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Development and Evaluation of a Probiotic-Derived Bioactive Biocompatible Gel Matrix for Topical Applications

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A B S T R A C T

Skin infections have always been a cause of concern for many years now, especially, infections caused by wounds. The current strategies largely involve use of antibiotics which add on to the current problem of antimicrobial resistance. Therefore, using biocompatible materials and bioactive molecules of probiotics can be used to treat the skin infections and to ensure skin care. This study explores the potential of bacteriocin-like substances extracted from probiotic strains namely, *Lactobacillus rhamnosus* LGG and *Lactobacillus plantarum* and isolate (IS1) obtained from commercial Kefir starter culture powder. The preliminary identification of the IS 1 revealed to belong to Lactobacillus strain (*Lactobacillus ferintoshensis*). Further, antioxidant potential of the three probiotic strains revealed that *Lactobacillus rhamnosus* possess the most antioxidant potential followed by *Lactobacillus plantarum.* Bacteriocins extracted from these strains demonstrated antimicrobial and antibiofilm activity against E. coli, S. aureus, and P. aeruginosa. These bacteriocins were successfully incorporated into a biocompatible pectin–chitosan hydrogel matrix formulated using pectin extracted from dried orange peels. The developed gel showed antimicrobial activity, indicating effective diffusion of bacteriocins from the matrix

Introduction

Skin has always been one of the most important part of humans. Skin accounts for about 15 percent of the total body weight of adults, with an average surface area of 1. 5–2 m^2. One of the main functions of the skin is its use as a mechanical barrier to disease-causing microorganisms and harmful substances; in fact, it could be viewed as one of the host's vital defenses against infections, as well as the innate and adaptive immune system. It is often hard to know whether skin issues, including skin pigmentation, skin wrinkles, skin aging, and skin dehydration, occur due to external elements or internal changes. Researchers have been experimenting a lot of synthetic and herbal products in order to cure the skin issues. Another major problem associated with skin the burn wounds, that cause major harm to the structure of the skin. In cases of wounds, certain skin diseases the

indigenous skin microbiome alters and one can observe increase in the pathogens leading to multiple problems. The skin microbiome includes bacteria, fungi, viruses, micro-eukaryotes (mites), archaea, and Phages. They can be found not only on the surface of the epidermis, but also in sweat, sebaceous glands and associated hair follicles. The most dominant species are *Staphylococcus epidermidis*, *Cutibacterium acnes* and *Corynebacterium*, which overall are estimated to constitute 45–80% of the skin microbiome. Among the fungi group, *Malassezia spp.* are present on the whole-body surface, predominant in oily sites (face, back); The viruses identified include Papplilpmaviridae, Pylomaviridae and Circoviridae families.

Skin microbiome alterations were found in the background of numerous dermatological diseases, including acne, atopic dermatitis (AD) and psoriasis, among others. Species of Staphylococcus bacteria are known to cause the most of the infectious diseases. *Pseudomonas aeruginosa* is another bacterium that causes skin infections especially diseases like folliculitis, dermatitis and burn wound infections. The administration of antibiotics to treat these infections is one of the most commonly and widely used method but long-term administration has now resulted in most of the bacteria being resistant to these drugs. Researchers tirelessly working on the problem have emphasised the use of Probiotics and Prebiotics to mitigate skin problems.

Probiotics are live microorganisms that provide health benefits when consumed in adequate amounts. They are commonly found in fermented foods such as yogurt, kefir, kimchi, sauerkraut, bread, wine, and traditional fermented soybean products like doenjang. Probiotics are mainly known for improving gut health, supporting digestion, and helping prevent certain diseases. Their antimicrobial activity is largely due to the production of postbiotics such as organic acids (lactic acid and acetic acid), bacteriocins, and hydrogen peroxide, which inhibit the growth of harmful microorganisms. Probiotics have shown effectiveness against several pathogenic bacteria, including *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Candida albicans*. They have also been used/1in managing burn wound infections and various intestinal disorders such as gastroenteritis, irritable bowel syndrome (IBS), Crohn's disease, peptic ulcers, and even colon cancer. *(*Arshad, T., *et al.,* 2024)

In addition to gut health, probiotics play an important role in skin health. Atopic dermatitis (eczema) is an inflammatory skin condition associated with asthma, allergic rhinitis, and food allergies. It is characterized by impaired skin barrier function, increased transepidermal water loss (TEWL), dry skin, itching, and colonization by pathogens like *Staphylococcus aureus*. Studies have shown that supplementation with strains such as *Lactobacillus rhamnosus* and *Lactobacillus reuteri* can significantly reduce eczema severity in children. probiotics support wound healing. Wounds, which may involve torn skin or tissue damage, heal through stages including hemostasis, inflammation, and tissue proliferation. During healing, growth factors stimulate fibroblast activity and collagen production to repair the skin. Probiotics can help prevent infection in wounds and support the natural healing process by maintaining healthy skin microbiota and controlling harmful pathogens. *(*Arshad, T., *et al.,* 2024)

Probiotics are also widely used for the postbiotics they produce. Along with lactic acid, H_2O2, bacteriocin and bacteriocin like substances are also produced that are used for their antibacterial properties. Bacteriocins are small antimicrobial peptides made up of about 20–60 amino acids. They are usually positively charged (cationic) and partly hydrophobic in nature. Unlike antibiotics that are chemically synthesized, bacteriocins are produced naturally by bacteria through the ribosomal machinery. Bacteriocins are generally inducible, meaning their production is triggered under specific conditions. Induction often requires the secretion and accumulation of signaling peptides outside the bacterial cell. Environmental factors such as high cell density, nutrient availability, the presence of acetic acid, and competence-stimulating peptides can enhance bacteriocin production.

Bacteriocins are reported to be significantly more potent than many conventional antibiotics. To prevent self-damage, bacteriocin-producing bacteria synthesize specific immunity proteins that protect them by neutralizing the bacteriocin or blocking its binding to target receptors. An important advantage of bacteriocins is their effectiveness against pathogenic and opportunistic bacteria, including multidrug-resistant strains. They do not differentiate between antibiotic-resistant and sensitive bacteria. (Hernández-González. *et al.,* 2021). Bacteriocins mainly act by binding to specific receptors on the bacterial cell membrane, leading to membrane disruption and pore formation. These pores

increase membrane permeability, causing leakage of cellular contents and ultimately bacterial cell death. Class II bacteriocins primarily form pores in the cytoplasmic membrane, while Class I bacteriocins (such as lanthipeptides) increase membrane permeability through electrostatic interactions with membrane phospholipids. In addition to membrane disruption, some bacteriocins interfere with gene expression and protein synthesis. They can inhibit DNA and RNA replication, block transcription, and interfere with enzymes involved in protein production. These actions disrupt essential cellular processes and lead to bacterial death. Bacteriocins act differently against Gram-positive and Gram-negative bacteria. In Gram-positive bacteria, they form membrane pores and inhibit peptidoglycan synthesis. In Gram-negative bacteria, they disrupt both outer and inner membranes and may also interfere with protein synthesis. Compared to usual antibiotics, lactic acid bacteria (LAB) bacteriocins are less likely to induce resistance. However, resistance can still occur through membrane modifications or efflux pump activation. Combining bacteriocins with other antimicrobial agents may help reduce resistance development. (Chen, X. *et al.,* 2025)

Kefir is a traditional fermented milk beverage that originated in the Caucasus Mountains between the Black and Caspian Seas. The word "kefir" is derived from the Turkish word *keif*, meaning "good feeling, " showing its long-standing reputation as a health-promoting drink. Kefir is produced by fermenting milk using kefir grains, which are a symbiotic consortium of bacteria and yeasts embedded in a polysaccharide matrix. This complex microbial community makes kefir one of the most diverse natural probiotic sources. Unlike many commercial probiotic products that contain only a few bacterial strains, kefir contains a complex mixture of lactic acid bacteria (LAB), acetic acid bacteria, and yeasts. Kefir also usually contains *Lactiplantibacillus plantarum* and other Lactobacillus spp., along with beneficial yeasts. These microorganisms coexist in a stable symbiotic relationship and collectively contribute to Production of lactic acid and acetic acid, Formation of bacteriocins, generation of hydrogen peroxide, synthesis of exopolysaccharides such as kefiran. Because of this diverse microbial composition, kefir functions as a natural probiotic consortium, capable of modulating gut microbiota and enhancing host immunity. Kefir shows strong broad-spectrum antimicrobial activity mainly due to the production of organic acids (such as lactic and acetic acid), bacteriocins, hydrogen peroxide, and carbon dioxide. Bacteriocins produced by kefir-associated strains, particularly *Lactiplantibacillus plantarum*, are capable of inhibiting several pathogenic microorganisms including *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella* spp., and *Listeria monocytogenes*. (John, S. *et al.,* 2015)

In addition to its antimicrobial effects, kefir exhibits significant antioxidant activity by reducing oxidative stress and lipid peroxidation, mainly due to bioactive peptides, kefiran (an exopolysaccharide), vitamins, amino acids, and enzymatic actions of probiotic strains. Kefir also has immunomodulatory and anti-inflammatory properties, as it enhances IgA production, stimulates immune cells such as macrophages and T-cells, and reduces pro-inflammatory cytokines. Studies further suggest that kefir may possess anti-carcinogenic potential by stimulating immune-mediated tumor suppression. Moreover, kefir-associated Lactobacillus species can help lower cholesterol through bile salt hydrolase activity, improving lipid metabolism. It also improves lactose digestion due to β-galactosidase-producing microorganisms and has demonstrated wound healing properties through its antimicrobial, anti-inflammatory, and tissue-regenerating effects. (John, S, *et al.,* 2015)

The topical application of probiotics to the skin is crucial, as it is essential to ensure that the bacterial strains remain viable and effective against pathogens. This can be achieved by encapsulating the probiotics, prebiotics, certain organic acids, bacteriocins within hydrogels, gel matrix, films, capsules or beads made by natural polymers. These are usually referred to as biomaterials, usually made from biopolymers (alginate, pectin, chitosan), they are not harmful to humans and at the same time are environment friendly. Hydrogels have grown in popularity for their properties such as biocompatibility, biodegradability, and exclusive "soft-wet" nature in correlation to biological tissue.. Hydrogels have a high-water content, which could swell and adsorb liquid due to their porous nature, and an injectable hydrogel is highly efficient for clinical use. Biopolymers are composed of monomeric units covalently attached to form bigger biomolecules. Pectin is among the most widely preferred biopolymer, usually pectic substances are differentiated into four different types protopectin, pectic acid, pectinic acid, and Pec. (Han, S, *et al.,* 2022), (Benassi L, *et al.,* 2021) Pectin and chitosan are widely used biopolymers in the development of hydrogel-based delivery systems for

food and pharmaceutical applications. Pectin, a natural polysaccharide, readily forms hydrogels through ionotropic gelation with calcium ions and is valued for its biocompatibility, film-forming ability, stability, and suitability for encapsulating probiotics and bioactive compounds. Chitosan, a cationic biopolymer derived from chitin, possesses excellent biocompatibility, biodegradability, mucoadhesiveness, and film-forming properties. Its positively charged amino groups enable electrostatic interaction with negatively charged polymers such as pectin, leading to the formation of composite matrices or core–shell structures with improved mechanical strength and controlled swelling behavior. Importantly, chitosan exhibits inherent antimicrobial activity against a broad spectrum of bacteria and fungi, primarily due to its interaction with negatively charged microbial cell membranes, resulting in membrane disruption and leakage of intracellular components. It also demonstrates hemostatic, wound-healing, and barrier-enhancing properties. The combination of pectin and chitosan therefore offers a synergistic system with enhanced structural integrity, protection against premature release under gastric conditions, and added antimicrobial functionality, making it a promising platform for targeted and controlled delivery applications. (Benassi L, *et al.,* 2021)

The following study explores idea of incorporating the bacteriocins derived from two standard *Lactobacillus* cultures (*Lactobacillus. rhamnosus* and *Lactobacillus. plantarum*), along with an isolate obtained from a commercial kefir starter culture, into a gel matrix intended for topical application, in order to evaluate its antimicrobial efficacy against skin pathogens.

Materials and Methods

Isolation of Probiotic strains from Kefir

The Kefir starter culture powder was enriched in milk and was incubated for 24 hours under anaerobic conditions. /1Isolation was performed on de Man, Rogosa and Sharpe (MRS) agar (Proteose peptone - 10. 000, HM Peptone B # - 10. 000, Yeast extract - 5. 000, Dextrose [Glucose] - 20. 000, Tween 80 [Polysorbate 80] - 1. 000, Ammonium citrate -2. 000, Sodium acetate - 5. 000, Magnesium sulphate - 0. 100, Manganese sulphate - 0. 050, Dipotassium hydrogen phosphate - 2. 000, Final pH [at 25°C] - 6. 5±0. 2) plate. Isolated colonies with similar morphology were chosen and purified again on MRS agar plate.

Primary Identification

The colony characteristics were noted and further Gram Staining and Biochemical tests were performed. (Ahirwar, S., *et al.,* 2017)

Catalase Test

The pure isolated colony was exposed to 3% H_2O_2 and was checked for immediate release of effervescence (Ahirwar, S., *et al.,* 2017)

Gas Production from glucose

CO_2 production from glucose was assessed using MRS broth with broth with inverted Durham tubes inoculated with the isolate. Incubation was done at RT in anaerobic conditions. (Ahirwar, S., *et al.,* 2017)

Sugar Fermentation

Andrade's indicator broth base was used to test the fermentation of various sugars namely, cellobiose and xylose, maltose, mannitol. Inverted durham's tube were used to detect gas production.

Hemolytic analysis of the Isolate

The obtained isolate was streaked on Blood agar plate and incubated at 37° C for 24 hours. The isolate was checked for α, β and γ hemolysis. (Halder D, *et al.,* 2017)

Total Antioxidant Activity of Probiotic

Total antioxidant capacity of the cell free supernatant (CFS) was determined using phosphomolybdenum method. This method is based on the reduction of Mo (VI) to Mo(V) by the antioxidants present in the extracts, resulting in the formation of a green phosphate/Mo(V) complex at acidic pH, which can be measured spectrophotometrically. The standard stock solution of ascorbic acid was made ranging from 200 mcg/ml to 1000mcg/ml with intervals of 200 mcg/ml.. Briefly, 0. 3 mL of CFS was mixed with 3 mL of phosphomolybdenum reagent (0. 6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Uninoculated MRS broth was used as a

control. The mixture was heated at 95°C until the colour develops and then cooled to room temperature. Absorbance was measured at 695 nm against a reagent blank. Results were expressed as ascorbic acid equivalents (AAE)

Extraction of Bacteriocin (partially purified)

The probiotic cultures were inoculated into 50 mL of MRS broth and incubated for 24 hours. After incubation, the broth culture (50 mL) was transferred into a separating funnel, and an equal volume (50 mL) of ethyl acetate was added.

The mixture was shaken vigorously for 10 minutes and then allowed to stand undisturbed until clear separation of the organic and aqueous layers occurred. The upper organic layer along with the precipitate, which contained the bacteriocin, was carefully collected for further analysis. (Hassan, M. *et al.,* 2020)

Protein Estimation of the Extracted Bacteriocin

Protein estimation was done using Folin-Lowry method. Protein concentration was determined using the Folin–Lowry method. The assay is based on the reaction of proteins with alkaline copper reagent, followed by reduction of Folin–Ciocalteu reagent, resulting in a blue-colored complex measurable at 660 nm. /1Briefly, 1 mL of the bacteriocin sample was mixed with alkaline copper reagent (prepared by mixing sodium carbonate in alkaline solution with copper sulfate and sodium potassium tartrate) and incubated at room temperature for 10 minutes. Following incubation, diluted Folin-Ciocalteu phenol reagent was added rapidly and mixed thoroughly. The reaction mixture was then incubated in the dark at room temperature for 30 minutes for colour development. The absorbance was measured at 660 nm using a UV - Visible spectrophotometer. Bovine Serum Albumin (BSA) was used as the standard in the range of 40-200 mcg/ml with an interval of 40mg/ml, and protein concentration of the bacteriocin extract was determined from the standard calibration curve (Lowry *et al.,* 1951).

Antibacterial activity of Bacteriocin

The antibacterial activity of the extracted Bacteriocin was determined by Agar well diffusion and Minimum inhibitory concentration.

Agar well Diffusion method

A 24-hour old culture of the test organisms (*E. coli*, *S. aureus* and *P. aeruginosa*) were adjusted to 0. 5 McFarland standard. 1 ml of the standardized culture was mixed with 15mL of molten Mueller–Hinton agar and poured into sterile Petri plates. After solidification, 8mm wells were made using a sterile cork borer. 50 µl of the bacteriocin was added into each well, respectively. The plates were incubated at 37 °C for 18–24 hours, and the zones of inhibition were measured in mm. (Attwani. *et al.,* 2014) (Khalfallah, G, *et al*., 2021)

Minimum Inhibitory Concentration

The MIC was determined by the broth dilution method. Serial two-fold dilutions of the bacteriocin were prepared in sterile Nutrient broth, and 0. 5 Mc Farland bacterial suspension of the test pathogens was added to each well. Growth and sterility controls were included. After incubation at 37 °C for 18–24 hours, the lowest concentration showing no visible growth was recorded as the MIC. (Hassan, M., *et al.,* 2011)

Biofilm Inhibition Assay by Crystal Violet method

The biofilm inhibition capacity of the extracted partially purified bacteriocin was determined using crystal violet method against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Sterile Nutrient Broth (NB) was used for biofilm formation. All procedures were carried out under aseptic conditions. The suspension of potential pathogens was inoculated into NB broth and incubated at 37 °C for 24 hours. The culture suspensions were adjusted to 0. 5 McFarland turbidity standard to obtain a standardized inoculum. A total volume of 200 µL of the adjusted microbial suspension was dispensed into sterile polystyrene tubes instead of microtiter plates. The bacteriocin was added to the tube, while untreated tubes served as growth control. The tubes were incubated at 37 °C for 24 hours to allow biofilm formation on the inner surface of the tubes. After incubation, the contents were gently removed, and the tubes were washed three times with sterile distilled water to remove non-adherent cells. The tubes were then air-dried at room temperature. The attached biofilms formed on the inner walls of the polystyrene tubes were stained with 0. 4% (w/v) crystal violet solution and allowed to stand for 15 minutes at room temperature. Excess stain

was removed by washing three times with distilled water. After complete drying, the bound crystal violet was solubilized using absolute ethanol. The optical density (OD) of the solubilized stain was measured at 570 nm using a UV spectrophotometer. The percentage of biofilm inhibition was calculated relative to the untreated control using the following formula: $\frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$

Extraction of Pectin from Citrus Fruit Peel (orange) and its Characterization

Citric acid solution of pH 2. 5 was prepared. 5g/1of powdered peel was heated with the citric acid solution for 30 minutes at 65 °C with continuous stirring. The mixture was then cooled to room temperature and filtered through muslin cloth. The filtrate was treated with double its volume of ethanol and allowed to precipitate. A jelly-like precipitate formed, representing pectin. The precipitate was washed twice with ethanol and then air dried.

Characterisation of Pectin

Degree of Esterification

0. 2 g of dried pectin was first moistened with ethanol and then dissolved in 20 mL of distilled water. Three drops of phenolphthalein were added to the solution, and it was titrated with 0. 1N NaOH until a faint pink colour appeared. The initial titration volume was used to calculate the number of free carboxyl groups. To neutralize the polygalacturonic acid, 10 mL of 0. 1 N sodium hydroxide was added. The sample was sealed with a stopper, shaken vigorously, and left to stand at room temperature for 2 hours to allow de-esterification.

After this, 10 mL of 0. 1 N hydrochloric acid was added to neutralize the excess sodium hydroxide, and the solution was shaken until the pink colour disappeared. Three drops of phenolphthalein were again added, and the sample was titrated with 0. 1 N sodium hydroxide until a faint pink colour reappeared. The final titration volume was used to calculate the number of esterified carboxyl groups. (Hamed, R. *et al.,* 2025), The degree of esterification (DE %) was calculated using the following formula:

$$\text{DE \%} = \frac{final\ volume\ (ml)}{initial\ volume + final\ volume\ (ml)} \times 100$$

Equivalent weight Determination

5g of pectin was accurately weighed and moistened with 5 mL of ethanol. Subsequently, 100 mL of distilled water was added along with a few drops of phenol red indicator. The solution was then titrated slowly with 0. 1 M NaOH to avoid possible de-esterification of the pectin. /1Formula used.

$$\frac{weight\ of\ sample\ (g) \times 1000}{volume\ of\ NaOH\ (ml) \times Molarity of\ NaOH}$$

Chitosan-Pectin Biocompatible Gel Matrix

A 2% (w/v) chitosan solution was prepared by dissolving chitosan in 1% (v/v) acetic acid under gentle stirring until complete solubilization. Separately, 1% (w/v) pectin was dissolved in sterile distilled water with constant stirring and mild heating at approximately 60°C to ensure complete dissolution. The chitosan and pectin solutions were then mixed in equal proportions under continuous stirring to obtain a homogeneous polymeric blend. Subsequently, 5 mL of undiluted bacteriocin (10% v/v of the total formulation) was incorporated into the mixture and stirred uniformly. Calcium chloride (1% w/v) was added as a cross-linking agent to enhance matrix stabilization, while glycerol (1% w/v) was incorporated as a plasticizer to improve flexibility of the formed film. The final solution was cast onto sterile Petri plates and allowed to undergo cross-linking at room temperature until a semi-solid, gel-like matrix was obtained. (Dafe, A, *et al.,* 2017) (Alamineh, E, *et al.,* 2019),

Characterization of the biocompatible gel matrix

Swelling ratio determination

The samples were be immersed in the distilled and the weight was determined for 2hrs, 4 hrs and 6 hrs. Excess surface water wad carefully removed off using filter paper. The swelling rate is will be calculated using the following formula. Swelling rate/1% = $\frac{Wa - Wb}{Wb} \times 100$. The Wa represents the weight of the gel/1after immersion in water and Wb represents the initial dry weight.

Determination of pH of the Gel

The pH of the prepared gel formulation was determined to assess its compatibility and suitability for topical application on the skin. (Hamed, R. *et al.,* 2025

Physical appearance of the gel matrix.

Attributes such as colour change, homogeneity and cloudiness, were evaluated visually. The/1analysis was done at 4 °C and room temperature. /1(Hamed, R. *et al.,* 2025)

Antimicrobial Activity of the gel

A 24-hour-old culture of the test organisms (*E. coli*, *S. aureus*, and *P. aeruginosa*) was adjusted to 0. 5 McFarland turbidity standard. Sterile cotton swabs were used to uniformly inoculate the standardized bacterial suspensions onto sterile Nutrient Agar plates. Approximately 0. 1g-0. 2 g of the test sample was aseptically placed onto the surface of the inoculated agar plates. The plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured and recorded. This enables one to understand if the bacteriocin diffuses from the gel matrix and inhibits the pathogens or not. (Attwani. *et al.,* 2014)

Results and Discussion

Isolation from Kefir Starter culture powder

A pure isolate with pin point, off white in color and with smooth consistency was obtained. The gram nature was found to be gram positive while the morphology resembled that of short rods.

Primary identification

The Biochemical results are/1follows-/1

Based on the obtained biochemical results, the isolate was tentatively identified as belonging to the genus Lactobacillus, with characteristics closely resembling *Lactobacillus ferintoshensis.*

Hemolytic Property of the isolate

Hemolysis is an important characteristic used to determine a bacterium's ability to damage red blood cells (RBCs) and assess its potential pathogenicity. The isolate IS1 was streaked onto Blood agar to evaluate this property. After incubation, no zone of hemolysis was observed around the colonies, indicating γ-hemolysis. This absence of hemolytic activity suggests that the isolate does not possess RBC-lysing ability and can therefore be considered non-hemolytic in nature.

Antioxidant property of Probiotic cultures

The total antioxidant capacity of the probiotic cultures was determined using their cell-free supernatant (CFS). The 24-hour-old cultures were centrifuged at 2500 rpm for 15 minutes to obtain the CFS, and all samples were tested at a dilution of 1:5.

The results indicated that *L. rhamnosus* exhibited the highest antioxidant capacity (1142. 85 µg/ml AAE), followed by *L. plantarum* (238. 09 µg/ml AAE). No detectable antioxidant activity was observed in the isolate (IS1). These findings suggest that among the tested cultures, *L. rhamnosus* possesses the strongest antioxidant potential under given conditions.

Extraction of Bacteriocin-like Compounds from the Probiotic strains

Bacteriocins from *Lactobacillus. rhamnosus* and *Lactobacillus. plantarum* and IS 1 were extracted using ethyl acetate solvent extraction method.

The organic solvent enabled separation of bacteriocin into the upper phase due to its amphiphilic nature, allowing removal of water-soluble impurities and resulting in a partially purified bacteriocin-rich fraction.

Protein Estimation of the Bacteriocin

Bacteriocin or bacteriocin like compounds are generally peptide-based molecules which are proteinaceous in nature and hence protein estimation was carried out and the results indicated that, bacteriocin extracted from *L. plantarum* had the highest protein concentration (101. 2mg%), followed by *L. rhamnosus* (77. 3mg%), while the isolate IS1 exhibited the lowest protein content (53. 3mg%).

Antimicrobial Activity of Bacteriocin

Minimum Inhibitory Concentration of the obtained Bacteriocin

The results indicated that *L. plantarum* exhibited the

strongest inhibitory activity against *P. aeruginosa*, demonstrating effectiveness even at lower dilutions. The bacteriocin from IS 1 showed great potency towards both *E. coli* and *S. aureus* by inhibiting at lower concentrations comparatively.

Table. 2 MIC of Bacteriocin against pathogens.

Pathogen	*L. plantarum*	*L. rhamnosus*	IS1
E. coli	25. 3%	19. 3%	6. 6%
S. aureus	25. 3%	19. 3%	13. 3%
P. aeruginosa	6. 3%	9. 6%	13. 3%

Agar well Diffusion of Bacteriocin

The antimicrobial activity of the extracted bacteriocins from *Lactobacillus. plantarum*, IS1, and *Lactobacillus. rhamnosus* was evaluated against *P. aeruginosa* and *E. coli* using the agar well diffusion assay. Against *P. aeruginosa*, the bacteriocin extracted from L. plantarum exhibited the highest inhibition zone (18. 75 ± 1. 77 mm), followed by *Lactobacillus. rhamnosus* (16. 5 ± 0 mm) and IS1 (14. 5 ± 0. 71 mm). The low standard deviation values indicate good reproducibility of the assay. In the case of *E. coli, Lactobacillus. plantarum* again demonstrated the strongest activity (17. 5 ± 1. 41 mm), followed by *Lactobacillus*. *rhamnosus* (14. 5 ± 2. 83 mm) and IS1 (11 ± 0. 71 mm). These findings suggest that the bacteriocin from *Lactobacillus. plantarum* exhibited comparatively superior antimicrobial efficacy against both tested pathogens. No observable inhibition zones were detected. against *S. aureus*

Antibiofilm Assay

The bacteriocins derived from the probiotic strains demonstrated notable biofilm inhibitory activity against all tested pathogens, although the extent of inhibition varied across strains. Against *E. coli*, the highest biofilm inhibition was observed with IS1 (66%), followed by *Lactobacillus. plantarum* (55. 5%) and *Lactobacillus. rhamnosus* (52. 5%). In the case of *S. aureus*, *Lactobacillus. rhamnosus* exhibited the greatest inhibitory effect (65. 35%), whereas IS1 showed comparatively lower activity (23. 75%). Similarly, for *P. aeruginosa*, *Lactobacillus. plantarum* demonstrated the highest inhibition (64. 7%), followed by *Lactobacillus. rhamnosus* (55. 25%) and IS1 (37. 35%).

Extraction of Pectin from Orange Peel Powder

The extracted pectin appeared brown in colour and was completely air-dried, as preliminary oven drying trials resulted in degradation and loss of structural integrity. Air drying was therefore preferred to preserve its physicochemical properties. The dried material was obtained in the form of flakes, which retained slight stickiness, possibly due to partial drying and the presence of residual sugars and low-molecular-weight polysaccharides. The pectin was stored in a dark environment at room temperature to prevent photodegradation and moisture absorption, thereby maintaining its stability.

Characterization of Pectin

Degree of Esterification (DE)

Upon titration, the initial volume of NaOH was found to be 7. 5ml and the final volume was found to be 56. 5ml. Therefore, the obtained DE is 88. 2 %. The degree of esterification (DE) of the extracted pectin was determined to be 88. 2%. This indicates that the pectin is classified as high methoxyl (HM) pectin, as the DE value exceeds 50%. The high DE suggests that the majority of the galacturonic acid residues are present in esterified form, with only a small proportion of free carboxyl groups available for ionic interactions.

Equivalent weight Determination

Acid – Base titration was done to determine the value and the equivalent weight was found to/1be 297. 6g/mol.

Chitosan-Pectin bacteriocin loaded gel matrix.

The formulated pectin–chitosan biopolymer gel matrix exhibited a semi-solid and homogeneous appearance. The system maintained structural integrity upon handling, indicating successful formation of a three-dimensional polymeric network. The incorporation of calcium chloride contributed to enhanced structural firmness of the matrix, likely due to ionic crosslinking within the pectin component, resulting in improved gel stability. The presence of chitosan further supported network formation and therefore, contributed to the structural integrity of the gel.

Characterization of Gel Matrix

Swelling Ratio

The swelling ratio was determined by immersing it in distilled water and determining its weight at certain time intervals. The weight of the gel was measured at 2nd hour, 4th hour and 6th hour. The weights were measurable up until 4 hours after which the gel started disintegrating slowly likely due to hydrophilic nature of pectin and chitosan and due to extremely high DE of pectin.

Table. 3 Swelling Ratio of Gel Matrix

Gel Type	2 h (%)	4 h (%)	Fold Change
Lactobacillus. rhamnosus	Nil	197	Nil
Lactobacillus plantarum	283. 3	100	0. 35 (reduction)
IS 1	136. 8	323	2. 36 (increase)
Control	275	323	1. 17 (increase)

pH Determination of the Gel Matrix

The pH of the gel matrix is always expected to be in the range of 5- 7 as this pH is more suitable for skin and does not cause any kind of irritation. The pH of the gel matrixes was found to be around 6-6. 5 which is definitely a favorable pH for the skin.

Physical appearance of the gel

The freshly prepared pectin–chitosan biopolymer gel matrix appeared transparent to slightly brownish in color and exhibited a homogeneous appearance without visible cloudiness. Upon incorporation of bacteriocin, the formulation developed a mildly turbid appearance, which may be due to the dispersion of proteinaceous components within the polymer network. During dehydration at room temperature, the gel transitioned into a flexible and elastic film, accompanied by a slight increase in brown coloration. This change may be associated with enhanced polymer–polymer interactions and concentration of matrix components upon water loss. When stored at 4 °C, similar visual characteristics were observed; however, the gel retained its hydrated state and did not undergo drying, indicating temperature-dependent moisture retention. The hydrated formulation exhibited noticeable surface tackiness, likely due to the hydrophilic nature of chitosan and the high degree of esterification of pectin.

Antimicrobial Activity of Gel Matrix

Antimicrobial Activity of the Gel Matrix

The antimicrobial efficacy of hydrogels incorporated with bacteriocins from *L. plantarum* and IS1 was evaluated against *P. aeruginosa*, *S. aureus*, and *E. coli* using the agar well diffusion assay. A blank hydrogel composed of chitosan and pectin without bacteriocin served as the control. Against P. aeruginosa, the *Lactobacillus. plantarum* bacteriocin-loaded gel produced an inhibition zone of 23 ± 1. 41 mm, while IS1 showed 22. 25 ± 6. 72 mm. The control gel exhibited 29. 5 ± 0. 71 mm inhibition. For S. aureus, inhibition zones of 25. 5 ± 0. 71 mm and (17. 5±0mm) were observed for *Lactobacillus. plantarum* and IS1 gels, respectively, whereas the control showed 28 ± 4. 24 mm. Against E. coli, the L. plantarum gel demonstrated the highest inhibition (34 ± 2. 12 mm), followed by the control (32. 5 ± 3. 54 mm) and IS1 (22 ± 0. 71 mm).

The inhibitory activity of the gel containing *Lactobacillus. rhamnosus* was seen but it was but not as clearly as the other samples. The gel's inhibition was extremely contact based determining the diffusion of bacteriocin did not occur properly since the gel was comparatively dehydrated. Statistical analysis showed a significant effect of pathogen on inhibition zones ($p < 0.05$), while the effect of bacteriocin source was marginal and not statistically significant ($p = 0.05$)

Probiotics have always been widely used in the current world to cure various diseases especially gut related issues. But apart from these applications of probiotics and postbiotics for topical applications are largely popular today. Kefir is also one of the most widely used probiotics, and the probiotics that it contains are widely used to treat skin infections. It contains organisms like LAB, *Leuconstoc, Pediococcus, Enterococcus, yeast like Saccharomyces boulardi, S. cerevrsisiae* certain Bifdidobacterium species.

The probiotic isolate (IS1) obtained from kefir starter culture powder showed colony morphology typical of lactic acid bacteria (LAB), Gram staining revealed Gram-positive short rods, which is consistent with members of the genus *Lactobacillus*. The isolate fermented multiple carbohydrates including glucose, mannitol, maltose, galactose, cellobiose, and xylose with acid and gas production. Gas production indicates heterofermentative metabolism, which is characteristic

of certain LAB species. The isolate was catalase negative, further confirming its classification as LAB, since these organisms lack catalase enzyme. Based on the results obtained, it can be said that the isolate might be *L. ferintoshensis*. To further confirm this, 16srRNA sequencing needs to be done in order to confirm the strain and species of the isolate.

Importantly, the isolate exhibited γ-hemolysis (no hemolysis) on blood agar. This is a critical safety parameter, as non-hemolytic behavior suggests that the isolate is non-pathogenic and safe for further application in antimicrobial formulations.

These are similar to results obtained by Li. Y., *et al.,* where in no zone of clearance was seen around the colonies streaked, which suggests that most of the Lactic acid bacteria or probiotic cultures in general possess no hemolytic property and are thus safe for use in various formulation be it for skin or gut.

The antioxidant capacity of the cell-free supernatant (CFS) revealed that *L. rhamnosus* showed the highest antioxidant activity (114. 2 mg%), followed by *L. plantarum* (238. 09mg%), while IS1 showed no detectable antioxidant activity. The high antioxidant activity observed in *L. rhamnosus* may be attributed to the production of bioactive metabolites such as exopolysaccharides, peptides, or organic acids that possess radical scavenging properties. The absence of antioxidant activity in IS1 suggests that although it possesses antimicrobial activity, it may not produce significant extracellular antioxidant compounds under the tested conditions. This variation highlights strain-dependent functional differences among probiotic bacteria. The ROS species generated by various aspects such exposure to pollution, UV rays, smoking, pathogens can cause serious harm to skin as they can breakdown the collagen which causes the skin to age. These ROS species cause DNA damage, lipid damage and also alter the proteins, delay wound healing, prolong infections, increase inflammation, etc. Various studies have shown results, where in the probiotics have shown enormous antioxidant capability. Although the study by Wu. S. *et al.,* contradicts the results obtained here. *L. plantarum* as well as *L. rhamnosus* produce varying results. The ATBS radicle scavenging assay showed that *L. plantarum* has a higher scavenging ability of 61. 26% whereas the DPPH assay reveals 99. 8% which is higher than that of LGG. Therefore, the study suggests that overall *L. plantarum* has a comparatively greater than that of LGG which is different from that obtained in our study which might be due to different strains used, the incubation time, difference of metabolites released and the different parameters assessed, that is the Phosphphomolybdenum assay measures the total antioxidant capacity, whereas the DPPH and ABTS assays measure a specific radical scavenging potential. (Wu. S, *et al.,* 2023)

Protein estimation revealed that bacteriocin extracted from *L. plantarum* showed the highest protein concentration (101. 2mg%), followed by *L. rhamnosus* (77. 3 mg%) and IS1 (53. 3 mg %). Since bacteriocins are proteinaceous antimicrobial compounds, higher protein concentration may indicate greater bacteriocin yield. However, antimicrobial efficacy does not depend solely on total protein content but also on the specific activity of the bacteriocin molecule. The presence of measurable protein concentration in IS1 confirms that it also produces bacteriocin-like inhibitory substances. The highest Protein concentration was found in the bacteriocin extracted from *L. plantarum* and the least in the bacteriocin extracted from IS 1 which may have influenced/1the observed inhibitory effects. The study by Wang Y. *et al.,* reports a concentration of 256mcg/ml of bacteriocin obtained from *L. plantarum* which is comparatively lesser to the obtained bacteriocin concertation in our study, and the observed differences might be due to the growth conditions, different strains used and extraction method use.

The MIC results demonstrated differential antimicrobial activity among the bacteriocins. The bacteriocin derived from IS1 exhibited the highest inhibitory effectiveness against *Escherichia coli* and *Staphylococcus aureus*, requiring only 6. 6% and 13. 3% concentration respectively for inhibition, compared to higher concentrations needed for *L. plantarum* and *L. rhamnosus*. This suggests that IS1 produces bacteriocin with stronger activity against both Gram-negative and Gram-positive organisms. In contrast, against *Pseudomonas aeruginosa*, *L. plantarum* demonstrated the lowest MIC (6. 3%), indicating superior effectiveness against this comparatively resistant opportunistic pathogen, followed by *L. rhamnosus* (9. 6%) and IS1 (13. 3%). Overall, the findings highlight that bacteriocin efficacy is highly strain- and pathogen-dependent, with IS1 showing broader potency against *E. coli* and *S. aureus*, while *L. plantarum* appears more effective against *P. aeruginosa*. A study by Li. Y. *et al.,* reports and MIC value of 25. 6% of bacteriocin from *L.*

plantarum, against *S. aureus* which is in consistent with our results. (Li. Y. *et al.,* 2023)

The agar well diffusion reveals that the bacteriocin from *L. plantarum* demonstrated the highest inhibitory effect against *P. aeruginosa*, producing an inhibition zone of 18. 75 ± 1. 77 mm, followed by *L. rhamnosus* (16. 5 ± 0 mm) and IS1 (14. 5 ± 0. 71 mm). Similarly, against *E. coli*, *L. plantarum* exhibited the strongest antimicrobial activity with an inhibition zone of 17. 5 ± 1. 41 mm, whereas *L. rhamnosus* and IS1 showed comparatively lower zones of 14. 5 ± 2. 83 mm and 11 ± 0. 71 mm, respectively. These results suggest that the bacteriocin derived from *L. plantarum* possesses comparatively better antimicrobial efficacy against the tested Gram-negative pathogens. However, no observable inhibition zones were detected against *Staphylococcus aureus* for any of the tested bacteriocins.

This enhanced activity may be associated to higher bacteriocin production, better stability during ethyl acetate extraction, or intrinsic structural characteristics that enable stronger interaction with bacterial membranes. The relatively consistent inhibition patterns and low standard deviation values suggest that the extraction and assay procedures were reproducible and reliable. Differences observed between *L. rhamnosus*, IS1, and *L. plantarum* may reflect strain-specific differences in bacteriocin type, molecular weight, spectrum of activity, expression levels or ability to diffuse into the agar. The absence of detectable activity against *S. aureus* could be due to several factors.

It may indicate strain-specific resistance mechanisms, limited diffusion of the bacteriocin through agar, or insufficient concentration following partial purification. Additionally, purification via organic solvent extraction may have resulted in partial loss or reduced activity of bacteriocins that are more effective against Gram-positive organisms. Statistical analysis indicated that the differences in inhibition zones among the tested groups were significant effect of pathogen ($p < 0.05$) and no significant effect of bacteriocin source ($p > 0.05$). Similar study done by Nwajiobi, F. *et al.,* reported the antimicrobial activity of bacteriocin from *L. rhamnosus* against *E. coli* to be of 27±1mm, *S. aureus* 28±2mm and against *P. aeruginosa* 31±2mm. This suggests that the yield of bacteriocin, purity of bacteriocin extracted, molecular weight of the bacteriocin effects the way the bacteriocin behaves.

Biofilm inhibition results further supported the antimicrobial findings. Against *E. coli*, the highest biofilm inhibition was observed with IS1 (66%), followed by *Lactobacillus. plantarum* (55. 5%) and *Lactobacillus. rhamnosus* (52. 5%). In the case of *S. aureus*, *Lactobacillus. rhamnosus* exhibited the greatest inhibitory effect (65. 35%), whereas IS1 showed comparatively lower activity (23. 75%). Similarly, for *P. aeruginosa*, *Lactobacillus. plantarum* demonstrated the highest inhibition (64. 7%), followed by *Lactobacillus. rhamnosus* (55. 25%) and IS1 (37. 35%). The study by Carvalho FM. *et al.,* reported that After 24 hours of exposure, *L. plantarum* and *L. rhamnosus* demonstrated substantial biofilm reduction, achieving 70% and 76% inhibition against *E. coli*, and 77% and 63% inhibition against *S. aureus*, respectively whereas Drumond MM. *et al.,* 2023 reported an inhibition by 71. 7% against *P. aeruginosa*. The higher values observed here and our result suggests the antibiofilm potential of the probiotic strains and serves as a proof of concept for the same (Drumond MM. *et al.,* 2023) (Carvalho FM. *et al., 2021)*

The extracted pectin exhibited a degree of esterification (DE) of 88. 2%, classifying it as high methoxyl (HM) pectin. High DE pectin contains a majority of esterified galacturonic acid residues and fewer free carboxyl groups. High methoxy pectin forms gels primarily through hydrophobic interactions and hydrogen bonding, especially in the presence of sugar and acid. The high DE may also influence swelling behavior and moisture retention, as fewer ionic crosslinking sites are available. Most of the studies state that/1the Pectin obtained from orange peels are usually classified as high methoxy pectin, although the degree of esterification might range from 5 -80 % based on the extraction method employed, temperature, pH, etc (Benassi L. *et, al*, 2021)

The pectin–chitosan gel matrix showed successful formation of a semi-solid, homogeneous structure with good structural integrity. The addition of calcium chloride likely contributed to ionic crosslinking within the pectin matrix, enhancing firmness and stability.

Table.1 Results of Biochemical Analysis

Biochemical Test	Observation
Catalase	No effervescence
Mannitol fermentation	Acid + Gas
Glucose fermentation	Acid + Gas
Maltose fermentation	Acid + Gas
Galactose fermentation	Acid + Gas
Cellobiose fermentation	Acid + Gas
Xylose fermentation	Acid + Gas

Figure. 1 IS1 showing γ hemolysis on Blood Agar plate

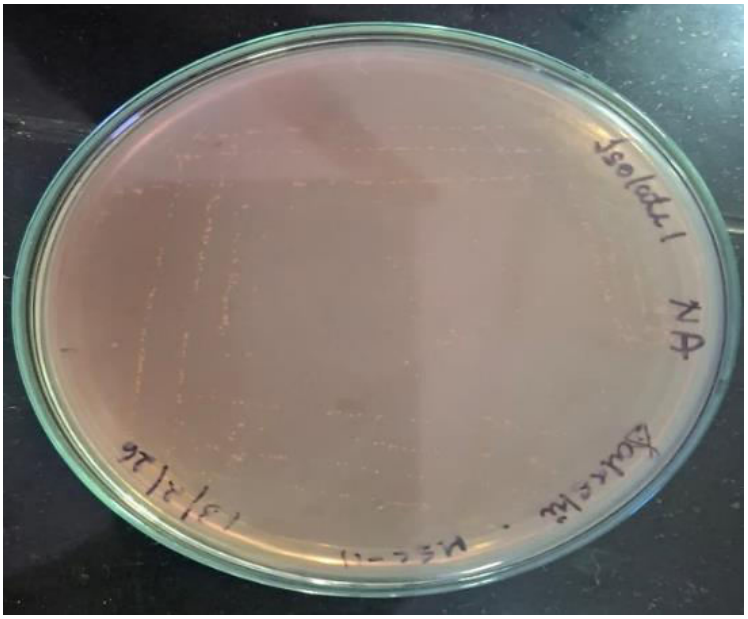
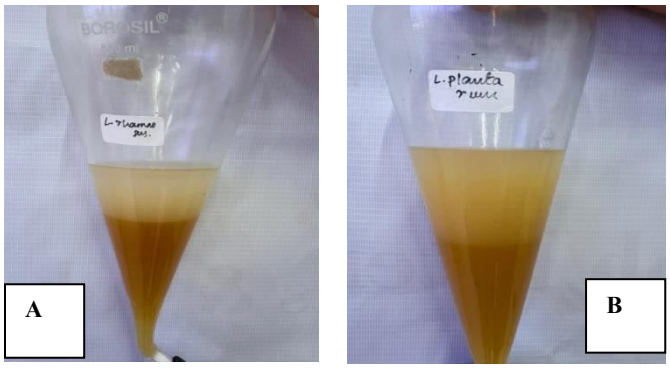
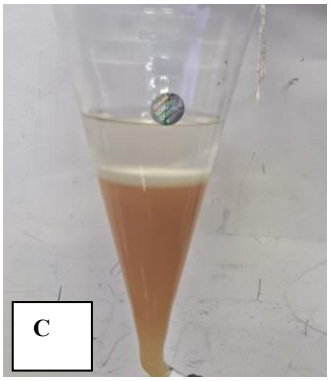
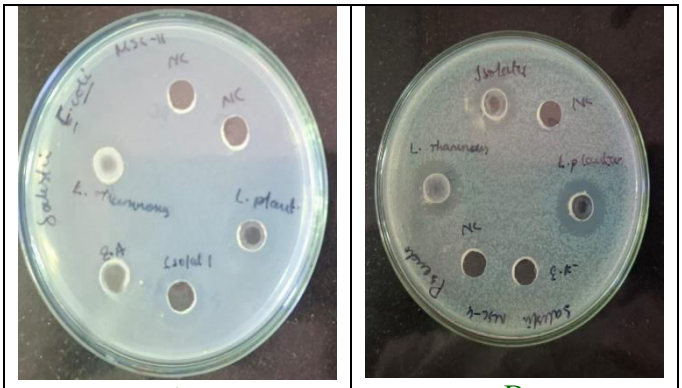


Figure. 2 Partially purified bacteriocins extracted from probiotic cultures using ethyl acetate: (A) *Lactobacillus rhamnosus* (B) *Lactobacillus plantarum* (C) IS1 isolate







A B

Figure. 3 Antibacterial Activity of Bacteriocin against A- *E. coli*, B-*P. aeruginosa*

Figure. 4 Dried Pectin Flakes

Figure. 5 A : gel matrix incorporated with bacteriocin of *Lactobacillus. plantarum*, B: gel matrix incorporated with bacteriocin of IS 1, C : gel matrix without bacteriocin, D : gel matrix incorporated with bacteriocin of *Lactobacillus. rhamnosus*

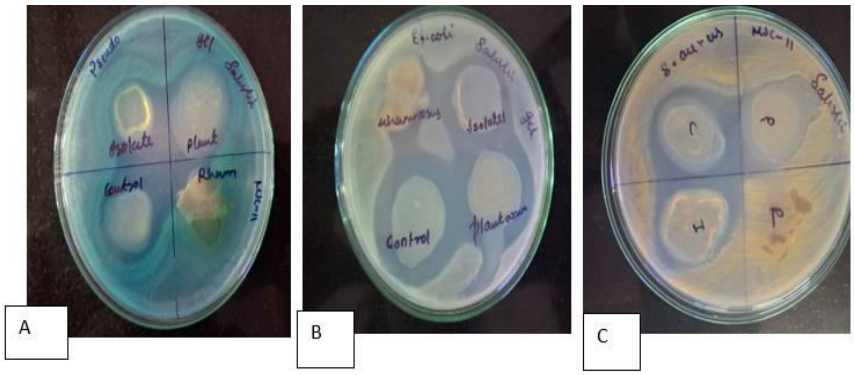
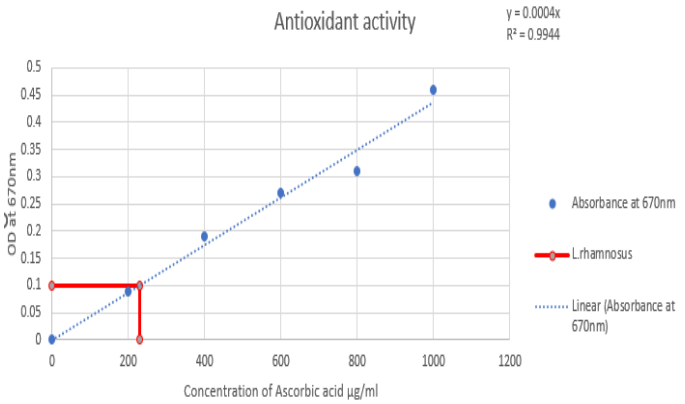
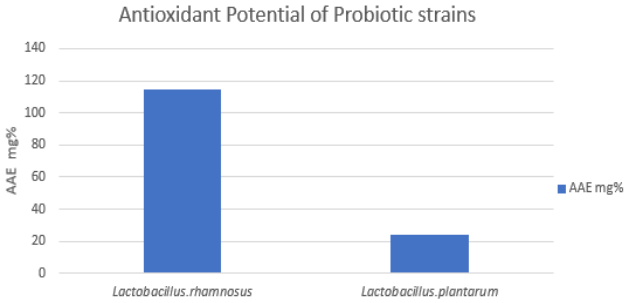


Figure 6 : Antimicrobial Activity of Gel Matrix against A – *P.aeruginosa* , *B- E.coli*, *C- S.aureus*.

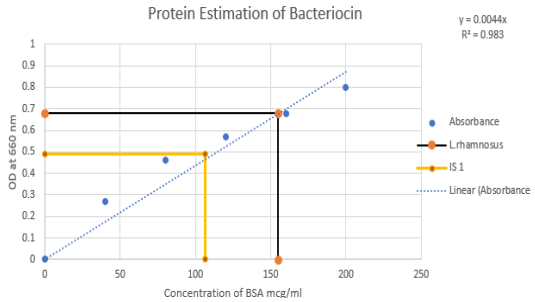
Graph. 1 Standard Ascorbic Acid Curve



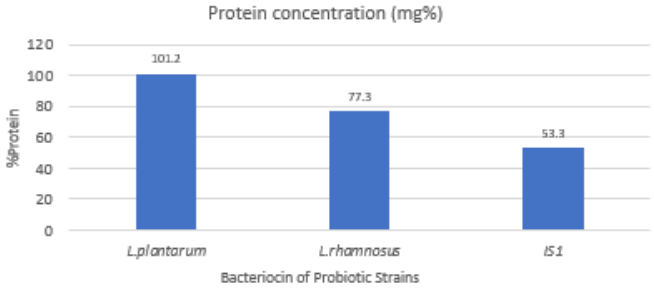
Graph. 2 Antioxidant Capacity of Probiotic Strains



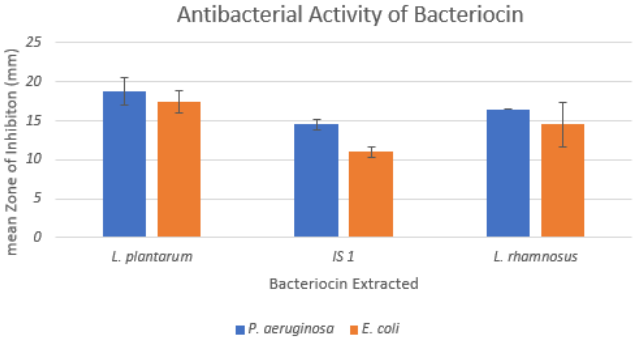
Graph. 3 Standard Curve for Protein Activity



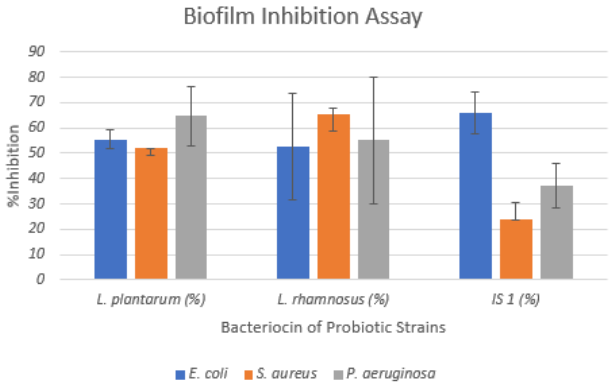
Graph. 4 Estimated Percent Protein



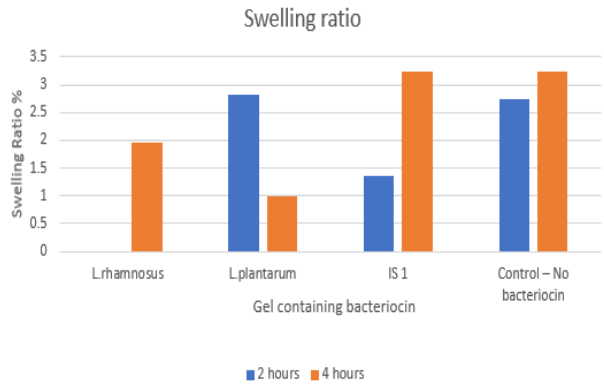
Graph. 5 Antibacterial Activity of Bacteriocin Extracted



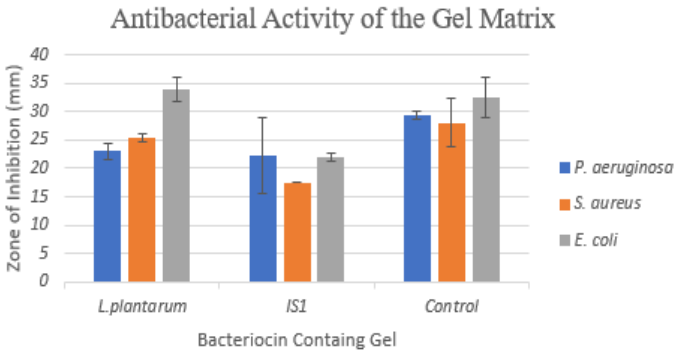
Graph. 6 Biofilm Inhibition by Extracted Bacteriocin



Graph. 7 1Swelling Ratio of Gel Matrix



Graph. 8 Antibacterial Activity of Gel Matrix



Chitosan, being positively charged, can interact electrostatically with negatively charged pectin residues, further strengthening the polymer network. This interaction results in formation of a stable three-dimensional gel matrix capable of encapsulating bacteriocin.

The gel matrix exhibited a pH range of 6–6. 5, which falls within the acceptable range for topical skin applications. Maintaining near-neutral pH is important to prevent skin irritation and maintain compatibility with the natural skin barrier. This pH range also supports stability of bacteriocins, as extreme pH conditions may

lead to protein denaturation. Similar results were seen in the study by Gonsalves A. *et al.,* where in hydrogels for wound healing and topical applications are usually used at pH 5. 4-7. 4 (Gonsalves A. *et al.,* 2021)

The freshly prepared gel appeared transparent to slightly brownish and homogeneous. After bacteriocin incorporation, mild turbidity was observed, likely due to dispersion of protein molecules within the polymer matrix. Upon drying at room temperature, the gel formed a flexible elastic film. This behavior suggests strong polymer–polymer interactions and indicates potential for wound dressing or film-based topical applications. At 4 °C, the gel retained moisture and did not dry, indicating temperature-dependent water retention. Surface tackiness was observed, which may be attributed to the hydrophilic nature of chitosan and the high DE pectin, both of which contribute to moisture affinity and adhesive properties.

All formulations showed swelling ratios greater than 100%, indicating substantial water uptake capacity. This is expected for hydrophilic polymers such as pectin and chitosan. The gel containing *L. plantarum* bacteriocin showed 283. 3% swelling at 2 hours, while IS1 and control gels showed high swelling at 4 hours (323%). High swelling indicates a porous polymer network capable of absorbing moisture, which is beneficial for wound healing applications where exudate management is required. However, gradual disintegration after 4 hours suggests that excessive water uptake may weaken structural integrity, possibly due to the hydrophilic nature of polymers and the high DE of pectin, which limits crosslinking. Thus, the swelling behavior confirms good hydration capacity but indicates that optimization of crosslinking density may further improve long-term stability. The swelling studies are in consistence with Azimi, S. G. *et al.,* who reports swelling % ranging from 160-205% while Puspitasari, D. *et al.,* has reported swelling greater that 200% upto 380% which again aligns with our study where in the gels show high swelling capacity. The swelling of the gel is assumed to ensure effective release of the bioactive molecule and thus is a characteristic quality of biocompatible The antimicrobial assessment of bacteriocin-incorporated hydrogels demonstrated that the inhibitory activity was influenced not only by the incorporated bacteriocin but also by the intrinsic properties of the hydrogel matrix. Statistical analysis (ANOVA, $p < 0.05$; n = 2) confirmed that the differences observed among the treatment groups were statistically significant, indicating that the antimicrobial effects were formulation-dependent. The study by Soltani, S, *et al.,* reported the zone of certain known bacteriocin, microcin and pediocin in hydrogels of chitosan being 13mm, 18mm, whereas the zone of chitosan alone being 15mm, which differs from our study, but serves as a proof of concept for the study (Azimi, S. G. *et al.,* 2025).

The hydrogel containing bacteriocin from *L. plantarum* exhibited comparatively stronger antimicrobial performance than the IS1-loaded gel across the tested pathogens. This enhanced activity may be attributed to higher bacteriocin potency, improved compatibility with the chitosan–pectin matrix, or more effective interaction with bacterial cell membranes. The comparatively lower activity observed with IS1 could reflect reduced bacteriocin concentration, structural differences, or limited release efficiency within the gel system. The blank chitosan–pectin hydrogel also displayed notable antimicrobial activity. This effect can be primarily attributed to chitosan, which possesses inherent antibacterial properties due to its polycationic nature.

Therefore, the observed inhibition in bacteriocin-loaded gels may represent a combined or synergistic effect of chitosan and bacteriocin. The antimicrobial action of the formulated gels containing the bacteriocin of *L. rhamnosus* appeared to work contact-based. The comparatively dehydrated state of the gel likely restricted adequate diffusion of bacteriocin into the surrounding agar medium. As a result, the inhibition zones may not fully represent the total antimicrobial potential of the incorporated bacteriocin, but rather reflect surface interaction between the gel and bacterial lawn. Limited hydration can reduce matrix porosity and slow-release kinetics, thereby affecting diffusion based antimicrobial activity. The results although not entirely, but are consistent with various studies that have proven chitosan and pectin as a good biocompatible matrix capable of delivering the drug, here bacteriocin, efficiently. (Mishra, S, *et al.,* 2025). Overall, the study highlights the antimicrobial, antibiofilm, antioxidant properties of the used probiotic strains *L. rhamnosus* and *L. plantarum* and the obtained isolate IS1. It also indicates that incorporating bacteriocin in the chitosan-pectin based gel matrix shows significant antimicrobial properties provided the hydration is maintained. The pH of the gel matrix remained favourable for topical applications and with gel showing good swelling index further strengthens its use in topical applications.

In conclusion, the study highlights the potential of

probiotic derived bacteriocins for topical applications. The kefir derived IS 1 was identified as a safe Lactobacillus strain. While *Lactobacillus rhamnosus* and *Lactobacillus plantarum* demonstrated notable antioxidant activity, the antioxidant potential of IS 1 could not be detected. emphasizing strain-specific functional variability. All strains produced bacteriocins, with *L. plantarum* showing the highest yield. MIC and inhibition studies revealed pathogen-dependent efficacy, where IS1 was more potent against *E. coli* and *S. aureus*, while *L. plantarum* was more effective against *P. aeruginosa*. Significant antibiofilm activity was also observed across strains. Incorporation of bacteriocins into a pectin–chitosan gel resulted in a stable, biocompatible formulation with suitable pH and high swelling capacity, supporting its potential for topical delivery. The intrinsic antimicrobial properties of chitosan further enhanced efficacy. Overall, the findings support the use of probiotic bacteriocins within biopolymer-based hydrogels as promising candidates for future wound care and skin infection management. Although the developed formulation showed promising results, further studies are required to enhance its practical applicability. Molecular identification of IS1 using 16S rRNA sequencing needs to be performed for precise strain confirmation.

Optimization of gel formulation should be done to improve flexibility, elasticity, and long-term structural stability. Detailed release kinetics studies should be performed to better understand bacteriocin diffusion from the gel matrix. Evaluation of cytotoxicity and biocompatibility of the gel needs to be carried out through in vitro cell line studies.

Performance of wound healing assays such as scratch assay and in vivo models needs to be done to validate therapeutic potential. Stability studies under different storage conditions needs to be checked to determine shelf life. With further optimization and characterization, the bacteriocin-loaded hydrogel system may serve as a safe and effective alternative to conventional antimicrobial treatments.

Author Contributions

Sakshi S. Narasapur: Investigation, formal analysis, writing—original draft. S. V. Raut: Validation, methodology, writing—reviewing.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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