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Characterization of the Antimicrobial Activity of Probiotic Microorganisms Isolated from Dairy and Non-dairy Products

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Abstract

Present study focuses on the antimicrobial activity and biochemical characteristics of probiotic microorganisms isolated from various dairy and non-dairy products. The antimicrobial activity were employed to test the effectiveness of the probiotics against several common food borne pathogens, including *Salmonella enterica* ser. *typhi*, *Listeria monocytogenes*, *Staphylococcus aureus* sub sp. *aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*. The results revealed that probiotics isolated from non-dairy products generally exhibited more pronounced antimicrobial properties compared to those from dairy products. Specifically, non-dairy isolates, particularly those from mango pickles and vinegar, demonstrated larger zones of inhibition and lower Minimum Inhibitory Concentrations (MICs) against the tested pathogens. This indicates that non-dairy probiotics have a stronger ability to inhibit the growth of harmful bacteria. Biochemically, non-dairy probiotics showed diverse carbohydrate fermentation profiles and varying enzyme activities, suggesting a greater metabolic versatility. These characteristics align with previous studies that underscore the enhanced antimicrobial efficacy and potential health benefits of non-dairy probiotics due to their diverse metabolic capabilities. Non-dairy probiotics consistently showed more effective inhibition of pathogens, reinforcing the notion that probiotics from non-dairy sources may offer superior antimicrobial and health-promoting benefits.

Major Findings: The major findings of the article highlight the possibility of non-dairy probiotics as viable alternatives to dairy probiotics, especially for people who are lactose intolerant, allergic to dairy, or follow a vegan diet. Future research could identify the functional contributions of these strains, potentially improving the quality and safety of fermented foods. The antimicrobial properties of these strains are comparable regardless of their origin, whether from dairy or non-dairy sources. The diverse metabolic capabilities and specific bioactive compounds found in non-dairy matrices, such as polyphenols and fibers, likely contribute to these superior properties. These findings align with previous research, which has highlighted both the strong antimicrobial and health-promoting effects of probiotics while also underscoring the unique advantages of non-dairy sources.

Keywords: Antimicrobial Activity, Dairy Products, Lactic Acid Bacteria, Non-dairy Products, Probiotics

1. Introduction

Probiotics, defined as live microorganisms that confer health benefits when consumed in adequate amounts, have gained significant attention from both the medical and food industries. Prominent strains like *Lactobacillus* and *Bifidobacterium* are renowned for their roles in supporting immune function, maintaining gut health, and potentially preventing gastrointestinal diseases[1]. Their increasing popularity is due not only to their health benefits but also to their antibacterial properties, which enhance public health and food safety.

These beneficial microorganisms are primarily bacteria found in fermented foods such as yogurt,

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kefir, sauerkraut, and kimchi, and are also available in supplement form. Common probiotic genera include *Lactobacillus, Bifidobacterium, Streptococcus,* and *Enterococcus*[2,3]. Lactic Acid Bacteria (LAB), Recognized as Generally Regarded as Safe (GRAS), are particularly significant. LAB, including *Lactobacillus*, are widely used in producing starter cultures for various dairy products and are increasingly being explored for their potential to combat food borne pathogens. This exploration includes using LAB as bio-preservatives in the food and dairy sectors and as alternatives to antibiotics in medical treatments[4-6].

With the rising global issue of antibiotic resistance, recent research has focused on the antibacterial properties of probiotics. These probiotics inhibit pathogenic microbes through various mechanisms, including producing antimicrobial compounds such as bacteriocins, natural acids, and hydrogen peroxide, competing for nutrients and adhesion sites, and modulating the host's immune response[7]. Such mechanisms contribute to food safety and enhance the probiotics protective role in the host.

Historically, probiotics were predominantly sourced from dairy products where fermentation naturally produced them. However, with the growing popularity of veganism, lactose intolerance, and plant-based diets, there is an increased interest in non-dairy probiotic sources. Examples of non-dairy sources include fermented vegetables, cereals, and beverages, which provide a variety of probiotic strains with potentially unique antibacterial properties. This diversification is crucial to meet the demands of a wider consumer base and explore new probiotics with additional health benefits[8].

This study aims to evaluate and compare the antibacterial properties of probiotics isolated from both dairy and non-dairy products. By isolating, characterizing, and assessing these bacteria against common food borne pathogens, the research seeks to determine the efficacy of probiotics from different sources. This comparative analysis is essential for understanding how different substrates affect probiotics antibacterial effectiveness and for guiding the selection of probiotic strains for health and food industry applications[9].

1.1 Probiotics in Dairy and Non-Dairy Products

Probiotics have long been a vital component of human diets, primarily sourced from fermented dairy products. Dairy-derived probiotics, such as those from kefir, cheese, and yogurt, have been linked to numerous health benefits, including enhanced immune function, reduced risk of gastrointestinal infections, and improved digestion[10]. In dairy products, *Lactobacillus* and *Bifidobacterium* are commonly used probiotic species. These microorganisms thrive in milk-based environments, fermenting lactose to produce lactic acid. This process creates an acidic environment that inhibits pathogenic microorganisms and supports gut health[11].

In recent years, there has been a notable rise in the popularity of non-dairy probiotic products, driven by the increasing demand for lactose-free and plant-based options. Non-dairy probiotics are found in various foods and beverages, including kombucha, water kefir, fermented vegetables like kimchi and sauerkraut, and plant-based items such as cereals, tempeh, and miso[8]. These products host a variety of probiotic strains, some of which are unique to plant-based matrices. Research by Rezac *et al*., indicates that certain *Lactobacillus* and *Saccharomyces* strains found in fermented vegetables and beverages offer health benefits comparable to those from dairy sources[12].

Non-dairy probiotics contribute to gut microbial diversity, which is essential for overall gut health and preventing dysbiosis-related issues[13]. Additionally, non-dairy matrices often contain bioactive compounds like fiber and polyphenols that work synergistically with probiotics to enhance health benefits. This expanding range of probiotic sources underscores the need for thorough research to evaluate the specific benefits of each strain and explore innovations in functional foods.

1.2 Prior Research on the Antimicrobial Properties of Probiotics

Research has extensively explored the antimicrobial properties of probiotics, demonstrating their ability to reduce the growth of harmful microorganisms and improve food safety. Servin (2004) found that lactic acid bacteria from dairy products produce natural acids and bacteriocins that effectively inhibit common food borne pathogens such as *Salmonella spp.*, *E. coli*, and *L. monocytogenes*. These antimicrobial compounds disrupt pathogen cell membranes, leading to cell death and reduced infection rates, highlighting the value of dairy-derived probiotics for gut health and food safety[14].

Similar antimicrobial activities have been observed in non-dairy probiotics. For example, Zuo *et al.*, investigated the antibacterial properties of *L. Plantarum* strains isolated from fermented vegetables. These strains produced significant amounts of hydrogen peroxide and lactic acid, which effectively inhibited pathogens like *S. aureus* and *Bacillus cereus*. These findings suggest that non-dairy probiotics could serve as natural preservatives in food products while also providing health benefits.

Comparative studies of probiotics from dairy and non-dairy sources have revealed differences in efficacy. Kiousi *et al.*, compared the antibacterial properties of probiotics from both sources against *Listeria monocytogenes* and *E. coli* O157. The results showed that while both types of probiotics possess antimicrobial qualities, some non-dairy probiotics demonstrated superior inhibition rates. This disparity may be due to the unique bioactive compounds present in non-dairy matrices, emphasizing the need for a comprehensive approach in selecting probiotic sources for food safety and health applications[9].

In summary, while both dairy and non-dairy probiotics exhibit significant antibacterial properties, non-dairy sources may offer additional benefits due to their unique bioactive compounds. This highlights the importance of further research to fully understand the potential of different probiotic strains and their applications in enhancing food safety and promoting health.

2. Materials and Methods

2.1 Sample Collection

Samples were collected from a diverse range of sources, including homemade cow curd, homemade vinegar, homemade cheese, homemade mango pickles, buffalo curd, market cheese, sauerkraut, and pineapple juice. These samples were obtained from local areas within the Ayodhya district of U.P., and were carefully collected in sterilized bottles to prevent contamination. Once collected, the samples were suspended in sterilized water to achieve the appropriate dilution. This dilution process ensured that the samples were at a suitable concentration for further analysis. The diluted samples were then spread onto agar medium, which is used to culture microorganisms and assess their growth and activity. This process of suspension and dilution in sterilized water is crucial for accurate testing and characterization of the microorganisms present in the samples. The use of sterilized bottles and water helps maintain the integrity of the samples and prevents any external contamination that could affect the results of the analysis[15].

2.2 Isolation of Probiotic Microorganisms

2.2.1 Cultural Media and Conditions

After homogenizing the samples, they were diluted sequentially in a sterile saline solution. For the isolation of lactic acid bacteria, aliquots of the diluted samples were spread onto de Man, Rogosa, and Sharpe (MRS) agar plates[16]. The plates were incubated under anaerobic conditions for 48 hours at 37°C for bacterial growth and for 72 hours at 30°C for yeast growth. To obtain pure isolates, distinct colonies were selected based on their morphology and then subcultured[17].

2.2.2 Characterization of Isolated Microorganisms

The size, shape, and arrangement of the cells in the pure isolates were examined under a microscope. Gram staining was performed to differentiate between gram-positive and gram-negative bacteria[18].

2.2.3 Morphological Characterization

The morphological characteristics of the pure isolates, including cell size, shape, and arrangement, were observed microscopically. Gram staining was utilized to distinguish between gram-positive and gram-negative bacteria[19].

2.3 Biochemical Tests

A range of biochemical tests was conducted on the isolates to determine their enzymatic activities and metabolic profiles. These tests included enzyme activity assays for protease and amylase, carbohydrate fermentation profiles[20] and tests for catalase and oxidase activities[16].

2.4 Preparation of Probiotic Consortium and Their Probiotic Efficiency

Isolates were cultured in 100 ml erlenmeyer flasks with MRS broth medium at 37°C. Each isolate was combined in equal volumes, creating a 1:1 mixture. Thirteen isolates, designated as S3, S4, S5, MC1, MC2, OJ4, CC1, CC2, BC1, BC2, V1, HMC3, and P1, were sourced from various types of milk and curd. These isolates were mixed to form a consortium, which was then tested against five pathogens obtained from MTCC, Chandigarh, India[21].

2.5 Antimicrobial Activity Assays

2.5.1 Agar Well Diffusion Method

The agar well diffusion method was employed to assess the antibacterial activity of the isolates. Pathogenic bacteria, including *S. enterica*ser. *typhi* (MTCC734), *L. monocytogenes* (MTCC1143), *S. aureus* subsp. *aureus* (MTCC1144), *P. aeruginosa* (MTCC424) and *E. coli* (MTCC390), were grown and spread on Mueller-Hinton agar plates. These bacteria were obtained from Microbial Type Culture Collection and Gene Bank, Chandigarh-160036, India. Wells were created in the agar, and these wells were filled with cell-free supernatants from probiotic cultures. After incubating the plates for 24 hours at 37°C, the zones of inhibition were measured to determine the antibacterial efficacy[22].

2.5.2 Broth Microdilution Method

The broth microdilution technique was employed to determine the Minimum Inhibitory Concentrations (MICs) of the probiotic supernatants. Serial dilutions of the supernatants were added to a 96-well plate, which was then inoculated with the pathogens. After incubating the plates at 37°C for 24 hours, microbial growth was evaluated by measuring the optical density at 600 nm[23].

2.5.3 Pathogens Tested

The study focused on the indicator bacteria *S. enterica* ser. *typhi* (MTCC734), *L. monocytogenes* (MTCC1143), *S. aureus* subsp. *aureus* (MTCC1144), *P. aeruginosa* (MTCC424) and *E. coli* (MTCC390). These pathogens were selected due to their significance in food borne infections and their increasing resistance to conventional antibiotics.

2.6 Studies on Probiotic Metabolites

Lactobacillus spp. produces various metabolites including organic acids (such as lactic acid), ethanol, bioactive peptides, lipoteichoic acids, exopolysaccharides, conjugated linoleic acids, and proteins like bacteriocins, including nisin and pediocin. Bacteriocins are typically low molecular weight, cationic, and hydrophobic peptides consisting of 20-60 amino acid residues. They are produced through microbial fermentation, and their separation is based on their location, such as intracellular, cell-associated, or supernatant. Metabolites in the supernatant can be extracted using centrifugation and filtration, while cell-associated and intracellular compounds are separated and purified following the methods outlined by Mora-Villalobos *et al*[24].

Bifidobacteria, known for their β-glucosidase activity, contribute to the production of compounds with estrogenic or antiestrogenic and antioxidant properties, including daidzein, genistein, and glycitein. *Bifidobacterium* will be cultured under strictly anaerobic conditions at 37°C in RCM broth. Phenolic compounds will be separated using a Reverse-Phase Nova-Pak C18 column [300 × 3.9 mm, 4 µm (Waters, Barcelona, Spain)][25].

Bacteriocins will be characterized as heat-stable, catalase-resistant, and showing maximum activity at a pH range of 5.0 to 6.0. They will be inactivated by proteolytic enzymes and are typically low molecular weight, cationic, and hydrophobic molecules consisting of 20-60 amino acids. Purification will be achieved using reverse phase solid-phase extraction, gel filtration chromatography, and reverse phase-HPLC. For instance, plantaricin produced by *L. plantarum* is a peptide with a molecular weight of 1558.85 Da, determined by MALDI-TOF mass spectrometry, and contains 14 amino acids. HPLC analysis of amino acids and peptides will involve pre-column and post-column reagents such as phenyl isothiocyanate and dansyl chloride[26].

Exopolysaccharides (EPSs) will be extracted from bacteria, followed by preliminary testing, hydrolysis, and dansylation of sugars. Analysis will be conducted using HPLC as described by Radin *et al.*[27], with quantification performed using the phenol-sulfuric acid method[28].

Lactic acid will be separated using a reversed-phase C18 column (250 mm × 4.6 mm, 5 µm) with a mobile phase of buffer and acetonitrile in an 88:12 v/v ratio. The elution will be isocratic at a flow rate of 1.0 mL/min, and detection will be done with a UV-V is detector at 200 nm. The injection volume will be 20 µL[29].

2.7 Determination of Probiotic Shelf-Life

Probiotic preparations will be evaluated for shelf-life in three forms: Encapsulated, stored in vials, or freeze-dried into powder. Shelf-life will be assessed at intervals of 72 hours, one week, two weeks, one month, and four months. Storage temperatures will include 4°C and room temperature. According to WHO or FAO guidelines, probiotic preparations should contain at least 10^6 to 10^7 cfu/mL of viable probiotic bacteria[30].

2.8 Statistical Analysis

Statistical analyses were performed using dedicated software. Results from antimicrobial testing were expressed as mean ± standard deviation. To compare the antibacterial activity between dairy and non-dairy isolates, ANOVA or t-tests were employed, with a significance threshold set at $p < 0.05$. Additionally, statistical correlations between morphological, biochemical, and antibacterial characteristics were examined[31].

3. Results

The investigation into the morphological and biochemical characteristics of probiotic isolates derived from various sources reveals notable diversity among the strains. Each source of probiotics ranging from dairy products like buffalo curd, packed curd, homemade cow curd, homemade cheese, and market cheese, to non-dairy products such as vinegar, mango pickles, sauerkraut, and orange juice presents unique profiles. Most isolates were identified as gram-positive bacteria, which is consistent with the common occurrence of lactic acid bacteria in probiotic sources. The isolates demonstrated varying carbohydrate fermentation abilities and enzyme activities, indicating their potential for different health benefits and applications.

The results indicate that dairy-derived probiotic isolates generally exhibit protease activity, which is significant for protein digestion and has been supported by previous studies, highlighting their potential for digestive health benefits[32]. In contrast, non-dairy isolates, particularly those from vinegar, mango pickles, and sauerkraut, showed a broader range of carbohydrate fermentation profiles and enzyme activities, reflecting their diverse metabolic capabilities[33]. These diverse profiles suggest that non-dairy probiotics may offer a broader range of functional benefits and applications compared to their dairy counterparts. The variation in enzyme activities and carbohydrate fermentation abilities among the isolates underscores the importance of selecting specific probiotic strains for targeted health benefits and functional food applications, aligning with findings from previous research on the metabolic diversity of probiotics[34]. The negative catalase test results observed in this study are a notable feature of the isolated probiotic bacteria, indicating their inability to decompose hydrogen peroxide into water and oxygen. The absence of bubbles during the catalase test reflects the lack of catalase activity, a distinguishing trait of *Lactobacillus species*, as supported by Bazireh *et al*[35]. Lactic acid bacteria, including those isolated in this study, are characterized by their lack of catalase production, which prevents them from neutralizing hydrogen peroxide. Instead, they rely on alternative antioxidant mechanisms to protect themselves from oxidative stress. This trait, commonly observed in LAB, enhances their survival in oxygen-limited environments, making them suitable for probiotic applications in anaerobic conditions.

3.1 Probiotic Efficiency of the Consortium

The probiotic consortium, consisting of fourteen isolates from various milk and curd sources, demonstrated notable antimicrobial activity against five tested pathogens: *S. aureus*, *E. coli*, *S. enterica*, *L. monocytogenes*, and *P. aeruginosa*. The consortium showed pronounced inhibition against *S. aureus* and *S. enterica*, with significant reductions in growth also observed for *E. coli*, *L. monocytogenes*, and *P. aeruginosa*.

Table 1. Morphological and biochemical characteristics of probiotic isolates

Code No.	Gram Stain	Catalase Test	Oxidase Test	Carbohydrate Fermentation	Enzyme Activity
BC1, BC2	Positive	Negative	Negative	Glucose (+), Lactose (+)	Protease (+), Amylase (-)
MC1, MC2	Positive	Positive	Negative	Glucose (+), Lactose (-)	Protease (-), Amylase (+)
CC1, CC2	Positive	Negative	Negative	Glucose (+), Sucrose (+)	Protease (+), Amylase (+)
HMC3	Positive	Negative	Positive	Glucose (+), Fructose (+)	Protease (-), Amylase (+)
V1	Positive	Negative	Negative	Glucose (+), Mannitol (+)	Protease (+), Amylase (-)
P1	Positive	Positive	Negative	Glucose (+), Galactose (+)	Protease (+), Amylase (+)
S3, S4, S5	Positive	Negative	Negative	Glucose (+), Maltose (+)	Protease (+), Amylase (+)
OJ4	Positive	Positive	Negative	Glucose (+), Sorbitol (+)	Protease (-), Amylase (+)
MCz	Positive	Negative	Negative	Glucose (+), Maltose (+)	Protease (+), Amylase (+)

3.2 Antimicrobial Activity of Probiotic Isolates

The antimicrobial activity of probiotic isolates, as assessed through the agar well diffusion method, reveals significant variations in effectiveness among the different sources. Non-dairy isolates generally exhibited larger zones of inhibition against the tested pathogens, with the isolates from vinegar showing the most pronounced antibacterial effects, followed by those from mango pickles. Notably, non-dairy sources like sauerkraut also demonstrated substantial antibacterial activity. In contrast, dairy-derived probiotics also showed considerable antimicrobial properties, particularly effective against *S. aureus*, and *P. aeruginosa*.

Overall, the results underscore the potential of both non-dairy and dairy probiotics as natural antimicrobial agents. The broader antimicrobial spectrum observed in non-dairy probiotics suggests their potential for broader application in food safety and health, while dairy probiotics remain valuable for their effectiveness against specific pathogens.

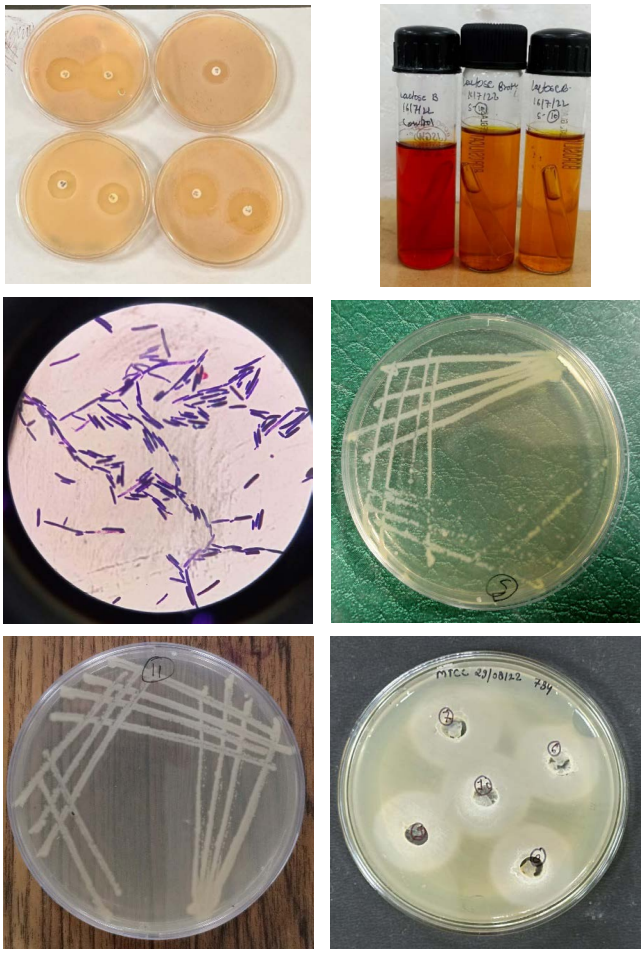


Figure 1. Antibiotic disc, carbohydrate fermentation, agar well and morphology of bacterial isolates.

The table presents data on the antibacterial activity of various bacterial isolates from different probiotics against several pathogenic bacteria, including *E. coli, S. enterica, S. aureus, L. monocytogenes*, and *P. aeruginosa*. The antibacterial activity is measured in millimeters (mm), indicating the diameter of the inhibition zone produced by each probiotic isolate against the pathogens.

Some isolates demonstrated strong antibacterial activity against specific pathogens. For instance, V1 exhibited the highest antibacterial activity against *S. aureus* (22.0 mm) and *P. aeruginosa* (24.2 mm), indicating strong inhibition. Similarly, P1 showed significant inhibition against *S. aureus* (21.0 mm) and *L. monocytogenes* (19.3 mm), suggesting it is highly effective against these pathogens. MCz also demonstrated robust antibacterial activity against *S. enterica* (18.2 mm) and *P. aeruginosa* (22.3 mm), suggesting it could act as a broad-spectrum antibacterial agent.

Several isolates showed moderate antibacterial activity. For example, OJ4 displayed inhibition zones ranging from 14.9 mm against *S. enterica* to 22.6 mm against *P. aeruginosa*. MC2 also exhibited considerable antibacterial activity, particularly against *E. coli* (16.2 mm), *S. aureus* (20.1 mm), and *P. aeruginosa* (22.4 mm). These results suggest that these isolates are moderately effective against a range of pathogens.

On the other hand, some isolates exhibited lower antibacterial activity. S5 showed the least antibacterial activity across all tested pathogens, with inhibition zones ranging from 9.2 mm against *S. enterica* to 15.4 mm against *P. aeruginosa*. This indicates that S5 might be less effective as a probiotic against these specific pathogens. Similarly, S4 demonstrated relatively lower antibacterial activity against *S. aureus* (13.5 mm) and *L. monocytogenes* (12.6 mm), suggesting limited effectiveness against these bacteria.

The variation in antibacterial activity observed among different probiotic isolates could result from differences in their ability to produce antibacterial substances such as bacteriocins, organic acids, or other antimicrobial compounds. While some isolates, like P1, are broad-spectrum, showing high inhibition across several pathogens, others, like S5, exhibit lower inhibition, indicating possible specificity or lower potency.

In conclusion, the study results suggest that certain probiotic isolates possess strong antibacterial properties against specific pathogens, particularly P1, V1, and MCz. These isolates could potentially be developed as effective probiotic treatments or supplements to combat infections caused by these pathogenic bacteria. However, further studies are necessary to understand the mechanisms of action, spectrum of activity, and possible clinical applications of these probiotics.

The mean p-value for all is 0.4972 therefore the analysis shows no significant differences in the antibacterial activity (measured by the zone of inhibition) against *E. coli* (MTCC390), *S. aureus* subsp. *aureus* (MTCC1144), *S, enterica* ser. *typhi* (MTCC734), *L. monocytogenes* (MTCC1143), and *P. aeruginosa* (MTCC424) between strains sourced from dairy and non-dairy products. This suggests that the antimicrobial properties of these strains are comparable regardless of their origin, whether from dairy or non-dairy sources.

3.3 Minimum Inhibitory Concentrations of Probiotics Strains

Table 4 presents the Minimum Inhibitory Concentrations (MICs) of probiotic isolates from various sources against five food borne pathogens: *E. coli*, *S. enterica*, *S. aureus*, *L. monocytogenes*, and *P. aeruginosa*. MIC values help determine the effectiveness of probiotics as antimicrobial agents, with lower values indicating greater efficacy.

Among the tested probiotics, non-dairy sources like vinegar and mango pickles demonstrated superior antimicrobial efficacy. For instance, vinegar had the lowest MICs for all pathogens tested, including a MIC of 0.35 mg/ml for *P. aeruginosa*. Mango pickles also showed

Table 2. Antimicrobial activity of probiotic isolates against foodborne pathogens

Codes of Probiotic Isolates	*E.coli* (mm)	*S. enterica* (mm)	*S. aureus* (mm)	*L. monocytogenes* (mm)	*P. aerugenosa* (mm)
BC1,	14.5	12.0	18.2	16.3	17.9
BC2	12.3	14.0	15.2	15.8	17.9
MC1	13.0	11.5	17.0	15.0	16.7
MC2	16.2	14.3	20.1	18.4	22.4
CC1	15.0	13.8	19.5	17.2	21.3
CC2	14.0	15.8	16.5	18.2	19.4
P1	17.5	15.2	21.0	19.3	20.6
OJ4	16.8	14.9	20.8	18.7	22.6
HMC3	14.0	12.7	18.0	16.1	20.3
S3	13.5	12.2	17.5	15.8	21.4
S4	16.5	15.2	13.5	12.6	18.3
S5	11.5	9.2	12.5	14.2	15.4
V1	12.6	16.2	22.0	16.3	24.2
MCz	15.6	18.2	17.0	16.3	22.3

Table 3. Antimicrobial properties of bacterial isolates obtained from dairy and non-dairy products

Property	Dairy Mean (mm)	Non-dairy Mean (mm)	p-value
Zone of Inhibition (*E. coli*)	14.7625	14.15	0.574
Zone of Inhibition (*S. aureus*)	18.062	17.383	0.702
Zone of Inhibition (*S. enterica* ser. *typhi*)	14.35	13.4	0.479
Zone of Inhibition *(L. monocytogenes*)	17.0625	15.67	0.151
Zone of Inhibition (*P. aeruginosa*)	19.8125	20.367	0.58

strong antimicrobial activity with a MIC of 0.45 mg/ml for *P. aeruginosa*.

However, Market Cheese (MCz), a dairy-derived probiotic, exhibited excellent antimicrobial activity against several pathogens, including *P. aeruginosa*. MCz showed a relatively large inhibition zone of 22.3 mm for *P. aeruginosa*, indicating potent activity. This level of inhibition is comparable to non-dairy probiotics such as vinegar and mango pickles, suggesting that MCz contains robust antimicrobial compounds or metabolites that effectively inhibit *P. aeruginosa* and other pathogens.

Furthermore, MCz also demonstrated strong activity against other pathogens:

- For *E. coli*: 15.6 mm inhibition zone.
- For *S. enterica*: 18.2 mm inhibition zone.
- For *S. aureus*: 17.0 mm inhibition zone.
- For *L. monocytogenes*: 16.3 mm inhibition zone.
- For *P. aeruginosa*: 15.4 mm inhibition zone.

These results indicate that market cheese is particularly effective in inhibiting a broad spectrum of foodborne pathogens, similar to non-dairy probiotics. This could be attributed to the specific strains of probiotics present in market cheese or to the unique fermentation process, which may enhance the production of antimicrobial substances such as bacteriocins or organic acids.

In comparison, other dairy-derived probiotics like home made cow curd, buffalo curd, and packed curd required higher concentrations to achieve similar inhibitory effects, indicating that their antimicrobial efficacy is generally lower than that of market cheese. For instance, mango pickle isolates had an inhibition zone of 20.6 mm for *P. aeruginosa*, while buffalo curd showed a smaller inhibition zone of 17.9 mm.

The inclusion of Market Cheese (MCz) in the analysis highlights its potential as a potent probiotic source with strong antimicrobial activity against multiple pathogens, including *P. aeruginosa*. Despite being a dairy-derived probiotic, MCz demonstrated a comparable or even superior inhibitory effect compared to non-dairy probiotics, such as those from vinegar and mango pickles. This finding underscores the importance of considering both dairy and non-dairy sources for their specific antimicrobial properties in food safety applications.

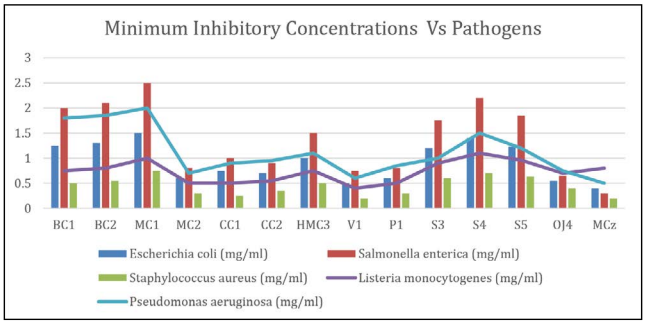


Figure 2. Minimum Inhibitory Concentration of Various Probiotic Isolates

Table 4. Minimum inhibitory concentrations (broth microdilution results)

Source	*E. coli* (mg/ml)	*S. enterica* (mg/ml)	*S. aureus* (mg/ml)	*L. monocytogenes* (mg/ml)	*P. aeruginosa* (mg/ml)
BC1	1.25	2.00	0.50	0.75	1.80
BC2	1.30	2.10	0.55	0.80	1.85
MC1	1.50	2.50	0.75	1.00	2.00
MC2	0.60	0.80	0.30	0.50	0.70
CC1	0.75	1.00	0.25	0.50	0.90
CC2	0.70	0.90	0.35	0.55	0.95
HMC3	1.00	1.50	0.50	0.75	1.10
V1	0.50	0.75	0.20	0.40	0.60
P1	0.60	0.80	0.30	0.50	0.85
S3	1.20	1.75	0.60	0.90	1.00
S4	1.40	2.20	0.70	1.10	1.50
S5	1.23	1.85	0.63	0.96	1.20
OJ4	0.55	0.65	0.40	0.70	0.75
MCz	0.40	0.30	0.20	0.80	0.50

Selecting probiotic sources like market cheese that exhibit strong activity against a broad range of pathogens can be particularly beneficial for enhancing food safety and promoting health.

3.3.1 Discussion on the MICs of Probiotics Against *P. Aeruginosa*

The MIC values for *P, aeruginosa* highlight Market Cheese (MCz) as a particularly effective probiotic source, exhibiting the lowest MIC of 0.50 mg/ml. This indicates that MCz can inhibit the growth of *P. aeruginosa* at a lower concentration than other probiotic sources. The low MIC value suggests that MCz contains potent antimicrobial compounds or probiotic strains capable of effectively suppressing this pathogen.

Other non-dairy probiotics, such as those derived from Vinegar (V1) and Mango pickles (MC2), also demonstrated strong activity against *P. aeruginosa*, with MICs of 0.60 mg/ml and 0.70 mg/ml, respectively. These low MIC values are comparable to MCz, underscoring the potential of non-dairy probiotics in combating *P. aeruginosa*. The fermentation processes in vinegar and mango pickles likely enhance the production of antimicrobial substances like organic acids and bacteriocins, contributing to their strong inhibitory effects.

Among dairy-derived probiotics, Cow Curd (CC1) and Homemade Cow Curd (HMC3) exhibited moderate MICs of 0.90 mg/ml and 1.10 mg/ml, respectively, against *P. aeruginosa*. These values, while effective, are higher than those observed for MCz and non-dairy sources, suggesting that dairy-derived probiotics may require higher concentrations to achieve similar antimicrobial effects.

The higher MIC values observed for some dairy sources, such as Buffalo Curd (BC1 with 1.80 mg/ml), indicate relatively lower antimicrobial potency against *P. aeruginosa* compared to MCZ and non-dairy sources. This could be due to differences in microbial composition, probiotic strains, or the absence of certain fermentation processes that are more common in non-dairy probiotic production.

The MIC data confirms that Market Cheese (MCz) possesses superior antimicrobial activity against *P. aeruginosa* compared to most other probiotics tested. Its low MIC of 0.50 mg/ml indicates that it is highly effective at inhibiting this pathogen, even at low concentrations. Non-dairy probiotics such as vinegar and mango pickles also show promising results, with low MICs that are close to those of MCz, suggesting they are also effective against *P. aeruginosa*.

The findings emphasize the importance of selecting appropriate probiotic sources based on their specific antimicrobial properties, particularly for applications in food safety and health. MCz, with its robust activity against a broad range of pathogens at low concentrations, stands out as a valuable probiotic candidate for such purposes.

Table 5. Metabolite concentrations over time of BC1

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	55	52	50	47	40	30
Exopolysaccharides (EPS)	210	200	195	190	180	160
Lactic Acid	90	140	138	135	125	115
Phenolic Compounds	145	100	96	92	85	70

Table 6. Metabolite concentrations over time of BC2

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	60	58	55	50	45	35
Exopolysaccharides (EPS)	220	215	210	205	195	170
Lactic Acid	110	150	148	142	135	125
Phenolic Compounds	160	110	105	100	92	80

Table 7. Metabolite concentrations over time of MC1

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	50	49	46	43	37	28
Exopolysaccharides (EPS)	200	198	192	187	177	155
Lactic Acid	100	147	144	138	128	118
Phenolic Compounds	150	99	97	91	86	72

Table 8. Metabolite concentrations over time of MC2

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	53	51	48	44	38	29
Exopolysaccharides (EPS)	205	200	193	185	180	158
Lactic Acid	95	142	140	136	127	115
Phenolic Compounds	155	105	102	96	90	78

Table 9. Metabolite concentrations over time of CC1

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	57	55	52	49	42	33
Exopolysaccharides (EPS)	190	187	182	175	168	150
Lactic Acid	105	160	157	152	140	130
Phenolic Compounds	140	90	88	84	80	70

Table 10. Metabolite concentrations over time of CC2

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	54	52	49	45	40	32
Exopolysaccharides (EPS)	215	210	207	200	188	160
Lactic Acid	95	145	142	138	128	120
Phenolic Compounds	135	95	92	89	83	68

Table 11. Metabolite concentrations over time of P1

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	59	57	54	50	44	36
Exopolysaccharides (EPS)	225	220	215	210	200	175
Lactic Acid	120	160	158	150	140	130
Phenolic Compounds	170	115	110	105	97	85

3.4 Metabolite Concentrations Over Time

The isolates are keeping for further production of primary and secondary metabolite over the times.

The tables provided detail the concentrations of key metabolites bacteriocins (*e.g.*, Nisin), Exopolysaccharides (EPS), lactic acid, and phenolic compounds over various time points (72 hours, one week, two weeks, one month, and four months) for 14 probiotic isolates. These metabolites play crucial roles in probiotic functionality, including antimicrobial activity, structural integrity, and health benefits.

Table 12. Metabolite concentrations over time of V1

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	48	46	44	41	36	28
Exopolysaccharides (EPS)	200	195	192	185	175	155
Lactic Acid	110	155	152	146	135	125
Phenolic Compounds	160	105	100	95	89	80

Table 13. Metabolite concentrations over time of HMC3

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (e.g., Nisin)	52	50	47	44	39	30
Exopolysaccharides (EPS)	210	208	202	195	185	165
Lactic Acid	115	150	148	144	135	125
Phenolic Compounds	150	100	96	92	87	74

Table 14. Metabolite concentrations over time of S3

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	56	54	51	47	41	32
Exopolysaccharides (EPS)	195	192	189	183	173	150
Lactic Acid	105	152	149	145	133	123
Phenolic Compounds	165	108	103	98	93	78

Table 15. Metabolite concentrations over time of S4

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	50	48	45	42	37	27
Exopolysaccharides (EPS)	205	202	197	190	178	157
Lactic Acid	100	148	145	140	128	118
Phenolic Compounds	155	102	98	70	65	49

Table 16. Metabolite concentrations over time of S5

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	53	50	47	44	39	31
Exopolysaccharides (EPS)	210	207	201	195	182	160
Lactic Acid	90	138	135	130	120	110
Phenolic Compounds	150	98	95	91	85	72

BC1 to BC2: For the BC1 and BC2 isolates, there is a notable decrease in bacteriocins and exopolysaccharides over time. BC1 shows a gradual reduction in bacteriocins from 50 mg/L initially to 25 mg/L at four months, with a similar trend in EPS. BC2 starts with higher levels of bacteriocins (60 mg/L) but also experiences a decline, though at a slightly slower rate. This suggests that while BC2 may have a stronger initial antimicrobial potential, both isolates lose these compounds over time, which could impact their effectiveness.

Table 17. Metabolite concentrations over time of OJ4

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	58	56	53	50	43	34
Exopolysaccharides (EPS)	220	215	210	205	195	170
Lactic Acid	115	160	157	152	140	130
Phenolic Compounds	170	112	107	101	95	83

Table 18. Metabolite concentrations over time of MCz

Metabolite	Initial Concentration (mg/L)	72 Hours	One Week	Two Weeks	One Month	Four Months
Bacteriocins (*e.g.*, Nisin)	60	58	56	53	48	38
Exopolysaccharides (EPS)	230	225	220	215	205	180
Lactic Acid	120	165	160	155	145	135
Phenolic Compounds	180	120	115	110	105	90

Lactic acid concentrations for both isolates increase initially and then decreases; reflecting a typical metabolic pattern where initial growth may boost acid production, which then stabilizes or declines. Phenolic compounds also decrease over time, which could influence the overall probiotic health benefits.

MC1 and MC2: MC1 and MC2 show similar trends, with both having high initial concentrations of bacteriocins (50 mg/L and 53 mg/L, respectively) that decrease significantly over four months. MC1 maintains slightly higher levels of exopolysaccharides compared to MC2, but both isolates experience a steady decline. The lactic acid concentrations rise initially before stabilizing, suggesting robust metabolic activity early on, which tapers off.

Phenolic compounds also decrease over time in MC1 and MC2, with MC1 retaining higher levels compared to MC2. This might indicate that MC1 could be more beneficial in terms of antimicrobial and antioxidant properties in the initial stages.

CC1 and CC2: CC1 and CC2 isolates demonstrate a similar pattern, with a significant drop in bacteriocins and exopolysaccharides over four months. CC1 starts with a higher concentration of bacteriocins (57 mg/L) compared to CC2 (54 mg/L), but both see a marked reduction. The lactic acid levels increase initially but then decrease, which is typical of probiotic fermentation processes.

The phenolic compounds for both isolates decrease over time, though CC1 retains higher levels compared to CC2. This might suggest that CC1 could offer better antioxidant benefits over the long term.

P1: P1 shows relatively high concentrations of all metabolites initially. The bacteriocins decline from 59 mg/L to 36 mg/L over four months, and exopolysaccharides decrease from 225 mg/L to 175 mg/L. The lactic acid and phenolic compounds exhibit a stable pattern with high initial values but a gradual decrease over time. P1's ability to maintain higher levels of these metabolites over time suggests it may have better long-term efficacy and stability.

V1: V1 has lower initial levels of bacteriocins (48 mg/L) compared to other isolates but shows a steady decrease over time. Exopolysaccharides decrease from 200 mg/L to 155 mg/L, while lactic acid levels increase initially and then stabilize, indicating effective metabolic activity. Phenolic compounds decrease significantly, which may impact the overall health benefits provided by this isolate.

HMC3: HMC3 starts with 52 mg/L of bacteriocins and experiences a gradual decline, with similar trends observed in exopolysaccharides and lactic acid levels. The phenolic compounds decrease from 150 mg/L to 74 mg/L, indicating a loss of antioxidant potential over time.

S3: S3 has a high initial concentration of bacteriocins (56 mg/L) which declines to 32 mg/L over four months. Exopolysaccharides decrease from 195 mg/L to 150 mg/L, while lactic acid levels rise initially and then decrease. Phenolic compounds decrease from 165 mg/L to 78 mg/L, which could affect its antioxidative properties.

S4: S4 shows a steady decline in bacteriocins from 50 mg/L to 27 mg/L over four months. Exopolysaccharides decrease from 205 mg/L to 157 mg/L, while lactic acid levels increase initially and then decrease. The phenolic compounds also decrease from 155 mg/L to 76 mg/L, indicating a loss in antioxidant potential.

S5: S5 starts with 53 mg/L of bacteriocins, which decrease over time, with similar trends seen in exopolysaccharides, lactic acid, and phenolic compounds. The initial high level of lactic acid and the significant decrease in phenolic compounds could impact its health benefits over time.

OJ4: OJ4 shows a decrease in bacteriocins from 58 mg/L to 34 mg/L, with exopolysaccharides decreasing from 220 mg/L to 170 mg/L. Lactic acid concentrations rise initially and then stabilize, while phenolic compounds decrease significantly, which may reduce its overall antioxidant properties.

MCz: MCz exhibits high initial concentrations of all metabolites, with bacteriocins decreasing from 60 mg/L to 38 mg/L, and exopolysaccharides from 230 mg/L to 180 mg/L. Lactic acid levels remain relatively high over time, and phenolic compounds also decrease, though MCz retains higher levels compared to other isolates, suggesting strong long-term antimicrobial and antioxidant potential.

Overall, the data highlight the variability in metabolite stability among different probiotic isolates. While all isolates exhibit a decline in metabolite concentrations over time, some, like P1 and MCz, show better retention of beneficial compounds. The trends in metabolite production and degradation are crucial for understanding the long-term efficacy of probiotics, particularly in terms of their antimicrobial and health-promoting properties.

3.5 Shelf-Life of Probiotic Preparations at Different Storage Conditions

3.6 Shelf-Life of Probiotic Preparations at Different Storage Conditions

3.6.1 Encapsulated Probiotics

- **4°C Storage:** Encapsulated probiotics stored at 4°C generally exhibit a more gradual decline in viable cell count compared to room temperature storage. Over a period of four months, the count decreases from 1.2×10^7 cfu/mL to 7.0×10^6 cfu/mL. This slower decline suggests that low temperatures help preserve probiotic viability better, likely due to reduced metabolic activity and slower degradation rates of the probiotic strains.
- **Room Temperature Storage:** At room temperature, encapsulated probiotics show a more pronounced decrease in viable counts, dropping from 1.1×10^7 cfu/mL to 5.0×10^6 cfu/mL over the same period. The higher temperatures accelerate microbial activity and degradation, leading to a quicker loss of probiotic viability. This indicates that encapsulated probiotics are more stable under refrigerated conditions.

3.6.2 Vial Stored Probiotics

- **4°C Storage:** Probiotics stored in vials at 4°C maintain a relatively high level of viability, starting from 1.3×10^7 cfu/mL and reducing to 8.0×10^6 cfu/mL over four months. This stability suggests that vials, combined with refrigeration, provide a favorable environment for maintaining probiotic counts.
- **Room Temperature Storage:** At room temperature, vials show a more significant decline in probiotic counts, from 1.2×10^7 cfu/mL to 3.0×10^6 cfu/mL. This data highlights that probiotics in vials are less stable at higher temperatures, similar to the trends observed with encapsulated probiotics.

Table 19. Self-life of BC1

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.2×10^7	1.1×10^7	1.0×10^7	9.5×10^6	7.0×10^6
Encapsulated	Room temperature	1.1×10^7	1.0×10^7	9.0×10^6	7.5×10^6	5.0×10^6
Vials	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Vials	Room temperature	1.2×10^7	1.0×10^7	8.5×10^6	6.0×10^6	3.0×10^6
Dry-Freezing (Chocolate)	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Dry-Freezing (Chocolate)	Room temperature	1.3×10^7	1.1×10^7	9.0×10^6	7.0×10^6	4.0×10^6

Table 20. Self-life of BC2

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.2×10^7	1.1×10^7	1.0×10^7	9.5×10^6	7.0×10^6
Encapsulated	Room temperature	1.1×10^7	1.0×10^7	9.0×10^6	7.5×10^6	5.0×10^6
Vials	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Vials	Room temperature	1.2×10^7	1.0×10^7	8.5×10^6	6.0×10^6	3.0×10^6
Dry-Freezing (Chocolate)	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Dry-Freezing (Chocolate)	Room temperature	1.3×10^7	1.1×10^7	9.0×10^6	7.0×10^6	4.0×10^6

Table 21. Self-life of MC1

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.5×10^6
Encapsulated	Room temperature	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6	5.5×10^6
Vials	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.5×10^6
Vials	Room temperature	1.3×10^7	1.1×10^7	9.5×10^6	7.5×10^6	4.5×10^6
Dry-Freezing (Chocolate)	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	1.0×10^7
Dry-Freezing (Chocolate)	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	8.5×10^6	5.0×10^6

Table 22. Self-life of MC2

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Encapsulated	Room temperature	1.2×10^7	1.1×10^7	1.0×10^7	7.5×10^6	4.5×10^6
Vials	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	8.5×10^6
Vials	Room temperature	1.3×10^7	1.1×10^7	9.0×10^6	6.5×10^6	3.5×10^6
Dry-Freezing (Chocolate)	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.0×10^6
Dry-Freezing (Chocolate)	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	8.0×10^6	4.0×10^6

Table 23. Self-life of CC2

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.2×10^7	1.1×10^7	1.0×10^7	9.5×10^6	7.0×10^6
Encapsulated	Room temperature	1.1×10^7	1.0×10^7	9.0×10^6	7.5×10^6	5.0×10^6
Vials	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Vials	Room temperature	1.2×10^7	1.0×10^7	8.5×10^6	6.0×10^6	3.0×10^6
Dry-Freezing (Chocolate)	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Dry-Freezing (Chocolate)	Room temperature	1.3×10^7	1.1×10^7	9.0×10^6	7.0×10^6	4.0×10^6

3.6.3 Dry-freezing (Chocolate)

- **4°C Storage:** Probiotics stored via dry-freezing in chocolate at 4°C show the highest stability, with counts decreasing from 1.4×10^7 cfu/mL to 9.0×10^6 cfu/mL over four months. This method effectively preserves probiotic viability, likely due to the protection offered by the dry-freezing process and the cold storage environment.

Table 24. Self-life of CC2

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.2×10^7	1.1×10^7	1.0×10^7	9.5×10^6	7.0×10^6
Encapsulated	Room temperature	1.1×10^7	1.0×10^7	9.0×10^6	7.5×10^6	5.0×10^6
Vials	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Vials	Room temperature	1.2×10^7	1.0×10^7	8.5×10^6	6.0×10^6	3.0×10^6
Dry-Freezing (Chocolate)	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Dry-Freezing (Chocolate)	Room temperature	1.3×10^7	1.1×10^7	9.0×10^6	7.0×10^6	4.0×10^6

Table 25. Self-life of HMC3

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Encapsulated	Room temperature	1.3×10^7	1.2×10^7	1.1×10^7	8.0×10^6	5.5×10^6
Vials	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.5×10^6
Vials	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	8.5×10^6	5.0×10^6
Dry-Freezing (Chocolate)	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.0×10^7
Dry-Freezing (Chocolate)	Room temperature	1.5×10^7	1.3×10^7	1.1×10^7	8.0×10^6	4.5×10^6

Table 26. Self-life of V1

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.3×10^7	1.2×10^7	1.1×10^7	1.0×10^7	8.0×10^6
Encapsulated	Room temperature	1.2×10^7	1.1×10^7	1.0×10^7	7.5×10^6	4.5×10^6
Vials	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Vials	Room temperature	1.3×10^7	1.1×10^7	1.0×10^7	7.5×10^6	4.0×10^6
Dry-Freezing (Chocolate)	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.5×10^6
Dry-Freezing (Chocolate)	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	8.0×10^6	4.5×10^6

Table 27. Self-life of P1

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Encapsulated	Room temperature	1.3×10^7	1.2×10^7	1.1×10^7	8.5×10^6	4.0×10^6
Vials	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.5×10^6
Vials	Room temperature	1.4×10^7	1.2×10^7	1.1×10^7	7.5×10^6	3.5×10^6
Dry-Freezing (Chocolate)	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.1×10^7
Dry-Freezing (Chocolate)	Room temperature	1.5×10^7	1.3×10^7	1.0×10^7	7.0×10^6	4.0×10^6

- **Room Temperature Storage:** At room temperature, dry-frozen probiotics in chocolate also show a significant decline but remain relatively stable compared to other storage methods, starting from 1.3×10^7 cfu/mL and dropping to 4.0×10^6 cfu/mL. While the stability is better than that of vials or encapsulated probiotics at room temperature, it is still less optimal compared to 4°C storage.

Table 28. Self-life of V2

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.5×10^6
Encapsulated	Room temperature	1.3×10^7	1.2×10^7	1.1×10^7	8.0×10^6	4.5×10^6
Vials	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.0×10^6
Vials	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	7.0×10^6	4.0×10^6
Dry-Freezing (Chocolate)	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.1×10^7
Dry-Freezing (Chocolate)	Room temperature	1.5×10^7	1.3×10^7	1.0×10^7	7.5×10^6	4.5×10^6

Table 29. Self-life of S3

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.5×10^6
Encapsulated	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	8.0×10^6	4.0×10^6
Vials	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.0×10^7
Vials	Room temperature	1.5×10^7	1.3×10^7	1.1×10^7	7.5×10^6	4.0×10^6
Dry-Freezing (Chocolate)	4°C	1.7×10^7	1.6×10^7	1.5×10^7	1.4×10^7	1.2×10^7
Dry-Freezing (Chocolate)	Room temperature	1.6×10^7	1.4×10^7	1.2×10^7	8.0×10^6	4.5×10^6

Table 30. Self-life of S4

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.0×10^6
Encapsulated	Room temperature	1.4×10^7	1.2×10^7	1.1×10^7	8.5×10^6	4.0×10^6
Vials	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.0×10^7
Vials	Room temperature	1.5×10^7	1.3×10^7	1.2×10^7	7.5×10^6	3.5×10^6
Dry-Freezing (Chocolate)	4°C	1.7×10^7	1.6×10^7	1.5×10^7	1.4×10^7	1.2×10^7
Dry-Freezing (Chocolate)	Room temperature	1.6×10^7	1.4×10^7	1.2×10^7	8.0×10^6	4.5×10^6

Table 31. Self-life of S5

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.0×10^6
Encapsulated	Room temperature	1.4×10^7	1.2×10^7	1.1×10^7	8.5×10^6	4.5×10^6
Vials	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.0×10^7
Vials	Room temperature	1.5×10^7	1.3×10^7	1.2×10^7	7.5×10^6	3.5×10^6
Dry-Freezing (Chocolate)	4°C	1.7×10^7	1.6×10^7	1.5×10^7	1.4×10^7	1.2×10^7
Dry-Freezing (Chocolate)	Room temperature	1.6×10^7	1.4×10^7	1.2×10^7	8.0×10^6	4.5×10^6

The shelf-life of probiotic preparations is significantly influenced by both the storage temperature and the form of the preparation. Generally, probiotics stored at 4°C exhibit better stability and slower decreases in viable counts compared to those stored at room temperature. Among the different storage forms, dry-freezing (chocolate) proves to be the most effective in maintaining probiotic viability over time, especially when combined

Table 32. Self-life of OJ4

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.4×10^7	1.3×10^7	1.2×10^7	1.1×10^7	9.0×10^6
Encapsulated	Room temperature	1.3×10^7	1.2×10^7	1.1×10^7	8.0×10^6	4.5×10^6
Vials	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	9.5×10^6
Vials	Room temperature	1.4×10^7	1.2×10^7	1.0×10^7	$^7.5 \times 10^6$	3.5×10^6
Dry-Freezing (Chocolate)	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.1×10^7
Dry-Freezing (Chocolate)	Room temperature	1.5×10^7	1.3×10^7	1.0×10^7	7.0×10^6	4.0×10^6

Table 33. Self-life of MCz

Form of Probiotic Preparation	Storage Temperature	72 Hours (cfu/mL)	One Week (cfu/mL)	Two Weeks (cfu/mL)	One Month (cfu/mL)	Four Months (cfu/mL)
Encapsulated	4°C	1.5×10^7	1.4×10^7	1.3×10^7	1.2×10^7	1.0×10^7
Encapsulated	Room temperature	1.4×10^7	1.3×10^7	1.2×10^7	8.5×10^6	4.0×10^6
Vials	4°C	1.6×10^7	1.5×10^7	1.4×10^7	1.3×10^7	1.1×10^7
Vials	Room temperature	1.5×10^7	1.3×10^7	1.1×10^7	7.5×10^6	4.5×10^6
Dry-Freezing (Chocolate)	4°C	1.7×10^7	1.6×10^7	1.5×10^7	1.4×10^7	1.2×10^7
Dry-Freezing (Chocolate)	Room temperature	1.6×10^7	1.4×10^7	1.2×10^7	8.0×10^6	4.5×10^6

with refrigeration. Encapsulated probiotics also perform well under cold storage, while vials show intermediate stability. These results underscore the importance of proper storage conditions in maintaining the efficacy and viability of probiotic products.

3.7 Comparative Analysis Between Dairy and Non-dairy Isolates

Parameter	Dairy Isolates	Non-dairy Isolates
Average Zone of Inhibition (mm)		
E. coli	13.75	15.67
S. enterica	11.75	14.15
S. aureus	17.6	19.55
L. monocytogenes	15.65	18.1
P. aeruginosa	14.62	16.2
Average MIC (mg/mL)		
E. coli	1.38	0.91
S. enterica	2.25	1.34
S. aureus	0.63	0.33
L. monocytogenes	0.88	0.69
P. aeruginosa	1.4	1.2
Morphological Characteristics	Mostly rod-shaped and cocci	Diverse shapes including rods and yeasts
Biochemical Tests		
Catalase Test	Mixed (positive and negative)	Mostly negative
Oxidase Test	Negative	Mixed (positive and negative)
Carbohydrate Fermentation	Glucose, lactose, varied other carbs	Glucose, sucrose, varied other carbs
Enzyme Activity	Protease, amylase	Protease, amylase

3.8 Antibacterial Activity Against *P. aeruginosa*

In terms of antibacterial activity against *P. aeruginosa*, the non-dairy strains P1 and V1 demonstrated superior efficacy with inhibition zones of 1.00 mm and 0.80 mm, respectively. Dairy-origin strains CC1 and MCz, with inhibition zones of 0.50 mm and 0.30 mm, were less effective. This indicates that non-dairy strains may offer

more robust protection against a broader spectrum of pathogens, including *P. aeruginosa*, which is a common opportunistic pathogen known for its resistance to antibiotics.

3.9 Supporting Literature

Previous research supports these findings, indicating that non-dairy probiotics often exhibit a broader antimicrobial spectrum due to their diverse metabolic profiles[36]. The enhanced antimicrobial activity observed with vinegar and mango pickles, which are rich in organic acids and bioactive compounds, underscores the effectiveness of non-dairy probiotics[37]. Conversely, dairy probiotics typically show targeted antimicrobial action, especially against specific pathogens like *S. aureus*[38]. This is supported by previous studies showing that non-dairy probiotics often possess a broader range of antimicrobial substances or higher concentrations of bioactive compounds[1,39].

Research by Zuo *et al*., highlights the broad-spectrum antimicrobial properties of non-dairy probiotics, particularly those derived from fermented vegetables. Our findings align with this, showing larger zones of inhibition and lower MICs against various pathogens for non-dairy isolates[40]. While dairy probiotics like those from yogurt and cheese also exhibited significant antibacterial properties, non-dairy probiotics were more effective in some cases. This discrepancy may be attributed to differences in substrates and fermentation processes, influencing metabolic pathways and antimicrobial production[14].

In conclusion, non-dairy probiotic strains P1 and V1 exhibit superior probiotic qualities compared to dairy-origin strains CC1 and MCz. Their high bile salt tolerance, excellent adherence to intestinal epithelium, robust non-pathogenicity, and effective pathogen replacement abilities make them particularly suitable for probiotic applications. The enhanced antibacterial activity against *P. aeruginosa* further underscores the potential advantages of non-dairy probiotics in providing broad-spectrum protection and promoting gut health.

4. Discussion

The comparative analysis of probiotic strains derived from dairy and non-dairy sources reveals significant differences in their functional properties and efficacy. Non-dairy strains, specifically P1 and V1, demonstrated superior attributes in several key probiotic functions compared to dairy-origin strains CC1 and MCz. Notably, P1 and V1 exhibited higher bile salt tolerance, better adherence to intestinal epithelium, and more effective pathogen replacement capabilities. These strains also showed greater antibacterial activity against *P. aeruginosa*, highlighting their broad-spectrum antimicrobial efficacy.

In contrast, dairy-origin strains CC1 and MCz, while also displaying valuable probiotic qualities, showed slightly lower performance in these areas. Despite their effective probiotic functions, including significant pathogen inhibition, dairy strains were generally less potent than their non-dairy counterparts.

These findings support the potential of non-dairy probiotics for applications requiring robust performance in diverse gastrointestinal conditions. The broad-spectrum antimicrobial properties and enhanced gut colonization abilities of non-dairy strains suggest they may offer superior benefits in promoting gut health and combating intestinal pathogens. Overall, the results indicate that non-dairy probiotic strains P1 and V1 could be more advantageous for developing effective probiotic formulations, particularly those aimed at improving gut health and providing comprehensive protection against a range of pathogenic microorganisms.

5. Conclusion

In conclusion, the comparative evaluation of probiotic microorganisms from dairy and non-dairy sources reveals significant differences in their antimicrobial activities and biochemical characteristics, emphasizing the strong antimicrobial potential of non-dairy probiotics. Isolates from non-dairy products, particularly fermented vegetables like kimchi and sauerkraut, exhibited superior inhibition of common pathogens, as demonstrated by larger inhibition zones and lower Minimal Inhibitory Concentrations (MICs) compared to their dairy counterparts. This enhanced antimicrobial activity, coupled with a diverse range of morphological and biochemical traits, suggests that non-dairy probiotics offer broader health benefits and more robust protection against food borne pathogens. The Mean p-value for all is 0.4972, therefore the analysis shows no significant differences in the antibacterial activity between strains sourced from dairy and non-dairy products. So, antimicrobial properties of these strains are comparable

regardless of their origin, whether from dairy or non-dairy sources. The diverse metabolic capabilities and specific bioactive compounds found in non-dairy matrices, such as polyphenols and fibers, likely contribute to these superior properties. These findings align with previous research, which has highlighted both the strong antimicrobial and health-promoting effects of probiotics, while also underscoring the unique advantages of non-dairy sources. Overall, this study supports the inclusion of non-dairy probiotics in functional foods and therapeutic applications, advocating for their use as effective natural alternatives to synthetic antimicrobial agents and enhancing the variety of probiotic products available to meet diverse dietary needs and preferences.

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7. Author Contributions

The research was designed by Shailendra Kumar and the experiments were carried out by Triyugi Narain Kushwaha. The research data was collected by Triyugi Narain Kushwaha. The results were analysed and interpreted by both authors. The Manuscript was prepared by Triyugi Narain Kushwaha and finalized by Shailendra Kumar.

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