier reports [31]. Moreover, the inhibition zone of *Lactobacillus, Streptomyces enteri and Lactococcus lactis* by nisin was slightly higher than that of pediocin Z-1 ($P<0.05$). In the test of *S. staphylococci* and *L. monocytogenes*, there was no significant difference between pediocin Z-1 and nisin ($P>0.05$). Notably, pediocin Z-1 showed a stronger antibacterial activity than nisin when using *S. aureus* as an indicator strain ($P<0.05$). It is indicated that pediocin Z-1 and nisin can both achieve the inhibitory effect of the growth of Gram-positive bacteria, however, the bacteriostaticability was dependent on the species of the detected bacteria.

Physicochemical properties of Pediocin Z-1

The effect of temperature on Pediocin Z-1 activity was presented in Fig. 3A. When heat treatments of bacteriocin gradually increased from 50℃ to 110℃, the antibacterial activity of both nisin and pediocin Z-1 significantly decreased ($P<0.05$). The antibacterial activity of nisin was significantly higher than that of pediocin Z-1 at room temperature (shown in control treatment) and the range of 50-80℃ ($P<0.05$, Fig. 3A). There was no significant difference in antibacterial activity between the two groups at 90℃.

Nevertheless, pediocin Z-1 showed a higher diameter of bacteriostatic zone than nisin when the treatment temperature was 100℃. Moreover, as the temperature processed to 110℃, the inhibition zone of pediocin Z-1 group was 11.52±0.36 mm while no bacteriostatic of nisin was observed. It is suggested that pediocin Z-1 had a more heat stability than nisin, as it was a typical characteristic of class IIa bacteriocin.

As it was shown in Fig. 3B, the pediocin Z-1 presented the antibacterial activity between pH 2-10, having a wider pH tolerance range than that of nisin. The antibacterial activity of pediocin Z-1 was significantly lower than that of nisin between pH 2.0 and 4.3 (P<0.05). The bacteriostatic ability of nisin decreased between pH 4.3 and 7.0 while the antibacterial activity of pediocin Z-1 was significantly increased at that range. The antibacterial activity of pediocin Z-1 was strongest at pH 6.0 and the diameter of bacteriostatic zone was 24.74±0.33 mm. Compared to the report of Zhu et al. [26], a bacteriocin of Plantaricin ZJ008 produced by *Lactobacillus Platarum* ZJ008 isolated from fresh milk exhibited narrow pH stability at the range of 4.0-5.0. The antimicrobial activity of two bacteriocins isolated from Portuguese fermented sausage decreased obviously when pH values below 5.0 and above 7.0 [32]. Thus, the results in current study enabled pediocin Z-1 possessing antibacterial activity have a wide pH range when applied in food preservation.

Both nisin and pediocin Z-1 can be degraded by pepsin, proteinase K, trypsin and papain (Table 4). This was consistent with the view that the class IIa bacteriocin was sensitive to proteases and was not to α-amylase [33]. Similar effect was observed for acidocin LCHV, its anti-bacterial activity was lost after the treatment of proteolytic enzymes [34]. Nisin and pediocin Z-1 are not sensitive to lipase amylase and amylase, demonstrating that the essence of pediocin Z-1 was a protein, not a glycoprotein. It was indicated that the bacteriocin can be degraded by proteases in the human digestive system and possessed no adverse effects to the human health. Above all, the physicochemical properties of pediocin Z-1 was preliminarily determined, suggesting that the discovered bacteriocin in the current study, pediocin Z-1, can be applied as a potent food additive.

Conclusions

The primary outcome in this paper was to screen a bacteriocin-producing lactic acid bacteria from Jinhua ham. *Pediococcus pentosaceus* Z-1 was identified and evidenced to produce bacteriocin by eliminating the effect of hydrogen peroxide and organic acid and the protease test. Pediocin Z-1 was stepwise purified by the pH adsorption, cellulose DEAE-52 ion exchange and Sephadex G-50 chromatography columns. A purity of 96.62% of pediocin Z-1 was obtained from and determined as molucular weight of 8227.35 Da by MALDI-TOF-MS. Pediocin Z-1 showed an inhibitory effect on Gram-positive and Gram-negative bacteria and was stable at acidic and alkaline pHs as well as at high temperature. Our results suggest that pediocin-Z-1 had a great potential in applying as a novel food preservative.

Abbreviations

LAB: lactic acid bacteria; 16S rDNA: 16S ribosomal deoxyribonucleic acid; MALDI-TOF-MS: Matrix-assisted laser desorption ionization-time of flight mass spectrometry； kDa: kilodalton; *S. aureus*: *Staphylococcus aureus*； *E. coli*: *Escherichia coli*；MRS: de Man, Rogosa and Sharpe; DNA: deoxyribonucleic acid； PCR: Polymerase chain reaction; AU/mL: arbitrary unit per milliliter； AU/mg: arbitrary unit per milligram; BCA: bicinchoninic acid； PBS: phosphate buffer saline; Tricine-SDS-PAGE: Tricine-SDS polyacrylamide gel electrophoresis； SDS: Sodium dodecyl sulphate； DTT: dithiothreitol； TFA: trifluoroacetic acid; BLAST: Basic Local Alignment Search Tool; NCBI: National Center for Biotechnology Information； Tris-HCl: Tris(hydroxymethyl)aminomethane-hydrochloride.

Declarations

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Availability of data and material

All data generated or analysed during this study are included in this published article.

Author Contributions

Conceptualization, QG and MW; Data curation, ZY, KL and RL; Investigation, XY and HP; Methodology, XY; Software, KL, RL and GX; Supervision, QG, HY and M W; Visualization, HP; Writing – original draft, XY; Writing – review & editing, ZY, RL and QG..

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests of this paper.

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Tables

Table 1 The inhibition zone diameter (mm) was recoded as the anti-bacterial activity of bacteriocin-producing lactic acid bacteria by adjusting the pH to 6.0 and the catalase treatment of cell-free supernatant to exclusion anti-bacterial effect of organic acid and hydrogen peroxide, respectively.

Strain	Organic acid		H_2O_2	
	S.aureus	*E. coli*	*S.aureus*	*E. coli*
L_1	12.52 ± 0.61^{de}	-	-	-
L_2	10.82 ± 0.53^{g}	10.18 ± 0.24^{d}	10.02 ± 0.53^{d}	-
L_3	11.60 ± 0.52^{f}	-	-	-
L_4	12.91 ± 0.33^{c}	10.59 ± 0.28^{c}	12.91 ± 0.33^{b}	10.59 ± 0.28^{b}
L_5	12.73 ± 0.19^{d}	11.36 ± 0.54^{b}	12.73 ± 0.19^{b}	11.36 ± 0.54^{a}
L_6	13.54 ± 0.82^{b}	12.01 ± 0.51^{a}	10.54 ± 0.82^{c}	9.01 ± 0.51^{c}
L_7	13.55 ± 0.47^{b}	-	-	-
L_8	12.41 ± 0.30^{e}	12.01 ± 0.70^{a}	-	-
Lactococcus lactis	14.97 ± 0.35^{a}	-	14.97 ± 0.35^{a}	-

Note: Elimination of organic acid: "-" indicates no bacteriostatic effect, and "a-g" indicates significant difference between different strains of the same indicator ($P<0.05$); Excluding H_2O_2 test results: "-" indicates no bacteriostatic effect, and "a-d" indicates significant difference between different strains of the same indicator ($P<0.05$).

Table 2 Antibacterial activity and yield of pediocin Z-1 during purification steps.

Purification steps	Volume （mL）	Antibacterial activity（AU/mL）	Total vitality （AU）	Specific activity （AU/mg）	Yield （%）	Purification factor
CFS	1000	76.02	76024.41	217.21	100	1
Crude extract	23	2047.18	47085.28	1431.59	61.93	6.59
DEAE-52	5	8137.57	40687.87	4398.68	53.51	20.25
Sephadex G-50	2	16640.03	33280.06	8712.05	43.77	40.10

Note: CFS was indicated as cell-free fermentation supernatant.

Table 3 Antimicrobial activity spectrum of purified bacteriocin of pediocin Z-1 and nisin.

Strains	Nisin	Pediocin Z-1	P-value
Lactobacillus plantarum	26.16±0.27^{a}	24.26±0.15^{b}	*
Lactobacillus cerevisiae	27.02±0.31^{a}	25.03±0.38^{b}	*
Lactobacillus campylobacter	26.84±0.33^{a}	24.82±0.30^{b}	*
Streptomyces enteri	24.35±0.50^{a}	23.01±0.24^{a}	*
Saprophytic staphylococci	24.82±0.36^{a}	24.19±0.37^{a}	NS
Lactococcus lactis	26.41±0.23^{a}	23.82±0.24^{b}	*
Listeria monocytogenes	22.49±0.48^{a}	21.57±0.35^{a}	NS
Staphylococcus aureus	22.86±0.27^{b}	24.86±0.27^{a}	*
Salmonella typhimurium	-	19.22±0.31^{a}	
Salmonella Potsdam	-	18.23±0.28^{a}	
Escherichia coli	-	18.73±0.41^{a}	

Note: “-” indicated no bacteriostatic activity referring to the diameter zone was not significantly different with the blank well; “*” indicated there were significant differences in inhibition zone between pediocin Z-1 and nisin groups ($P<0.05$). “NS” showed that there were no significant differences between two bacteriocin groups ($P>0.05$).

Table 4 The inhibition zone diameter of pediocin Z-1 and nisin by enzymatic digestion.

bacteriocin	Pepsin	Proteinase K	Trypsin	Papain	Lipase	Amylase
Nisin	-	-	-	-	26.36±0.27*	25.82±0.24*
Pediocin Z-1	-	-	-	-	23.72±0.81	24.07±0.35
P-value						

Note: "-" means no bacteriostatic effect, “*” referred that there were significant differences in inhibition diameter between pediocin Z-1 and nisin groups after digestion.

Figures

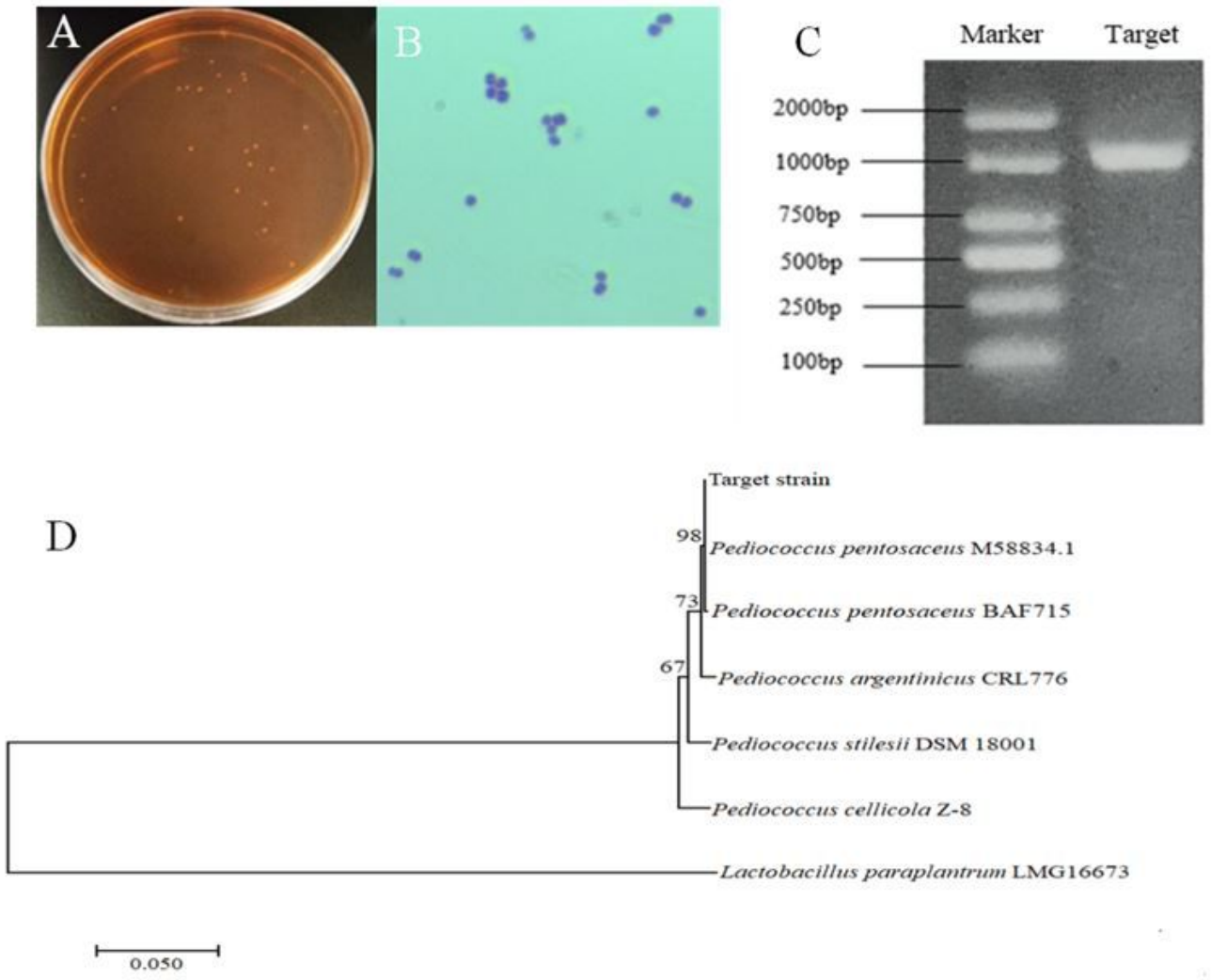

Figure 1

Morphological identification and sequence homology analysis of 16s rDNA gene of bacteriocin-producing strain L5 which was screened from Jinhua ham. Morphological characteristics of strain L5 (A, B), the PCR amplification product of the target strain (C) and the phylogenetic tree of the target strain based on 16S rDNA sequence analysis (D).

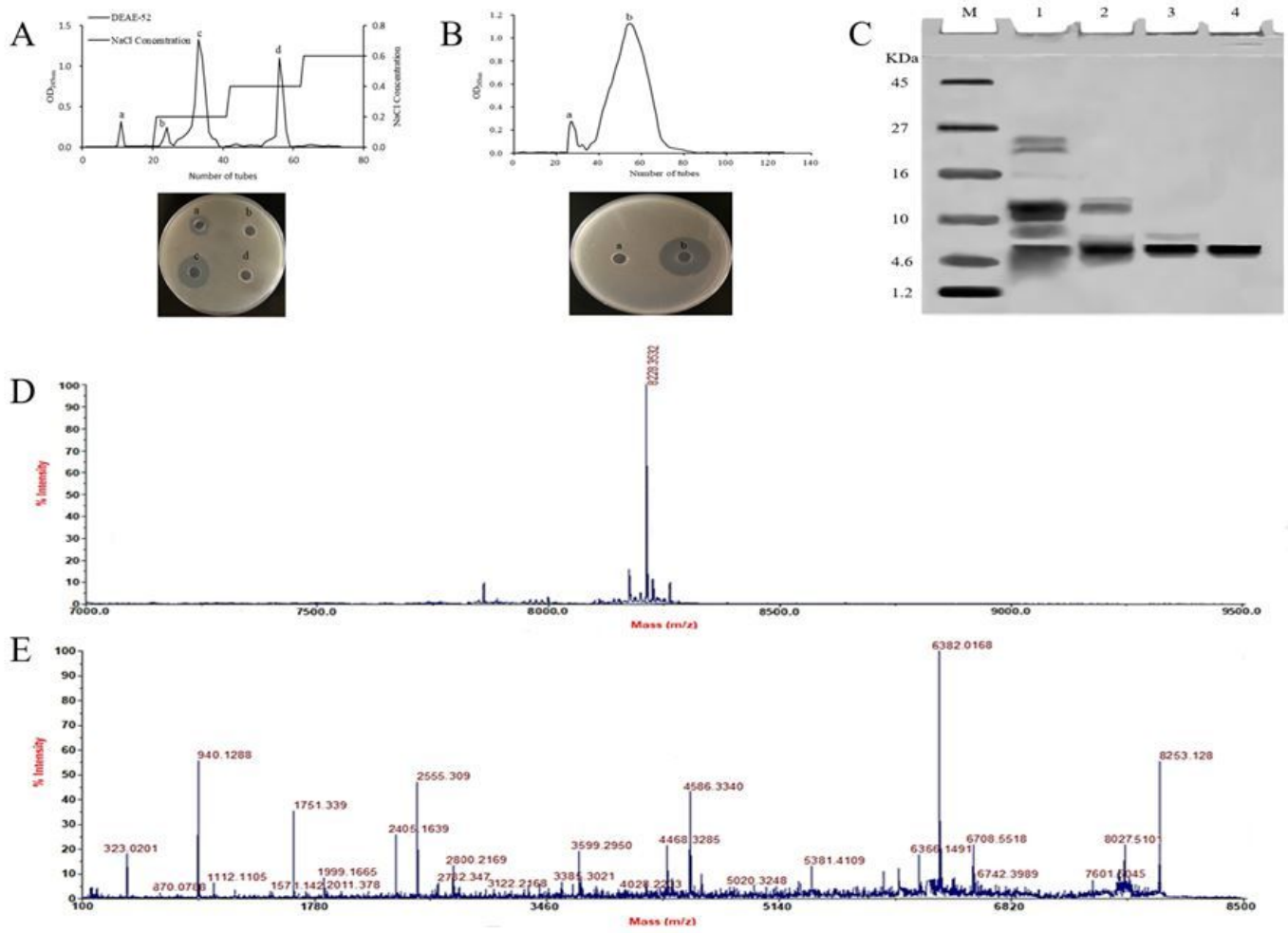

Figure 2

Purification and identification of bacteriocin. Purification of bacteriocin was performed using cellulose DEAE-52 exchange chromatography (A) and Sephadex G50 gel chromatography (B) and active fraction of each column was identified, respectively. Tricine-SDS-PAGE gel showed the initial purity of bacteriocin during the purification process (C, 1 referred to cell-free fermentation supernatant, 2 for crude protein extracted by cell adsorption, 3 for active faction c from DEAE-52 column, and 4 for active faction b from Sephadex G50 column). Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was used to identify the purified bacteriocin (D) and the fragment ions map of m/z indicated the molecular weight of the purified bacteriocin was 8.23 kDa (E).

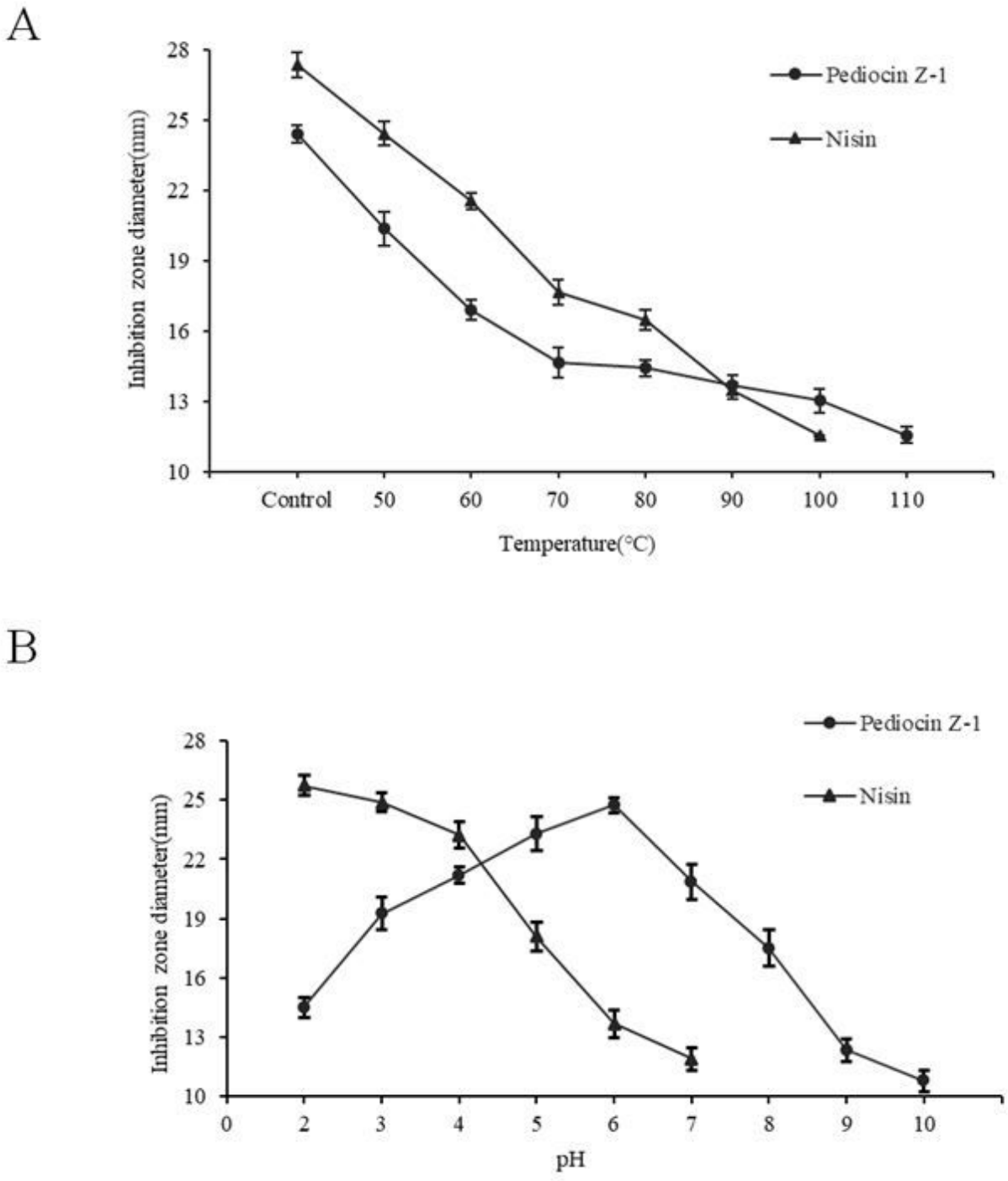

Figure 3

Effect of temperature (A), pH (B) on the anti-bacterial activity (inhibition zone) of pediocin Z-1 and nisin.

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