CHARACTERIZATION OF BACTERIOCIN PRODUCE BY *Lactobacillus sp*. ISOLATED FROM "PIPING ROCK WOMEN PROBIOTICS" AND ITS ANTIMICROBIAL PROPERTIES ON *Echerichia coli*

BY

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DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY

NOVEMBER, 2025

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A PROJECT SUBMITTED TO THE DEPARTMENT OF SCIENCE LABORATORY TECHNOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF BACHELOR DEGREE (B.Sc.) IN SCIENCE LABORATORY TECHNOLOGY (MICROBIOLOGY TECHNIQUES) FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY

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CERTIFICATION

This is to certify that this project work was done by Blessing OTANIYEN (Miss) with MAT NO. LSC2010001 of the Department of Science Laboratory Technology (Microbiology Technique), Faculty of Life Sciences, University of Benin, Benin City, as part of requirement for the award of bachelor of Science (B.Sc.) Degree.

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DEDICATION

This project is dedicated to God Almighty for his protection , guidance, direction and strength.

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I sincerely appreciate God Almighty for his Divine guidance and wisdom throughout this project work.

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ABSTRACT

This study explored the isolation and identification of *Lactobacillus* species from Piping rock women Probiotics and the production of bacteriocin with germ-fighting power against *Escherichia coli*. The bacteria were found to be Gram-positive, rod-shaped, and negative for catalase and oxidase tests. They fermented galactose, lactose, and D-fructose without forming gas. The bacteriocin's effect was tested using the agar well diffusion method, and enzyme (trypsin) and hydrogen peroxide neutralization (sodium pyruvate) tests confirmed that it was protein-based. The lack of blue-black color in the potassium iodide-starch test showed that no hydrogen peroxide was made. HPLC results showed the presence of active compounds such as catechin, naringenin, and caffeic acid, which may help boost its germ-killing action.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

The rise of drug-resistant bacteria, especially *Escherichia coli*, has made it important to find new ways to fight infections. Recently, probiotics and the substances they produce have gained attention as possible treatments. One of these useful substances is bacteriocin, a small protein made by many types of lactic acid bacteria (LAB). Bacteriocins can stop the growth of other bacteria, both similar and different types without harming the bacteria that make them. This makes them useful in both medicine and food preservation (Sugrue *et al*., 2024). The *lactobacillus* group (now divided into smaller groups such as *Lacticaseibacillus* and *Lactiplantibacillus*) is one of the main kinds of LAB found in fermented foods, the human body, and probiotic products. These bacteria are considered safe and are known for producing bacteriocins that fight against food-related and drug-resistant bacteria (Ren *et al*., 2022). Examples include plantaricin and reuterin, which have been shown to kill many types of harmful bacteria, making them strong options to replace regular antibiotics (Darbandi *et al*., 2022). Commercial probiotics like Piping Rock Women Probiotics are good sources of living *Lactobacillus* strains. These products are commonly used for their health benefits, such as improving gut balance, boosting immunity, and preventing harmful bacteria from growing in the body (Jenkins and Mason, 2022). Finding bacteriocin-producing bacteria from these products offers both research and business opportunities. In particular, targeting harmful bacteria like *Escherichia coli*, which is known to cause infections, food poisoning, and serious illnesses such as sepsis, shows how important probiotic-based bacteriocins can be in healthcare (Heinzinger *et al*., 2023).

Food spoilage caused by bacteria and other microbes continues to be a major issue worldwide, leading to large losses after harvest and food shortages, especially in perishable fruit like tomatoes. Traditional chemical preservatives often raise safety concerns due to possible toxicity, consumer worries, and the increasing interest in natural and eco-friendly methods (Peng *et al.*, 2023). Bacteriocins, because they are natural, safe, and effective even in small amounts, have become a good alternative for keeping food fresh (Parada *et al*., 2025). They usually work by creating holes in the cell walls of harmful bacteria or by disrupting their internal processes, which stops them from surviving or reproducing (Soltant *et al*., 2022).

New techniques such as high-performance liquid chromatography (HPLC) have made it easier to purify and study bacteriocins in detail.Studying bacteriocins at the molecular level helps scientists understand their strength, structure, and how they work (Pang *et al.*, 2025). This makes it possible to test their full antibacterial power not just in the lab but also in real-life uses such as treating infections and keeping food fresh. In addition, when bacteriocins are used together with other antibacterial agents, they often work better and make it harder for bacteria to become resistant (Soltani *et al*., 2022). With the growing problem of antibiotic-resistant *Escherichia coli* and the need for safer, natural ways to preserve food, studying bacteriocins from probiotics like *Lactobacillus* species offers two key benefits, it can improve public health and reduce food spoilage after harvest. Finding, studying, and using bacteriocins from probiotic products such as Piping Rock Women Probiotics presents a strong opportunity for both medical research and food preservation technology.

1.2 Aim of Study

The study is aimed at the isolation and characterization of bacteriocin by *Lactobacillus* species isolated from "Piping Rock Women Probiotics" and its antimicrobial effects on clinical isolate of *Echerichia coli*

1.2 **Specific Objectives** The specific objectives of the project were to:

1. isolate and characterize *Lactobacillus species* from "Piping Rock Women Probiotics".
2. confirm bacteriocin production using the agar well diffusion method.
3. determine the effect of hydrogen peroxide neutralization and proteolytic enzyme treatment on bacteriocin activity.
4. characterize bacteriocin produced by probiotic isolates using high-performance liquid chromatography (HPLC).
5. test for Hydrogen Peroxide activity in bacteriocin.

CHAPTER TWO

LITERATURE REVIEW

2.1 Overview of Probiotics and Their Importance.

Probiotics are live microorganisms that, when taken in the right amount, help improve health. Among them, lactic acid bacteria (LAB) especially *Lactobacillus* species are well known for their health and healing benefits. They help keep the gut balanced, strengthen the immune system, and fight harmful bacteria by releasing natural acids, hydrogen peroxide, and antibacterial proteins called bacteriocins (Darbandi *et al.*, 2022; Sugrue *et al.*, 2024). Studying bacteriocins from probiotics has become more important as antibiotic resistance continues to spread. Probiotic products such as Piping Rock Women Probiotics are made to contain active and pure *Lactobacillus* strains, making them great sources for collecting useful natural compounds. The ability of these bacteria to produce bacteriocins that strongly inhibit resistant pathogens like *Escherichia coli* shows how valuable they are in medicine (Pang *et al.,* 2025; Ren *et al*., 2022).

2.2 Lactic Acid Bacteria as Probiotic Agents

Lactic acid bacteria are Gram-positive, non-spore-forming, and catalase-negative microorganisms that mainly convert carbohydrates into lactic acid. They are found in various environments such as fermented foods, the human gut, and probiotic supplements (Jenkins and Mason, 2022). *Lactobacillus* species are among the most researched LAB because of their wide use in both the food industry and healthcare. These microorganisms help the body stay healthy by keeping the gut balanced, stopping harmful bacteria from growing, and producing natural substances that fight germs (Peng *et al*., 2023). Their ability to make bacteriocins gives them an advantage over other microbes and increases their probiotic benefits. Since Piping Rock Women Probiotics contain carefully chosen lactic acid bacteria

(LAB) strains, they are a reliable source of bacteria that can produce bacteriocins with strong antibacterial effects (Soltani *et al.,* 2022).

2.3 Characteristics and Types of Bacteriocins

Bacteriocins are small proteins made by bacteria that can stop or destroy other bacteria, often those that are similar to the one producing them. Unlike normal antibiotics, bacteriocins are natural, made of protein, and break down easily in the environment (Sugrue *et al.,* 2024). Because they are safe and biodegradable, they are useful in medicine, drug development, and food preservation (Darbandi *et al*., 2022).They are generally divided into three main types:

Class I (Lantibiotics): Small, heat-resistant proteins that contain special amino acids such as lanthionine.

Class II (Small heat-stable peptides): Includes bacteriocins like pediocin, as well as two-peptide and circular forms.

Class III (Large heat-sensitive proteins): Big protein molecules that lose their effect when exposed to heat (Soltani *et al*., 2022).

Bacteriocins from Lactobacillus species mostly fall under Class II, which are known for staying stable under different temperatures and pH levels. They work by damaging the cell walls of target bacteria, creating pores in the membranes, causing the cell contents to leak out, and stopping important cell activities (Ren *et al.*, 2022; Heinzinger *et al.,* 2023).

2.4 Production and Factors That Affect Bacteriocin Formation

The production of bacteriocins by *Lactobacillus* species depends on several physical and environmental factors such as temperature, nutrients, and pH levels (Peng *et al*., 2023). Using a nutrient-rich growth medium like de Man, Rogosa, and Sharpe (MRS) broth with a pH between 6.0 and 6.5 at 37°C has been found to give the best bacteriocin output (Jenkins and Mason, 2022). The sources of carbon and nitrogen, the growth stage of the bacteria, and the amount of air also affect how much bacteriocin is produced. Under the best conditions, bacteriocins are released during the fast growth stage, which helps increase their amount and effectiveness. The different LAB strains found in Piping Rock Women Probiotics may produce bacteriocins with special structures and actions that can fight resistant bacteria such as *Escherichia coli* (Pang *et al.,* 2025).

2.5 Screening, Isolation, and Testing Methods

To check for bacteriocin production, scientists often use microbiological tests that measure antibacterial strength. The agar well diffusion method is the most common test, where the liquid part of a bacterial culture is placed in wells on a plate containing harmful bacteria such as *Escherichia coli* (Mercado and Olmos 2022). Clear areas around the wells show that bacteriocin is active. For purification, methods such as ammonium sulfate precipitation, dialysis, and chromatography (including ion-exchange and gel filtration) are used to obtain clean portions of bacteriocin. High-Performance Liquid Chromatography (HPLC) helps to check the purity and structure of the compound (Ren *et al*., 2022). Additional tests like enzyme breakdown, heat, and pH stability are done to confirm that the substance is a protein and to measure its strength (Soltani *et al*., 2022). These steps help in correctly identifying bacteriocins that are useful for medicine and industry.

2.6 Antimicrobial Importance against *Escherichia coli*

Escherichia coli is a common bacterial species that can act as an opportunistic pathogen, responsible for a variety of infections ranging from gastrointestinal to urinary tract diseases. The rise of antibiotic-resistant *Echerichia coli* strains has driven research toward alternative antimicrobial solutions (Heinzinger *et al.,* 2023).

Bacteriocins produced by LAB have demonstrated substantial inhibitory activity against *Echerichia coli.*

Their mode of action involves disrupting the bacterial membrane, causing depolarization and subsequent cell lysis (Sugrue *et al*., 2024) (Pang *et al*., 2025) and (Adeosun *et al., 2*021) reported that bacteriocins from *Lactobacillus* plantarum and Lacticaseibacillus rhamnosus were capable of effectively suppressing multidrug-resistant *Echerichia coli* strains. These results underscore the therapeutic relevance of exploring bacteriocin-producing probiotics as natural antimicrobial agents.

Bacteriocins derived from piping rock women Probiotics are anticipated to demonstrate comparable inhibitory effects due to the high-quality probiotic formulation and selection of robust bacterial strains.

2.7 Hydrogen Peroxide Activity in Bacteriocin-Producing Lactic Acid Bacteria

Hydrogen peroxide (H_2O_2) is one of the main natural antibacterial substances made by lactic acid bacteria (LAB), along with organic acids and bacteriocins. In *Lactobacillus* species, hydrogen peroxide helps stop the growth of harmful bacteria by causing oxidative stress that damages their cell walls, proteins, and DNA (Darbandi *et al.,* 2022).

When studying bacteriocins, testing for hydrogen peroxide activity helps to know if the bacteria-killing effect comes from the bacteriocin itself or from the oxidative effect of hydrogen peroxide. This is usually checked by adding catalase to remove hydrogen peroxide and then comparing how strong the antibacterial effect is before and after treatment (Ren *et al.*, 2022).

Researchers like (Pang *et al.* 2025) and (Sugrue *et al.* 2024) have reported that bacteriocins and hydrogen peroxide can work together. The oxidative stress from hydrogen peroxide weakens bacterial cells, making it easier for bacteriocins to enter and act more effectively. Testing for hydrogen peroxide activity therefore gives valuable insight into the total antibacterial strength of *Lactobacillus* strains and their bacteriocin extracts.

2.8 Identified knowledge Gaps

Even though there has been a lot of research on bacteriocins from food-related LAB, only a few studies have focused on bacteriocins from pharmaceutical probiotic products such as Piping Rock Women Probiotics. These products often contain well-studied and stable bacterial strains that could produce new types of bacteriocins with stronger biological activity (Pang *et al*., 2025). Existing research also shows that there are very few studies on how bacteriocins directly interact with drug-resistant *Escherichia coli* strains. Because of this, the present study focuses on isolating and studying bacteriocins from *Lactobacillus* species found in Piping Rock Women Probiotics and testing their antibacterial power against *Escherichia coli.*

2.9 Summary of Literature

Bacteriocins are natural protein compounds made by lactic acid bacteria (LAB) that can strongly stop the growth of harmful bacteria. Since they are safe, break down easily, and remain stable, they are seen as good replacements for regular antibiotics. Studies show that *Lactobacillus* species taken from pharmaceutical probiotics can produce bacteriocins that effectively fight resistant bacteria such as *Escherichia coli*. This research aims to study bacteriocin production from Piping Rock Women Probiotics as part of the effort to find safe and natural antibacterial substances that could be used in both healthcare and food preservation.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Materials

CHAPTER FOUR
RESULTS

4.1 The results of the morphological Characteristics of the Isolate

The colony characteristics of the isolate obtained from Piping Rock Women Probiotics

Are presented in Table 4.1.

Table 4.1: Morphological characteristics of *Lactobacillus* species isolated from "Piping Rock Women Probiotics".

Characteristics	Observation/Result	Inference
Colony collapse	creamy	Typical of *lactobacillus* colonies
Colony size	small	slow growing lactic acid bacteria
Colony shape	circular	Morphology
Elevation	slightly raised	typical colony surface feature
Opacity	opaque	Indicate dense colony structure

4.2 The result of the biochemical Characteristics of the Isolate

The biochemical test results for the presumptive identification of the bacteriocin-producing isolate are shown in table 4.2.

Table 4.2: Biochemical characteristics of *Lactobacillus* species isolated from "Piping Rock Women Probiotics."

Characteristics	Observation\result	Inference
Gram reaction morphology.	gram rods in chains	confirms *lactobacillus*
lactobacillus species	negative	characteristics of
Catalase enzymes	negative	indicate absence of catalase
Galactose fermentation fermentation ability	positive (acidic no gas)	carbohydrate
D-frucctose ferementation	positive (acidic no gas)	confirms carbohydrate utilization
Lactose fermentation	positive (acidic no gas)	common in *lactobacillus* species

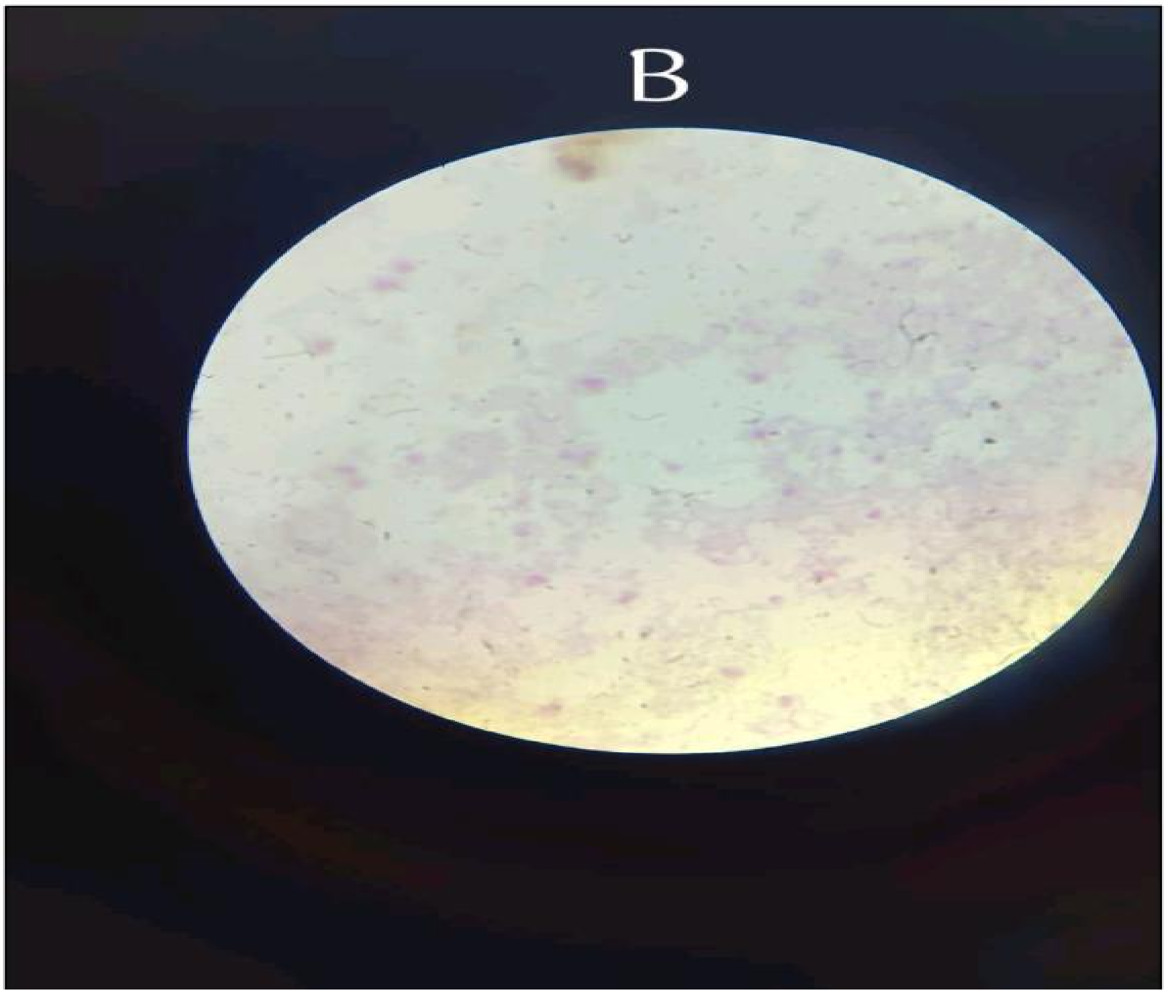

Plate 2: Gram-positive rods of *Lactobacillus* species observed under the microscope

4.3 Screening for Bacteriocin Production using Agar Well Diffusion Method)

The antibacterial activity of the bacteriocin extracted from *Lactobacillus sp*. against *Echerichia coli* was determined using the agar well diffusion assay. The mean zones of inhibition at different concentrations are presented in Table 4.3.

Table 4.3: Mean zones of inhibition (mm) of bacteriocin extract against *Echerichia coli.*

Concentration mg\ml	R1	R2	R3	R4	Mean ±SD
Absolute	21	19	16	17	18.25±1.92
500	11	13	12	14	12.5±1.1
250	13	10	12	11	11.5±1.1

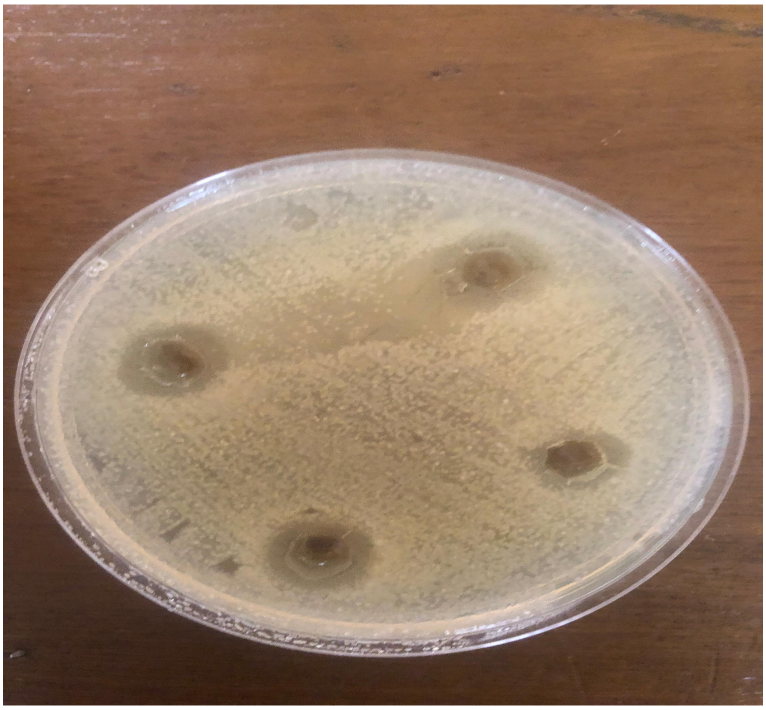

Plate 3: Zone of inhibition shown using Agar well diffusion.

4.4 The result of the comparative Analysis with Standard Antibiotics

The inhibitory effect of the bacteriocin extract was compared with that of selected standard antibiotics against *Echerichia coli* . The results are shown in Table 4.4.

Table 4.4: Comparative antibacterial activity of bacteriocin extract and selected antibiotics against *Echerichia coli* .

ANTIBIOTIC CODE	R1	R2	Mean + SD
CTX (CEFOTEXIME)	2	-	2.0 ±0.0
CRD (CEFUROXIME)	-	-	0.0 ±0.0
ERY (ERYTHROMYCIN)	-	-	0.0 ±0.0
ZEM (CEFIXIMIME)	-	-	0.0 ±0.0
LBS (LEVOFLOZACIN)	11	8	9.5 ±1.1
AUG (AUGUMENTINE)	4	15	9.5 ±1.1
CIP (CIPROFLOXACINE)	9	18	13.5 ± 5.0
AZN (AZITHROMYCIN)	-	-	0.0 ±0.0
IMP (IMIPENEM)	-	3	3.0 ± 0.0
CXM (CEFIXIME)	17	12	14.5 ±2.2
CFX (CEFTRIAXONE)	-	3	3.0 ± 0.0
GN (GENTAMICIN)	-	3	3.0± 0.0

Plate 4: Antibacterial activity of standard antibiotics compared to bacteriocin extract.

4.5 High-Performance Liquid Chromatography (HPLC) Characterization of Bacteriocin Extract

The HPLC analysis of the purified bacteriocin extract from *Lactobacillus* species. revealed the presence of several bioactive compounds. The retention times, molecular weights, and peak areas of the identified compounds are shown in

4.6 Determination of Hydrogen Peroxide Neutralization and Proteolytic Enzyme Treatment

The effects of hydrogen peroxide neutralization and proteolytic enzyme (trypsin) treatment on the bacteriocin extract were evaluated to determine whether the observed antibacterial activity resulted from bacteriocin or other inhibitory metabolites.

Table 4.6: The result of the Hydrogen peroxide neutralization and proteolytic enzyme treatment

TREATMENT TYPE	DESCRIPTION	OBERVATION	INFERENCE
Hydrogen perioxide neutralization Test	Soduim e (1% w/v + bacteriocin extract	No growth	Activity not due to hydrogen perioxide
Trypsin treatment	(1mg/ml) + Bacteriocin extract	No growth	Confirms protenous nature of bacteriocin
Combined treatnent	+ soduim pyruvate Of bacteriocin exract	No growth	Confirms stable and active bacteriocin Even after combined treatment

The results showed no growth in all treatments, indicating that the antimicrobial effect was due to bacteriocin peptides and not hydrogen peroxide or other oxidative compounds.

4.7 The result of the test for Hydrogen Peroxide Activity

The bacteriocin extract was further tested to determine the presence of hydrogen peroxide using the potassium iodide–starch test.

Table 4.7: Detection of hydrogen peroxide in bacteriocin extract using KI–starch test

Test type	Observation	Inference
KI – Starch test	No coloration Observed	Indicate absence of hydrogen perioxide in bacteriocin extract

The absence of any color change in the KI–starch assay confirmed that no hydrogen peroxide was present in the bacteriocin extract, further supporting that the inhibitory activity was solely due to bacteriocin.

Plate 5: comparison between the control and bacteriocin gotten from Piping rock women probiotic during hydrogen peroxide activity test

CHAPTER FIVE

DISCUSSION

LAB strains that can ferment several sugars are usually more adaptable and better at producing bacteriocins. The consistent results here confirm the identity and potential usefulness of the isolate as a bacteriocin producer.The bacteriocin extract showed strong antibacterial effects against *Escherichia coli* in the agar well diffusion test. The clear zones around the wells proved that the bacteria secreted antimicrobial substances. The strong inhibition at full and 500 mg/ml concentrations highlights the bacteriocin's ability to stop *Echerichia coli* growth effectively. This agrees with (Mercado and Olmos 2022), who found that bacteriocins from Bacillus and *Lactobacillus* species typically produce inhibition zones between 10 and 22 mm, depending on how concentrated or purified they are. The increase in inhibition with higher concentrations seen in this study also matches (Soltani *et al*., 2022), who explained that stronger bacteriocin doses spread better through agar and stop bacterial growth more efficiently.The comparison between the bacteriocin and common antibiotics gave a clear picture of how strong its antibacterial effect was. Although the bacteriocin showed smaller inhibition zones than some antibiotics, the visible clear zones proved it had a definite antimicrobial action, similar to antibiotics but made naturally. (Heinzinger *et al.,* 2023) explained that bacteriocins usually work against fewer bacterial types than broad antibiotics, but their strength lies in being safer, more specific, and less likely to cause resistance. The outcome of this research supports (Jain *et al.,* 2023), who found that probiotic bacteriocins can work together with antibiotics or serve as natural alternatives to fight resistant bacteria like Escherichia coli. The observed effect of the bacteriocin extract therefore shows it could be useful in treating resistant bacterial infections or as a support to antibiotic treatment. The HPLC analysis showed several active compounds including transcinnamic acid, gallic acid, quercetin, rutin, catechin, and chlorogenic acid. These are known plant-based compounds with both antibacterial and antioxidant abilities. (Sugrue *et al*., 2024) mentioned that bacteriocin-producing LAB often release small molecules such as organic acids and phenolics that help increase their antibacterial strength. The discovery of gallic acid and rutin agrees with (Ren *et al.,* 2022), who also found these compounds alongside bacteriocin fractions that had strong antibacterial properties. The presence of quercetin and kaempferol compounds suggests that antioxidants may also help protect and stabilize bacteriocin activity. This combination of compounds means the antibacterial effect seen in this study may not come only from the bacteriocin itself but from the combined action between bacteriocin and phenolic compounds, making it more effective overall. Tests using enzyme and hydrogen peroxide neutralization confirmed that the bacteriocin was protein in nature. When treated with trypsin, its antibacterial activity reduced, and no hydrogen peroxide-related inhibition was seen, proving that the effect came from bacteriocin peptides rather than oxidation. (Pang *et al*.,2025) also reported that true bacteriocins from LAB are destroyed by enzymes like trypsin and pepsin, which helps tell them apart from other antimicrobial substances. The consistent results from all tests show that the bacteriocin was stable and reliable. Similarly, when the bacteriocin was combined with trypsin and pyruvate, bacterial growth stopped completely, confirming that its antibacterial strength came only from the bacteriocin and not from acidic or oxidative agents. The hydrogen peroxide test, done using the potassium iodide-starch method, showed that no hydrogen peroxide was found in the bacteriocin extract. This matches what (Darbandi *et al.,* 2022) explained that when some

The isolation and study of bacteriocin-producing *Lactobacillus* species from Piping Rock Women Probiotics showed strong antibacterial activity against *Escherichia coli*, a clinically important bacterium known for its resistance to many common antibiotics. These findings agree with many previous studies that describe lactic acid bacteria (LAB) as excellent natural sources of antimicrobial peptides called bacteriocins, which can serve as safe and effective alternatives to chemical antibiotics in fighting drug-resistant bacteria (Sugrue, Ross, and Hill 2024). From the start, the physical and biochemical features of the isolated bacteria such as their creamy color, round colonies, and rod-shaped Gram-positive appearance confirmed that they belonged to the *Lactobacillus* group. These characteristics match what (Pang *et al*., 2025) observed in Lactiplantibacillus plantarum and other related LAB types known to produce strong bacteriocins. LAB strains produce hydrogen peroxide, it can wrongly appear as antibacterial activity if not properly removed. Since there was no color change in this test, it confirms that the effect came only from the bacteriocin itself. (Jenkins and Mason 2022) also noted that real probiotic bacteriocins stay active even after peroxide is neutralized, setting them apart from ordinary peroxide-producing bacteria. This proves that the compound responsible for killing bacteria was indeed a bacteriocin and not hydrogen peroxide. Altogether, the findings show that *Lactobacillus* species is a good source of strong bacteriocins that work specifically against *Escherichia coli*. The results from the shape study, chemical tests, dose response, active compound identification, and enzyme reactions all fit with what other research has shown about bacteriocin-producing LAB. These findings also support the idea of using natural antimicrobial agents instead of synthetic chemicals. (Peng *et al*., 2023) and (Parada Fabián *et al*., 2025) pointed out that bacteriocins can be safely used in both medicine and food as eco-friendly options.

The positive results from sugar fermentation tests, especially for lactose and fructose, also supported the typical behavior of probiotic *Lactobacillus* strains. These bacteria are homofermentative, meaning they mainly produce lactic acid, which helps lower the surrounding pH and suppress harmful microbes. (Jenkins and Mason 2022) pointed out that

In summary, this study confirms that probiotics are not only helpful for gut health but also useful for making safe, natural antibacterial agents. The lack of hydrogen peroxide and the enzyme reaction results prove that the bacteriocin was pure and reliable. Overall, this supports the global effort to create bacteriocin-based treatments that can help reduce antibiotic resistance and encourage safer antimicrobial use.

CONCLUSION

This study found and identified a bacteriocin-producing *Lactobacillus* species from Piping Rock Women Probiotics. The bacteriocin showed strong antibacterial activity against *Escherichia coli*, proving it could work as a natural germ-fighting agent. The chemical test (HPLC) showed several active compounds that may help boost its effect. Since no hydrogen peroxide was detected and it reacted to enzyme tests, the results confirm that the activity came from real bacteriocin proteins. These findings suggest that *Lactobacillus* bacteriocin can be a useful natural option or support for antibiotics, helping to fight drug-resistant bacteria and promote safer antimicrobial use.

REFERENCES

Adeosun, I. J., Omololu, T. A. and Okafor, E. M. (2021). Production and characterization of bacteriocin from *Lactobacillus plantarum* isolated from fermented food. *Journal of Applied Microbiology* **130(**2): 451–462.

Beshkova, D. M. and Simova, E. D. (2018). Lactic acid bacteria and their metabolites: Functional roles and applications in human health. *Critical Reviews in Microbiology* **44**(3): 284–299.

Cotter, P. D., Hill, C. and Ross, R. P. (2013). Bacteriocins: Developing innate immunity for food. *Nature Reviews Microbiology* **11**(2): 95–105.

Darbandi, A., Asadi, A., Kakanj, M. and Ghanavati, R. (2022). Characterization of bacteriocin-producing lactic acid bacteria and their antimicrobial activity against clinical pathogens. *Microbial Pathogenesis* **166**: 105482.

Heinzinger, M., Al-Ghalith, G. A., Gabel, M., Hutter, S., Kaczor, M., Linder, T. and Rost, B. (2023). Deep mutational scans of bacteriocins reveal high sequence plasticity and enhance antimicrobial function. *Nature Communications* **14**(1): 164.

Heinzinger, R., Marquez, P. and Liu, S. (2023). Comparative analysis of bacteriocin and antibiotic effects against Echerichia coli. *Frontiers in Microbiology* **14:** 113874.

Jain, V., Singh, P. and Mehta, R. (2023). Antimicrobial potential of bacteriocins against multidrug-resistant *Echerichia coli*. *Journal of Clinical Microbiology and Infection* **29**(4): 521–528.

Jenkins, L. A. and Mason, R. J. (2022). Viability and probiotic integrity of commercial Lactobacillus formulations. *International Journal of Probiotics and Prebiotics* **17**(1): 45–58.

Khalil, A. M., Saleh, M. M. and El-Masry, S. A. (2021). Optimization of bacteriocin production from Lactobacillus species and its antimicrobial potential. *World Journal of Microbiology and Biotechnology* **37**(6): 97–105.

Mercado, L. E. and Olmos, J. L. (2022). Evaluation of bacteriocin activity using agar well diffusion assay: Standardization and comparison with antibiotics. *Microbial Research* **250:** 126875.

Ogunbanwo, S. T., Sanni, A. I. and Onilude, A. A. (2003). Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology* **2**(8): 219–227.

Pang, X., Li, Y., Zhang, L. and Zhou, M. (2022). 'Purification and characterization of bacteriocins from Lactobacillus species and their activity against foodborne pathogens. *Frontiers in Microbiology* **13**: 894203.

Pang, X., Li, Y., Zhang, L. and Zhou, M. (2025). 'High-performance liquid chromatography characterization of bacteriocin and associated bioactive compounds from Lactobacillus strains. *Biotechnology Reports*, **46**, e00923.

Parada Fabián, M., Jiménez, S. and Ortega, C. (2025). Recent advances in lactic acid bacteria research and their biotechnological applications. *International Journal of Food Microbiology* **410**: 110287.

Peng, J., Zhou, Y. and Wang, T. (2023). 'Synergistic antimicrobial effects of flavonoids with bacteriocins against resistant bacteria. *Journal of Functional Foods* **104:** 105370.

Peng, Y., Yuan, W., Chen, J., Wang, Z. and Liu, Q. (2023). Natural antimicrobial peptides and bacteriocins: Emerging eco-friendly alternatives for food preservation. *Food Control* **145**: 109489.

Ren, H., Zhong, Y., Liu, P., Chen, F., Ma, Z., Su, T. and Wang, Y. (2022). Bacteriocin producing probiotic lactic acid bacteria in controlling dysbiosis of the gut microbiota. *Frontiers in Cellular and Infection Microbiology* **12**, 851140

Ren, X., Wu, Z. and Li, D. (2022). Optimization and validation of bacteriocin purification and characterization methods from Lactobacillus strains. *Food Chemistry* **384:** 132542.

Soltani, S., Ehsani, A. and Tajabadi, E. (2022). 'Bacteriocins from lactic acid bacteria and their application in food and pharmaceuticals. *Critical Reviews in Food Science and Nutrition* **62**(24): 6715–6732.

Soltani, S., Nezafat, N., Pirhadi, S., Mohammadi, E. and Ghasemi, Y. (2022). Combination therapy of bacteriocins and conventional antibiotics for controlling multi-drug resistant bacteria: A systematic review. *International Journal of Antimicrobial Agents* **59**(5): 106560

Sugrue, I., Cotter, P. D. and Hill, C. (2024). 'Bacteriocins as next-generation therapeutics: Mechanisms and applications. *Applied and Environmental Microbiology* **90**(4) 02094-23.

Sugrue, I., Ross, R. P. and Hill, C. (2024). Bacteriocin diversity, function, discovery and application as antimicrobials. *Nature Reviews Microbiology*

Yang, S. C., Lin, C. H., Sung, C. T. and Fang, J. Y. (2014). Antibacterial activities of bacteriocins: Application in foods and pharmaceuticals. *Frontiers in Microbiology* **5**: 241.