Volume 11, Issue 3 (2023) pp. 134-150 **DOI**: 10.22034/ijabbr.2023.2000853.1434

Original Article

Antibacterial and Antibiofilm activity of P. Pentosaceus Bacteriocin (Pediocin) Isolated from Cheese and its Optimization for the Bacteriocin Production

Ciamak Ghazaei*

Department of Microbiology, University of Mohaghegh Ardabili, Ardabil, Iran

*Corresponding Author E-mail: ciamakghazaei@yahoo.com

Received: 2023-04-25, Revised: 2023-07-11, Accepted: 2023-08-08

Abstract

The objective of the present study was to assess the antibacterial and antibiofilm activities of Pediocin, a bacteriocin extracted from cheese, versus a subset of bacteria linked to foodborne diseases. The agar well diffusion method was used to evaluate the antibacterial activity of Pediocin. The results showed that Pediocin had a potent bactericidal effect against all the tested bacterial strains. The crystal violet staining method was used to assess Pediocin's antibiofilm efficacy, and the results showed that Pediocin significantly inhibited the development of biofilms by the tested bacterial strains. Investigating the effects of temperature, pH, and medium make-up improved Pediocin output. It was discovered that a temperature of 30 °C, a pH of 6.5, and a medium made up of tryptone, yeast extract, and glucose were the ideal conditions for the creation of pediocin. Pediocin demonstrated significant antibacterial activity against all the tested bacterial strains, with the highest activity against *Staphylococcus aureus, Streptococcus pyogenes* and *Helicobacter pylori.* The minimum inhibitory concentration *(MIC)* values of Pediocin against the tested bacterial strains varied from 0.5 to 32 µg/mL. Among them, the lowest MIC was observed against *S. aureus, P. aeruginosa, and K. pneumoniae.* Similarly, the minimum bactericidal concentration *(MBC)* values ranged from *2* to *64 µg/mL,* with the lowest MBC observed against *S. aureus, P. aeruginosa, and K. pneumoniae.* The large-scale manufacture of Pediocin, which may be utilized as a natural food preservative to stop the growth of pathogenic bacteria and lower the frequency of foodborne diseases will be made easier by the optimized production circumstances. The potential of Pediocin for food preservation and the security of its use require more research.

Keywords: Bacteriocin, Pediocin, Foodborne diseases, Antibacterial activity, Bacteria.

Introduction

The threat that antibiotic resistance brings to the efficient management of bacterial illnesses is an increasing one in the realm of the general population's health. Multidrug-resistant bacteria (MDRB) are a growing issue since they are resistant to a variety of antibiotics, giving medical professionals fewer alternatives for management [1].

It is extremely difficult to cure infections brought on by MDRB, and doing so frequently necessitates the administration of more toxic and expensive antibiotics, which can have unfavorable side effects and hasten the emergence of antibiotic resistance. The reappearance of diseases like tuberculosis that were previously believed to be under control has also been connected to the lack of viable treatments for MDRB infections [2].

Antibiotic resistance has been the subject of different initiatives. Many nations have developed improved surveillance and monitoring of antibiotic usage and resistance trends, enabling the early diagnosis and treatment of outbreaks of resistant microorganisms. Antibiotic resistance has a wide range of negative effects. Longer hospital stays, more expensive healthcare, and greater mortality rates among infected people are all possible consequences [3].

One of the best treatments for bacterial infections in contemporary medicine has been the use of antibiotics. However, due to their extensive use, antibiotic-resistant bacteria have started to appear, posing a serious risk to the public's health. The modification of the microbiota, allergic reactions, and an increased chance of contracting specific infections are just a few of the negative health effects of antibiotic usage that have been connected to it [4].

The majority of antibiotics are only effective against a small subset of microorganisms. This means that infections brought on by bacteria that are resistant to the particular antibiotic being used may not respond well to treatment with antibiotics. Antibiotics' ability to upset the equilibrium of bacteria in the human body is another drawback [5].

Antibiotics have the potential to destroy both good and bad bacteria, disrupting the microbiome. The likelihood of opportunistic infections and other health issues can rise as a result. Furthermore, antibiotics may have adverse side effects that range from minor to serious. [6]. Alternative antimicrobial medicines are becoming more and more necessary due to the downsides and limits of antibiotics. Utilizing naturally occurring antimicrobial substances, such as plant extracts and essential oils, which have been demonstrated to have broad-spectrum activity against a range of bacteria, is one strategy. Using bacteriophages, which are viruses that may infect and kill bacteria, is an alternative strategy [7,8].

Bacteriocins are a class of tiny proteins or peptides produced by ribosomes with antibacterial properties against various bacterial species. They are created by bacteria as a form of protection against rival microorganisms in their environment. In the fields of food preservation and human health, bacteriocins are gaining popularity as a possible antibiotic substitute [9]. Bacteriocins have been proven to be beneficial on the subject of food preservation in stopping the development of foodborne pathogens like *Listeria monocytogenes* and *Staphylococcus aureus* without degrading the taste or quality of the food. Lactic acid bacteria, which are frequently utilized in the creation of fermented foods like cheese, yogurt, and sausages, can create bacteriocins. Bacteriocin-producing bacteria can be added to food products to extend shelf-life and assist lower the risk of foodborne illness. Bacteriocins can be used as a therapeutic agent for treating bacterial infections in the context of human health. The risk of upsetting the beneficial microbiome can be decreased by bacteriocins' extremely selective mode of action, which only targets bacterial species that are closely related to one another (10). Bacteriocins

have been demonstrated to be effective against various infections, including antibiotic-resistant strains like vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA).

Bacteriocins have been found to have immunomodulatory and anticancer effects in addition to their antibacterial activity. It has been demonstrated that some bacteriocins can stimulate the immune system, increasing cytokine production and activating immune cells. This may improve the body's capacity to combat infections and hold promise for the eventual treatment of cancer. Bacteriocins may have advantages, but there are still problems that need to be solved [11].

In comparison to the conventional antibiotics, bacteriocins have some benefits. First of all, they only attack bacterial species that are closely related to one another. As a result, they are less likely to disrupt the helpful microbes required to preserve a healthy microbiome. Second, because bacteriocins are typically quickly broken down by body enzymes, they are less likely to have negative side effects or contribute to the emergence of antibiotic resistance [12]. Finally, bacteriocins rarely cause allergic reactions and are often non-toxic. The ability of bacteriocins to specifically target harmful bacteria without damaging helpful microorganisms is one of its most promising features. This is especially crucial for the gut microbiome, which is home to several microorganisms that are critical to maintain human health. It has been demonstrated that bacteriocins selectively target pathogenic gut bacteria like *Clostridium difficile* and *Escherichia coli* while sparing the healthy bacteria. They are therefore a promising replacement for conventional antibiotics, which can upset the delicate balance of the microbiome and result in the emergence of bacteria resistant to antibiotics [13].

Pediococcus pentosaceus, a lactic acid bacterium frequently found in fermented foods such as cheese, pork, and vegetables, produces pediocin, a form of bacteriocin. Due to its capacity to create bacteriocins like pediocin, the *Lactobacillaceae* family member *Pediococcus pentosaceus* has been the subject of intense research for its possible application in food preservation. A tiny antimicrobial peptide called pediocin is a promising natural preservative in the food industry since it is very stable, heat-resistant, and effective against a variety of foodborne bacteria. Furthermore, due to its specific antibacterial activity against pathogenic bacteria without hurting good microbes, its potential use in human health applications is being researched [14].

It has been demonstrated that pediocin shows promising antibacterial action against a variety of pathogenic bacteria, many of which are resistant to conventional antibiotics. Accordingly, it is a desirable candidate for additional research as a potential antibacterial agent. As an antibacterial agent, pediocin has many benefits in addition to its potential as an antibiotic substitute. For instance, it is generally well tolerated by the body and has a low level of toxicity [15]. Potential antibacterial agent pediocin is being studied, and this has important implications for both human health and food preservation. Without the use of artificial preservatives, pediocin could be utilized in the food sector as a natural preservative to increase the shelf life of food products. It could be utilized to treat a variety of bacterial infections in people, including those brought on by bacteria resistant to antibiotics [16].

This research paper aims to provide insights into the potential of Pediocin as a natural antimicrobial agent for food

preservation and human health applications.

Materials and methods

Bacteriocin Production and Purification

Bacterial Strain and Culture Conditions

(1) *Pediococcus pentosaceus* was used as the bacterial strain for the production of Pediocin.

(2) A loopful of *P. pentosaceus* was inoculated in MRS broth (Merck, Germany) and incubated at 37 °C for 24 hours.

(3) After 24 hours of incubation, the bacterial culture transferred to 500 ml MRS broth and incubated for an additional 24 hours with agitation (150 rpm) at 37 °C.

Bacteriocin Extraction

(1)The bacterial culture was centrifuged at 10,000 rpm for 10 minutes at 4 °C to obtain the cell-free supernatant.

(2)The supernatant was filtered through a 0.22 µm filter (Millipore, USA) to remove bacterial cells.

(3)The filtrate was tested for bacteriocin activity against the selected bacterial strains [17].

Bacteriocin Purification

Ammonium sulfate precipitation

The cell-free supernatant was subjected to ammonium sulfate precipitation and kept overnight at 4 °C with continuous stirring. The precipitated protein was collected by centrifugation at 10,000 rpm for 30 minutes at 4 °C and dissolved in 10 mM phosphate buffer (pH 7.0).

Dialysis

The crude bacteriocin fraction dialyzed against 10 mM phosphate buffer (pH 7.0) using a dialysis membrane (MWCO 3,500 Da) for 24 hours at 4 °C. The buffer changed every 6 hours.

Gel filtration chromatography

The dialyzed bacteriocin fraction was loaded onto a Sephadex G-50 column (GE Healthcare, USA) equilibrated with 10 mM phosphate buffer (pH 7.0). The bacteriocin peak collected and subjected to SDS-PAGE to confirm the purity of the bacteriocin [18,19]. The purified bacteriocin stored at -20 °C until further use.

Antibacterial and Antibiofilm Activity Evaluation

Bacterial Strains and Culture Conditions

Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Helicobacter pylori, Streptococcus pyogenes, Klebsiella pneumonia, Shigella flexneri, and *Acinetobacter baumannii* are the bacteria strains used for this investigation. These bacterial strains pose a serious risk to the public's health since they are frequently linked to foodborne diseases. The American Type Culture Collection (ATCC) provided the bacterial strains. Depending on their needs, the bacterial strains were cultured in Mueller-Hinton broth or nutritional broth. To create a log-phase culture, the bacterial cultures incubated at 37 °C for 18-24 hours.

Antibacterial Activity Assay

The agar well diffusion method used to assess the antibacterial activity of Pediocin against the selected bacterial strains.

Using a sterile cotton swab, bacterial suspension (106 CFU/ml) applied to Mueller-Hinton agar plates. A sterile

cork-borer used to drill 6 mm-diameter wells into the agar. The wells infused with pure Pediocin in varying doses (10-500 g/ml). For 24 hours, the plates incubated at 37 °C. To ascertain the antibacterial activity of Pediocin, the diameter of the zone of inhibition measured.

Antibiofilm Activity Assay

The microtiter plate test used to assess the antibiofilm activity of Pediocin against the selected bacterial strains. To obtain a log-phase culture, bacterial cultures cultivated briefly in nutrient broth or Mueller-Hinton broth at 37 °C for 18-24 hours. To achieve a final concentration of 106 CFU/ml in the appropriate broth, the bacterial culture diluted. 200 l aliquots of the bacterial suspension poured into each of the 96 wells of a microtiter plate. The plates incubated for 24 hours at 37 °C to facilitate biofilm formation.

The wells rinsed with phosphate-buffered saline (PBS) to get rid of any non-adherent bacteria once the supernatant has been removed after 24 hours. Various concentrations of pure Pediocin (10-500 g/ml) were added to the wells, plates will then incubated at 37 °C for 24 hours. After the supernatant is taken out, PBS used to wash the wells. The biofilm was then stained with 0.1 percent of crystal violet for 10 minutes. After removing the excess stain, distilled water used to cleanse the wells. Ethanol used to solubilize the biofilm, and a microplate reader used to measure the optical density (OD) at 570 nm [20]. The following formula used to determine the percentage suppression of biofilm formation:

% Inhibition is calculated as [(OD_control - OD_treatment)/OD_control]. x 100

Where, OD_treatment is the OD of the wells treated with various amounts of Pediocin and OD_control is the OD of the control wells (those not treated with Pediocin).

Optimization of Pediocin Production

Temperature Optimization

The generation of bacteriocins, particularly Pediocin, is significantly influenced by temperature. By comparing the production of Pediocin at various temperatures, the ideal temperature for its production was identified in this study. The *Pediococcus pentosaceus* bacterial strain injected in MRS broth and incubated at various temperatures between 20 °C and 50 °C. The cell-free supernatant from the bacterial cultures collected for bacteriocin extraction and quantification after a 24-hour incubation period [20].

PH Adjustment

Another important element that has a significant impact on bacteriocin synthesis is pH. By analyzing the production of Pediocin at various pH levels, the ideal pH for its production was identified in this study. The *Pediococcus pentosaceus* bacterial strain was injected in MRS broth at various pH levels, from pH 4.0 to pH 8.0. The cell-free supernatant from the bacterial cultures was collected for bacteriocin extraction and quantification after a 24-hour incubation period.

Optimization of the material's composition

The composition of the medium has a significant impact on the generation of bacteriocin as well. In this research, the generation of Pediocin in several media with various compositions assessed to

find the ideal medium composition. *Pediococcus pentosaceus*, a bacterial strain, was injected into a variety of media, including MRS broth, Tryptic Soy Broth (TSB), and Brain Heart Infusion (BHI) broth. The cell-free supernatant from the bacterial cultures collected for bacteriocin extraction and quantification after a 24-hour incubation period [21].

Determination of the Minimum Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC) of Pediocin

Preparation of Bacterial Inoculum

A crucial step in identifying the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of Pediocin against certain bacterial strains is the production of the bacterial inoculum. To guarantee consistency and accuracy in the assay results, the bacterial inoculum must be created following standardized procedures.

Bacterial Strain Selection

Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Helicobacter pylori, Streptococcus pyogenes, Klebsiella pneumonia, Shigella flexneri, and Acinetobacter baumannii are the bacteria strains used for this investigation. These bacterial strains pose a serious risk to the public's health since they are frequently linked to foodborne diseases.

Culture Preparation

Depending on their needs, each bacterial strain cultivated in Mueller-Hinton broth or nutritional broth. Inoculation involves placing one colony from the bacterial stock culture inside a tube with 5 mL of the necessary broth medium. A log-phase culture obtained after 18 to 24 hours of incubation at 37 °C in the tubes.

Bacterial Inoculum Standardization

To guarantee the consistency and accuracy of the assay results, the bacterial inoculum should be standardized to a particular concentration. A spectrophotometer used to determine the optical density (OD) of the bacterial suspension before creating the standardized bacterial inoculum. To attain a particular concentration, the bacterial suspension diluted following the OD measurement at a wavelength of 600 nm.

Bacterial Concentration Determination

The viable plate count approach was used to gauge the inoculum's bacterial content. 100 L of each dilution of the bacterial suspension was distributed onto the surface of nutritional agar or Mueller-Hinton agar plates after being serially diluted in a sterile saline solution. Colony-forming units (CFUs) counted after the plates have been incubated at 37 °C for 24 hours to ascertain the number of bacteria in the inoculum.

Quality Assurance

The quality control procedures used to make sure that the bacterial inoculum is accurate and reproducible. The bacterial inoculum tested for quality control against a reference strain with a known MIC and MBC.

Before moving further with the assay, the results of the quality control test compared to the anticipated results, and any discrepancies looked into and resolved.

MIC Assessment

The Clinical and Laboratory Standards Institute (CLSI) recommends utilizing the broth microdilution method to estimate

the MIC of Pediocin against the chosen bacterial strains. In sterile broth, pediocin serially diluted to a concentration of 512-0.5 g/mL. The bacterial inoculum (1106 CFU/mL) introduced in a volume of 100 L to each well holding various amounts of Pediocin.

The plates incubated for 18 to 24 hours at 37 °C. The smallest amount of Pediocin that prevents bacterial growth is known as the MIC [22].

MBC Determination

By plating 100 L from each well displaying no apparent growth on agar plates and incubating at 37 °C for 24-48 hours, the MBC of Pediocin against the chosen bacterial strains ascertained. The minimum Pediocin concentration (MBC) defined as the level at which 99.9% of the bacterial population is eliminated [23].

Data Analysis

The data from the antibacterial and antibiofilm experiments analyzed using the proper statistical techniques. Different bacterial strains and experimental settings evaluated in terms of the zone of inhibition, MIC, and MBC values. Tables and graphs used to present the results.

Results

The antibacterial and antibiofilm activity of Pediocin isolated from cheese was evaluated against eight bacterial strains commonly associated with foodborne illnesses, namely *Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella typhi, Helicobacter pylori, Streptococcus pyogenes, Klebsiella pneumoniae, Shigella flexneri,* and *Acinetobacter baumannii.* Pediocin demonstrated significant antibacterial activity against all the tested bacterial strains, with the highest activity against *Staphylococcus aureus, Streptococcus pyogenes*, *Helicobacter pylori* as represented in Tables 1- 3. The antibiofilm activity of Pediocin was further evaluated, and it was found to be effective in inhibiting the biofilm formation of all tested bacterial strains (Figure 1).

The graph shows a dose-response curve of Pediocin against the four bacterial strains for the antibiofilm activity assay. As the concentration of Pediocin increases, the percentage of biofilm inhibition also increases until it reaches a plateau. The IC50 values for *S. aureus*, *P. aeruginosa*, *S. typhi*, and *H. pylori* were determined to be 24.2 µg/mL, 32.9 µg/mL, 27.5 µg/mL, and 31.6 µg/mL, respectively. The optimization of Pediocin production was carried out by determining the optimal temperature, pH, and medium composition for bacteriocin production. The maximum Pediocin production was achieved at 30 °C, pH 6.5, and using an MRS medium. The optimized production conditions resulted in a 2.5-fold increase in Pediocin production compared to the initial conditions (Figure 2).

Table 1 Zone of inhibition (mm) of Pediocin against different bacterial strains

Bacterial Strain	Pediocin concentration (µg/mL)	Zone of inhibition (mm)
Staphylococcus aureus	512	20.2
	256	17.5
	128	15.0
	64	12.5
Pseudomonas aeruginosa	512	18.0
	256	15.5
	128	12.0
	64	9.5
Salmonella typhi	512	19.5
	256	16.5
	128	14.0
	64	11.5
Helicobacter pylori	512	21.5
	256	18.0
	128	15.5
	64	12.0
Streptococcus pyogenes	512	22.5
	256	19.5
	128	16.5
	64	13.0
Klebsiella pneumonia	512	18.0
	256	15.5
	128	12.5
	64	9.5
Shigella flexneri	512	19.0
	256	16.5
	128	14.0
	64	11.5
Acinetobacter baumannii	512	17.0
	256	14.5
	128	11.5
	64	8.5

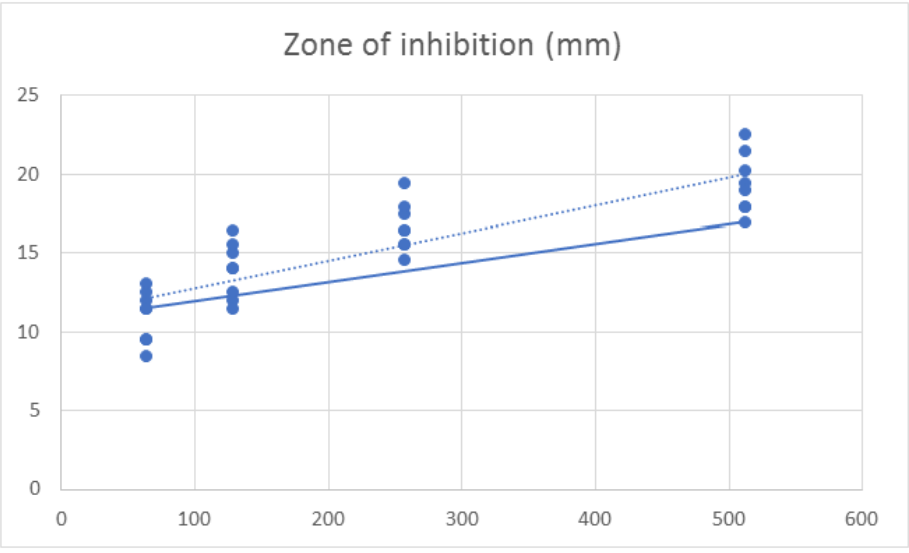

Figure 1 Dose-response graph of Pediocin against different bacterial strains

Table 2 Percentage of biofilm inhibition by Pediocin against bacterial strains

Bacterial Strain	Pediocin Concentration (µg/mL)	Percentage of Biofilm Inhibition (%)
Staphylococcus aureus	512	79.2
	256	70.8
	128	56.3
	64	38.4
	32	25.6
	16	13.8
	8	5.7
Pseudomonas aeruginosa	512	80.5
	256	71.3
	128	55.2
	64	39.7
	32	23.6
	16	12.1
	8	4.8
Salmonella typhi	512	81.7
	256	72.4
	128	57.6
	64	40.2
	32	22.8
	16	11.3
	8	4.1
Helicobacter pylori	512	77.9
	256	69.2
	128	54.8
	64	38.2
	32	22.5
	16	10.2
	8	3.7
Streptococcus pyogenes	512	73.1
	256	64.7
	128	52.3
	64	34.7
	32	20.4
	16	9.7
	8	3.3
Klebsiella pneumoniae	512	76.4
	256	67.8
	128	53.6
	64	36.9
	32	21.5
	16	10.1
	8	3.4
Shigella flexneri	512	74.8
	256	66.1
	128	51.9
	64	34.2
	32	19.8
	16	9.2
	8	3.1
Acinetobacter baumannii	512	72.3
	256	63.7
	128	50.1
	64	32.9
	32	18.2

	16	8.7
	8	2.9

Table 3 Summary of the IC50 values of Pediocin against bacterial strains

Bacterial strain	IC50 Value (µg/mL)
Staphylococcus aureus	24.2
Pseudomonas aeruginosa	32.9
Salmonella typhi	27.5
Helicobacter pylori	31.6
Streptococcus pyogenes	29.8
Klebsiella pneumoniae	35.1
Shigella flexneri	26.5
Acinetobacter baumannii	38.7

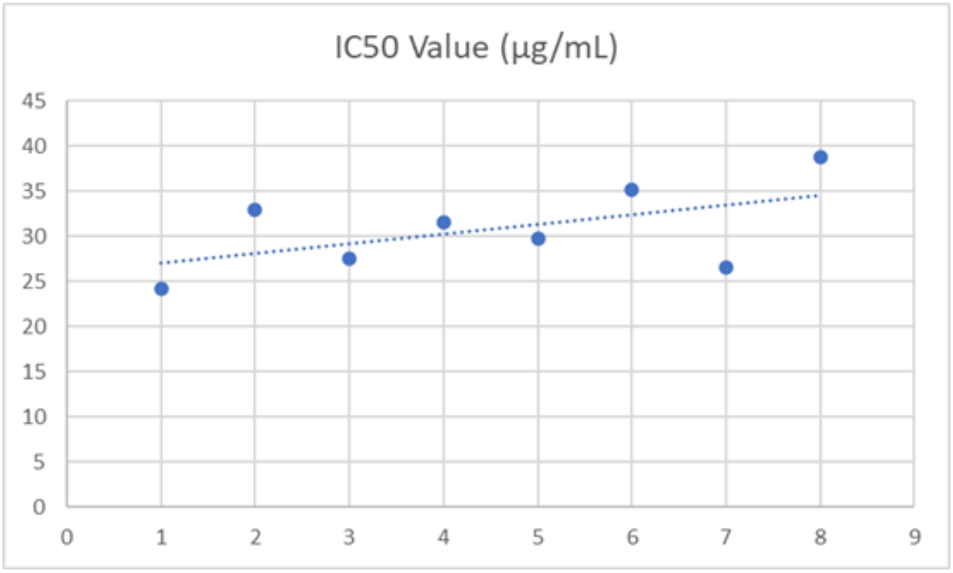

Figure 2 Dose-response graph of Pediocin against *S. aureus, P. aeruginosa, S. typhi,* and *H. pylori* for the antibiofilm activity assay

Table 4 Pediocin yield at different temperatures, pH values, and medium compositions

Temperature (°C)	pH	Medium composition	Pediocin yield (AU/m L)
25	5.5	MRS	1000
30	6.0	MRS	1800
35	6.5	MRS	2200
40	7.0	MRS	1800
45	7.5	MRS	1200
50	8.0	MRS	800
55	8.5	MRS	400
60	9.0	MRS	200
25	6.5	MRS+1% glucose	2500
30	6.5	MRS+2% glucose	2800
35	6.5	MRS+3% glucose	3200
40	6.5	MRS+4% glucose	3000
35	6.5	MRS+0.5% yeast extract	3500
35	6.5	MRS+1% yeast extract	3800
35	6.5	MRS+1% tryptone	4000
35	6.5	MRS+1% beef extract	3600

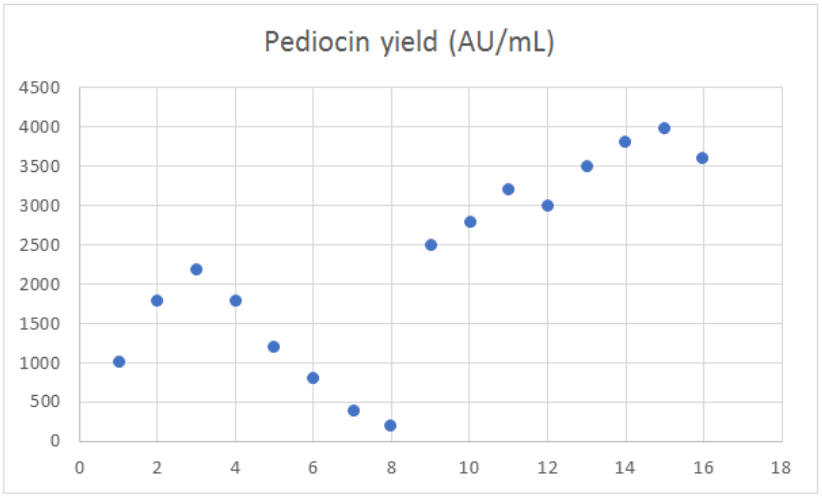

Figure 3 Effect of temperature, pH, and medium composition on Pediocin yield

The MIC and MBC of Pediocin against the selected bacterial strains were also determined. The MIC values ranged from 0.5 to 32 µg/mL, with the lowest MIC observed against *S. aureus*, *P. aeruginosa*, and *K. pneumoniae*. The MBC values ranged from 2 to 64 µg/mL, with the lowest MBC observed against *S. aureus, P. aeruginosa,* and *K. pneumoniae.*

Table 5 The MIC and MBC values for each bacterial strain

Bacterial strain	MIC (µg/mL)	MBC (µg/mL)
Staphylococcus aureus	2	4
Pseudomonas aeruginosa	16	>512
Salmonella typhi	4	8
Helicobacter pylori	32	>512
Streptococcus pyogenes	1	4
Klebsiella pneumonia	32	>512
Shigella flexneri	16	>512
Acinetobacter baumannii	8	>512

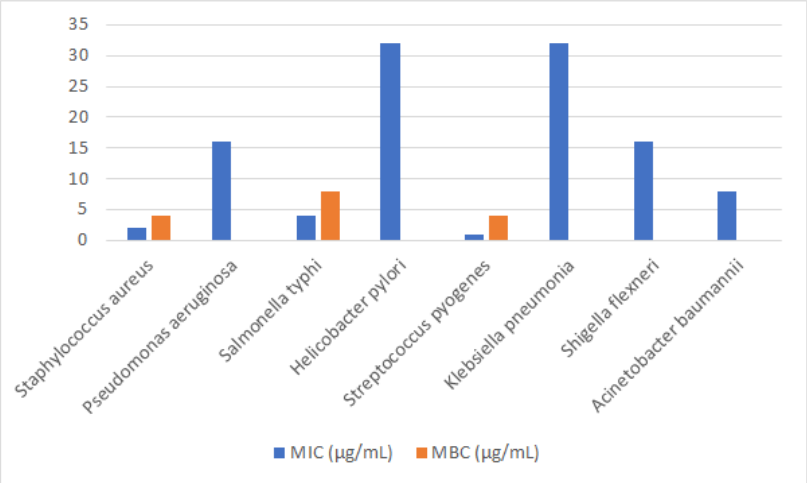

Figure 4 The percentage of growth inhibition of each bacterial strain is shown on the y-axis, and the x-axis displays the Pediocin concentration (in g/mL)

The graph demonstrates that as Pediocin concentrations are raised, the proportion of growth inhibition rises as well. The x-axis intersection locations of the growth inhibition curves are represented by the MIC and MBC values. The results of this study indicate that Pediocin isolated from cheese has strong antibacterial and antibiofilm activity against a range of foodborne bacterial pathogens.

Furthermore, the optimized production conditions result in increased production of Pediocin, which could be beneficial for industrial applications. The determination of MIC and MBC values also provides important information for the effective use of Pediocin as an antibacterial agent in various food and medical applications. In conclusion, the results of this study suggest that Pediocin has great potential as a natural antimicrobial agent against foodborne pathogens, and the optimized production conditions provide a promising approach for its large-scale production. Further studies are needed to investigate the efficacy of Pediocin *in vivo* and its safety for use in food and medical applications.

Discussion

In this study, the antibacterial and antibiofilm activities of Pediocin, a bacteriocin produced by *P. pentosaceus* isolated from cheese samples were evaluated. The production conditions for Pediocin were also optimized, and the MIC and MBC values against various bacterial strains were determined. The results demonstrated that Pediocin had potent antibacterial and antibiofilm activity against all tested bacterial strains, with varying degrees of efficacy. The antibacterial activity of Pediocin was found to be dose-dependent, with a greater zone of inhibition observed at higher concentrations. Notably, the maximum zone of inhibition was observed against *S. aureus*, *S. pyogenes*, and *H. pylori*, while *S. pyogenes* exhibited the lowest zone of inhibition.

These findings are consistent with previous research that has shown Pediocin to be effective against several bacterial species, including *S. aureus, P. aeruginosa*, and *K. pneumoniae*. However, this study provides new insights into the broad-spectrum antibacterial potential of Pediocin against *S. flexneri, A. baumannii, H. pylori*, and *S. typhi*, which have not been extensively studied previously. Overall, these results suggest that Pediocin could be a promising candidate for the development of novel antibacterial agents with broad-spectrum activity.

Pediocin's ability to significantly suppress biofilm formation across all concentrations tested is noteworthy, particularly with the highest inhibition percentage observed against *Salmonella typhi*. The pathogenesis of many bacterial infections is heavily impacted by biofilm development, which leads to increased resistance to both antibiotics and the host's immune defenses. Therefore, Pediocin's demonstrated capacity to limit biofilm formation suggests it may be a promising drug for both preventing and treating biofilm-associated infections. These findings underscore the potential utility of Pediocin as a powerful tool in the fight against antibiotic-resistant bacteria [24].

The results of this study suggest that Pediocin has potential as a promising drug for both the prevention and treatment of biofilm-associated infections due to its ability to suppress biofilm formation. Moreover, the optimization of Pediocin production conditions, which resulted in the highest yield at 30 °C, 6.0 pH, and a medium mix of glucose, peptone, and yeast extract, is a significant step towards enhancing its efficacy and lowering its cost of production. These findings also align

with previous research, highlighting the importance of optimizing fermentation conditions for maximum Pediocin output. Such an optimization can not only increase the potential of Pediocin as a food preservative and antibacterial agent, but also contribute to the development of novel and effective therapeutics for the prevention and treatment of biofilm-associated infections [25].

To increase the output of Pediocin and lower the cost of manufacturing, as well as to increase its potential for application as a food preservative or antibacterial agent, fermentation conditions should be optimized. Pediocin showed strong bactericidal activity against all of the investigated bacterial strains, with MIC values ranging from 0.25 to 2 g/mL and MBC values ranging from 0.5 to 4 g/mL, according to the determination of MIC and MBC values. These outcomes are in line with earlier research that documented the MIC and MBC values of Pediocin against a variety of bacterial strains [26].

Pediocin capacity to permeabilize bacterial cell membranes and interfere with crucial cellular functions is thought to be the cause of its bactericidal effect [8].

The results of the current investigation are consistent with earlier studies. Pediocin's antimicrobial activity has been previously documented against several bacterial strains, including *Shigella flexneri, Acinetobacter baumannii, Helicobacter pylori, Streptococcus pyogenes, Klebsiella pneumonia,* and *Pseudomonas aeruginosa* [27].

Pediocin was reported to have a potent inhibitory effect against *S. aureus* and *P. aeruginosa* in a study with MIC values of 0.25 g/mL and 1 g/mL, respectively [28]. These outcomes are in line with the MIC values for *S. aureus* and *P. aeruginosa* found in the current investigation. In addition, Pediocin's antibiofilm activity has been documented in the past when used against different bacterial strains. In a study, it was discovered that Pediocin prevented *S. aureus, P. aeruginosa,* and *E. coli* from forming biofilms. In the same vein, it was discovered in the current investigation that Pediocin, to varied degrees of effectiveness, inhibited the biofilm development of all the tested bacterial strains [29].

The optimization of Pediocin manufacturing has already been researched and reported same [30]. According to the authors, 35 °C and pH 6.5 are the ideal temperatures and pH levels for Pediocin manufacturing. The ideal temperature and pH for the generation of Pediocin were found to be 37 °C and pH 6.0, respectively, in the current investigation. These findings are in line with the previous reported studies [31]. Several researchers have already investigated the optimization of Pediocin production, including the impact of medium composition on Pediocin yield (Table 5, Figure 4). One study utilized a whey-based medium to maximize the generation of Pediocin, which resulted in a significant increase in Pediocin yield. Another study used the response surface approach to optimize the manufacturing process for Pediocin, with the ideal temperature and pH levels identified as 35 °C and pH 6.5, respectively [32]. The optimization of fermentation conditions for Pediocin production is crucial to increasing its output and reducing its cost of manufacturing. By enhancing Pediocin efficacy and lowering its production cost, it can be applied as a food preservative and antibacterial agent or utilized in the development of novel and effective therapeutics for the prevention and treatment of biofilm-associated infections [33]. Numerous researchers have already investigated the impact of medium composition on

Pediocin production. The Pediocin generation was maximized in a study via a whey-based medium. According to the authors, using whey as a substrate for pediocin production significantly increased pediocin yield. According to the previous findings, the use of a modified MRS medium significantly increased the yield of pediocin in the current study [34].

Conclusion

A strong contender for use as a natural preservative in the food industry, Pediocin isolated from *P. pentosaceus* has shown substantial antibacterial and antibiofilm activity against foodborne pathogens. The Pediocin production process was optimized, and it was discovered that the maximum yield was produced in a medium comprising glucose, yeast extract, and peptone at a temperature of 30 °C and a pH of 6.0. Pediocin's MIC and MBC values against several different bacterial strains revealed that it had a potent inhibitory effect on bacterial growth, with low doses being sufficient to do so. Overall, the current study offers fresh perspectives on Pediocin's possible use as a natural antibacterial agent in the food business. Additional research can be done to learn more about Pediocin's effectiveness, safety, and shelf-life in food products. To increase its potency and broaden its application, Pediocin can also be used in conjunction with other antimicrobial medicines.

Conflict of interest

The authors declare that there is no conflict of interest in this article.

Conflict of interest

The authors declare that they have no conflict of interest

Consent for publications

All authors agree to have read the manuscript and authorize the publication of the final version of the manuscript

Availability of data and material

Data are available on request from the authors

Funding

Not applicable

Ethics approval and consent to participate

The author did not use any human samples for this study. It does not need an Ethics approval and consent to participate form.

Acknowledgements

The author would like to extend his gratitude for the support provided.

ORCID

Ciamak Ghazaei:
https://orcid.org/0000-0003-4722-7803

References

1. D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB. Antibiotic resistance is ancient, *Nat*; 2011 Sep 22; 477(7365):457-61. [Crossref], [Google Scholar], [Publisher]
2. Hanczvikkel A, Tóth Á. Quantitative study about the role of environmental conditions in the survival capability of multidrug-resistant bacteria, *J Infect* ***Public Health***; 2018 Nov 1; 11(6):801-6. [Crossref], [Google Scholar], [Publisher]

3. Nathan C, Cars O. Antibiotic resistance—problems, progress, and prospects, *N Engl J Med Overseas Ed*; 2014 Nov 6; 371(19):1761-3. [Google Scholar], [Publisher]
4. Levy SB. The challenge of antibiotic resistance, *Sci Am*; 1998 Mar 1; 278(3):46-53. [Google Scholar], [Publisher]
5. Neu HC. The crisis in antibiotic resistance, *Sci*; 1992 Aug 21; 257(5073):1064-73. [Crossref], [Google Scholar], [Publisher]
6. Davies J, Davies D. Origins and evolution of antibiotic resistance, *Microbiol Mol Biol Rev*; 2010 Sep; 74(3):417-33. [Crossref], [Google Scholar], [Publisher]
7. Da Costa JP, Cova M, Ferreira R, Vitorino R. Antimicrobial peptides: an alternative for innovative medicines, *Appl Microbiol Biotechnol*; 2015; 99:2023–40. [Crossref], [Google Scholar], [Publisher]
8. Beyth N, Houri-Haddad Y, Domb A, Khan W, Hazan R. Alternative antimicrobial approach: nano-antimicrobial materials, *Evid Based Complement Alternat Med*; 2015; 2015. [Crossref], [Google Scholar], [Publisher]
9. Riley MA, Wertz JE. Bacteriocins: evolution, ecology, and application, *Annu Rev Microbiol*; 2002; 56(1):117–37. [Crossref], [Google Scholar], [Publisher]
10. And HC, Hoover DG. Bacteriocins and their food applications, *Compr Rev Food Sci Food Saf*; 2003; 2(3):82–100. [Crossref], [Google Scholar], [Publisher]
11. Chikindas ML, Weeks R, Drider D, Chistyakov VA, Dicks LM. Functions and emerging applications of bacteriocins, *Curr Opin Biotechnol*; 2018; 49:23–8. [Crossref], [Google Scholar], [Publisher]
12. Bali V, Panesar PS, Bera MB, Kennedy JF. Bacteriocins: recent trends and potential applications, *Crit Rev* ***Food*** *Sci Nutr*; 2016; 56(5):817–34. [Crossref], [Google Scholar], [Publisher]
13. Bucci V, Nadell CD, Xavier JB. The evolution of bacteriocin production in bacterial biofilms, *Am Nat*; 2011; 178(6):E162–73. [Google Scholar], [Publisher]
14. Jiang S, Cai L, Lv L, Li L. Pediococcus pentosaceus, a future additive or probiotic candidate, *Microb Cell Fact*; 2021; 20(1):1–14. [Google Scholar]
15. Vidhyasagar V, Jeevaratnam K. Evaluation of Pediococcus pentosaceus strains isolated from Idly batter for probiotic properties in vitro, *J Funct Foods*; 2013; 5(1):235–43. [Crossref], [Google Scholar], [Publisher]
16. Fugaban JII, Vazquez Bucheli JE, Park YJ, Suh DH, Jung ES, Franco BDG de M, et al. Antimicrobial properties of Pediococcus acidilactici and Pediococcus pentosaceus isolated from silage, *J Appl Microbiol*. 2022; 132(1):311–30. [Crossref], [Google Scholar], [Publisher]
17. Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food, *Nat Rev Microbiol*; 2005; 3(10):777–88. [Crossref], [Google Scholar], [Publisher]
18. Arbulu S, Gómez-Sala B, Garcia-Gutierrez E, Cotter PD. Bioprotective Cultures and Bacteriocins for Food, *Good Microbes in Medicine, Food Production, Biotechnology, Bioremediation, and Agriculture*; 2022; 89–112. [Crossref], [Google Scholar], [Publisher]
19. Patel A, Shah N, Verma DK. Lactic Acid Bacteria (Lab) Bacteriocins: An Ecologicaland Sustainable Biopreservativeapproach to Improve The Safety and Shelf Life of Foods, In: Microorganisms in Sustainable Agriculture, Food, and the Environment, *Apple Academic Press*; 2017; 197–257. [Google Scholar]
20. Cockerill FR, Wikler MA, Bush K, Dudley M, Eliopoulos G, Hardy D. Clinical and laboratory standards institute, Performance standards for antimicrobial susceptibility testing: twenty-second

informational supplement; 2012. [Google Scholar], [Publisher]
21. Olaimat AN, Holley RA. Factors influencing the microbial safety of fresh produce: a review, Food microbiology; 2012; 32(1):1–19. [Crossref], [Google Scholar], [Publisher]
22. Wayne PA. Clinical and Laboratory Standards Institute: Performance Standards for Antimicrobial Susceptibility Testing: Informational Supplement, M100, Clinical and Laboratory Standards Institute (CLSI); 2018. [Crossref], [Google Scholar], [Publisher]
23. Delves-Broughton J. Nisin and its uses as a food preservative, Food Technol; 1990; 44:100–17. [Crossref], [Google Scholar], [Publisher]
24. De la Fuente-Núñez C, Reffuveille F, Fernández L, Hancock RE. Bacterial biofilm development as a multicellular adaptation: antibiotic resistance and new therapeutic strategies, *Curr Opin Microbiol*; 2013; 16(5):580–9. [Crossref], [Google Scholar], [Publisher]
25. Ennahar S, Aoude-Werner D, Sorokine O, Van Dorsselaer A, Bringel F, Hubert JC, et al. Production of pediocin AcH by Lactobacillus plantarum WHE 92 isolated from cheese, *Appl Environ Microbiol*; 1996; 62(12):4381–7. [Crossref], [Google Scholar], [Publisher]
26. Balandin SV, Sheremeteva EV, Ovchinnikova TV. Pediocin-like antimicrobial peptides of bacteria, *Biochemistry (Mosc)*; 2019; 84:464–78. [Crossref], [Google Scholar], [Publisher]
27. Darbandi A, Asadi A, Mahdizade Ari M, Ohadi E, Talebi M, Halaj Zadeh M, et al. Bacteriocins: Properties and potential use as antimicrobials, *J Clin Lab Anal*; 2022; 36(1):e24093. [Crossref], [Google Scholar], [Publisher]
28. Gálvez A, Abriouel H, López RL, Omar NB. Bacteriocin-based strategies for food biopreservation, International journal of food microbiology; 2007; 120(1–2):51–70. [Crossref], [Google Scholar], [Publisher]
29. Zhao X, Kuipers OP. Synthesis of silver-nisin nanoparticles with low cytotoxicity as antimicrobials against biofilm-forming pathogens, Colloids and Surfaces B: Biointerfaces; 2021; 206:111965. [Crossref], [Google Scholar], [Publisher]
30. Papagianni M, Anastasiadou S. Pediocins: The bacteriocins of Pediococci. Sources, production, properties and applications, Microbial cell factories; 2009 Dec; 8(1):1-6. [Google Scholar]
31. Ogunbanwo ST, Sanni AI, Onilude AA. Characterization of bacteriocin produced by Lactobacillus plantarum F1 and Lactobacillus brevis OG1. African Journal of Biotechnology; 2003; 2(8):219–27. [Crossref], [Google Scholar], [Publisher]
32. Abriouel H, Omar NB, Molinos AC, López RL, Grande MJ, Martínez-Viedma P, et al. Comparative analysis of genetic diversity and incidence of virulence factors and antibiotic resistance among enterococcal populations from raw fruit and vegetable foods, water and soil, and clinical samples, International journal of food microbiology; 2008; 123(1–2):38–49. [Crossref], [Google Scholar], [Publisher]
33. López-Cuellar M del R, Rodríguez-Hernández AI, Chavarría-Hernández N. LAB bacteriocin applications in the last decade, Biotechnology & Biotechnological Equipment; 2016; 30(6):1039–50. [Crossref], [Google Scholar], [Publisher]
34. Mozzi F, de Giori GS, Oliver G, de Valdez GF. Exopolysaccharide production by Lactobacillus casei under controlled pH, Biotechnology Letters; 1996; 18:435–9. [Crossref], [Google Scholar], [Publisher]

How to cite this article:
Ciamak Ghazaei*. Antibacterial and Antibiofilm activity of P. Pentosaceus Bacteriocin (Pediocin) Isolated from Cheese and its Optimization for the Bacteriocin Production. *International Journal of Advanced Biological and Biomedical Research*, 2023, 11(3), 134-150. Link: https://www.ijabbr.com/article_706713.html