



Journal of the SCIENCE OF FOOD AND AGRICULTURAL DEVELOPMENT

<https://journals.kwasu.edu.ng/index.php/jsfad>



Dual role of lactic acid bacteria cultures for fermentation and control of pathogenic bacteria in fruit enriched fermented milk

¹Majekodunmi, R., Adedayo and ¹Kate R. Imeje

¹Department of Microbiology, Faculty of Pure and Applied Sciences, Kwara State University, Malete, Nigeria

*Corresponding author: Kate Rachael Imeje Kateimeje22@gmail.com

ABSTRACT

The use of metabolites from lactic acid bacteria to improve fermentation, safety and shelf-life of dairy products is gaining increasing attention. This study evaluated the fermentative and antibacterial potential of lactic acid bacteria isolated from cheese whey for the production of pineapple-enriched fermented milk. Lactic acid bacteria were isolated from wara-derived cheese whey and identified using standard morphological and biochemical tests. The isolates were used as starter cultures in powdered milk enriched with pineapple juice and incubated for four hours under laboratory conditions. The physicochemical and sensory properties of the fermented milk samples were determined, while crude bacteriocin extracts from the isolates were screened for antibacterial activity against selected foodborne pathogens using the agar well diffusion method. The isolates were identified as *Lactobacillus* sp. 1 and *Lactobacillus* sp. 2. The fermented milk samples had moisture content ranging from 72.28 to 72.42%, ash content from 0.99 to 1.09%, carbohydrate from 22.26 to 22.56%, lipid from 0.09 to 0.12%, crude protein from 3.92 to 4.21%, and calorific value from 445.86 to 447.13 kJ/100 g. Titratable acidity ranged from 7.21 to 7.25 mg/ml. The sensory acceptability scores ranged from 66.40 to 84.80%, with the combined starter culture sample showing higher acceptability. Crude bacteriocin from the isolates inhibited selected pathogens, with zones of inhibition recorded against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The findings show that cheese whey-derived lactic acid bacteria possess promising fermentative and bacteriocinogenic activity and may serve as starter and bioprotective cultures in pineapple-enriched fermented milk production.

Keywords: Lactic acid bacteria; *Lactobacillus*; cheese whey; pineapple-enriched fermented milk; bacteriocin; biopreservation.

INTRODUCTION

Dairy manufacture continues to be a large-scale problem for food safety due to the continual presence of pathogenic microorganisms in the manufacturing process. Pathogenic microorganisms cause foodborne diseases, and diarrhoeal diseases cause public health concerns, food insecurity and decreased economic growth. This problem is found all around the world, including Nigeria (Anyogu *et al.*, 2021; Uzeh and Imafidon 2022; Chukwu *et al.*, 2023). In Nigeria specifically, common contaminants identified in traditional fermented dairy products such as wara

and nono include *Escherichia coli*, *Listeria monocytogenes*, *Bacillus cereus*, and *Salmonella* spp. Pathogenic microorganisms are typically associated with the use of unpasteurized milk, inadequate personal hygiene during milk-handling, lack of adherence to temperature and time control during milk processing (Fayyaz *et al.*, 2022; Focardi, 2022; Sessou *et al.*, 2023). In addition, antibiotic therapy for foodborne infections is contributing to resistance, during the past several years, in areas where antibiotics were indiscriminately administered to livestock as a result of their veterinary uses (Yakubu *et al.*, 2020; Adeiza *et al.*, 2022; Yusuf *et al.*, 2023; Aworh *et al.*,

2024). Consequently, the effectiveness of antibiotic therapy is limited and contributes to prolonging recovery from foodborne infection (Yakubu *et al.*, 2020; Adeiza *et al.*, 2022; Yusuf *et al.*, 2023; Aworh *et al.*, 2024).

To enhance both the nutritional safety and quality of fermented foods, Lactic Acid Bacteria offer a sustainable and non-antibiotic alternative (Ibrahim *et al.*, 2021; Noguerol *et al.*, 2021; Sidhu and Nehra, 2021; Sharma *et al.*, 2022; Ahansaz *et al.*, 2023). They are considered to be generally regarded as safe (GRAS) and produce antimicrobial agents like organic acid, hydrogen peroxide and bacteriocins that can inhibit unwanted micro-organisms from growing in food systems (Ibrahim *et al.*, 2021; Noguerol *et al.*, 2021; Sidhu and Nehra, 2021; Sharma *et al.*, 2022; Ahansaz *et al.*, 2023). Several strains of lactic acid bacteria also possess probiotic properties which may promote gut health and aid in immune function (Noguerol *et al.*, 2021; Sharma *et al.*, 2022; Ahansaz *et al.*, 2023). The ability of lactic acid bacteria to enhance the fermentation of dairy products while at the same time providing a protective effect against foodborne illness, makes these bacteria important in the production of dairy products and in the development of functional foods (Ibrahim *et al.*, 2021; Sidhu and Nehra, 2021; Ahansaz *et al.*, 2023).

Fermented dairy drinks can be made more appealing to the senses, nutritious, and better functional products by adding pulp from tropical fruits such as pineapple (Byresh *et al.*, 2022; Terzoglu *et al.*, 2022; Bakar *et al.*, 2024; Priyashantha *et al.*, 2025). The addition of pineapple gives foods a pleasant taste and smell, like fiber, and nutrients with antioxidant properties (Byresh *et al.*, 2022; Terzoglu *et al.*, 2022; Bakar *et al.*, 2024; Priyashantha *et al.*, 2025). Additionally, adding pineapple to yogurt will increase diversity and approval of consumers toward fermented dairy products (Byresh *et al.*, 2022; Bakar *et al.*, 2024; Priyashantha *et al.*, 2025). This provides an area worth studying especially where tropical fruit and dairy products are both local resources (Bakar *et al.*, 2024; Priyashantha *et al.*, 2025).

The under-utilization of cheese whey generated during the production of Wara in Nigeria is due to a lack of awareness about the many ways it can be used as an economical source of viable lactic acid bacteria for technological use (Ajibola *et al.*, 2021;

Melia *et al.*, 2022; Oyedokun *et al.*, 2023). Additionally, although Wara can provide direct employment and household income, the way cheese whey is treated is that it is usually discarded or under-utilised as a by-product of Wara production (Ajibola *et al.*, 2021; Oyedokun *et al.*, 2023). As such, incorporating cheese whey into the process of producing local fermented milks and other fermented milk products would benefit local communities by providing new sources of starter cultures with practical uses and commercial potential for producing safe, high-quality dairy products by using indigenous methods; reducing waste from cheese whey and creating new opportunities for local employment. In conclusion, further carry out investigation into the potential uses of cheese whey as a source of bacteria is needed (Ajibola *et al.*, 2021; Melia *et al.*, 2022; Oyedokun *et al.*, 2023).

Many studies have been conducted on the use of probiotic lactic acid bacteria in fruit-enhanced fermented milk beverages (Shukla, 2012; Baba *et al.*, 2016; Barat and Özcan, 2017; Islam *et al.*, 2021). However, few studies have investigated the potential for utilising lactic acid bacteria recovered from Nigerian cheese whey as both starter and protective cultures for the production of pineapple-enhanced fermented milk (Sangoyomi *et al.*, 2010; Ayeni *et al.*, 2011; Ajibola *et al.*, 2021; Oyedokun *et al.*, 2023). In contrast to previous work, this study will focus on identifying locally sourced cheese whey as a low-cost reservoir of lactic acid bacteria that might be used as an ingredient in local dairy products (Ayeni *et al.*, 2011; Oyedokun *et al.*, 2023). This research is significant because if large amounts of cheese whey are produced during the making of wara, they typically are underutilised and contain many valuable microorganisms with potential for use in making food (Sangoyomi *et al.*, 2010; Garcia *et al.*, 2020; Ajibola *et al.*, 2021). Hence, this research offers regionally applicable proof regarding the fermentative and anti-bacterial abilities of lactic acid bacteria obtained from cheese whey in aiding the safe production of fruit-enhanced fermented milk products (Osuntoki and Korie, 2010; Ajibola *et al.*, 2021; Melia *et al.*, 2022). The goal of this research was to assess the fermentative and anti-bacterial capabilities of cheese whey-derived lactic acid bacteria in relation to the production of pineapple-flavoured fermented milk. The aim of this study was to evaluate the fermentative and antibacterial potentials of lactic acid bacteria isolated from cheese whey for the production of pineapple-enriched fermented milk.

Materials and Methods

Sample collection

The samples of cheese whey were collected into pre sterilized sample bottles from retailers at Ipata market in Ilorin, Kwara State, aseptically transported to the lab on ice pack, and used within 4 hours of collection. Pineapple juice (*Ananas comosus*) was bought in Frootify, Ilorin. Powdered milk was locally sourced from a local vendor for the fermentation experiment (Ana Claudia *et al.*, 2019). The test Foodborne pathogenic bacteria used were *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Salmonella enterica* and *Pseudomonas aeruginosa* obtained from the culture collection of Department of Microbiology, Kwara State of University, Nigeria.

Isolation of Lactic Acid Bacteria from Cheese Whey

The cheese whey samples were subjected to a ten-fold serial dilution, 0.1 ml of appropriate dilution was plated into a molten de Mann Ragusa Sharpe Agar (MRSA) using pour plate method. The plates were incubated at 37 degree celsius for 24 hours. Distinct colonies were sub-cultured repeatedly to obtain pure culture. The isolates were maintained on agar slants for further research (Fawole and Oso, 2007; Misganaw and Teketay, 2016).

Characterization and Identification of the LAB Isolated

The LAB isolates were subjected to Gram's reaction (Oladeji and Odelade, 2016), motility test and biochemical tests for identification. Isolates were screened for their ability to produce indole, oxidase, catalase, and urease production following standard methods described and used by previous authors (Aborisade *et al.*, 2024). The LAB isolates were also subjected to sugar fermentation test to determine their ability to utilize standard sugar to produce acid with or without gas (Aborisade *et al.*, 2024).

Confirmation Tests on the Biochemical Characteristics of the Test Bacteria

The test Foodborne pathogenic bacteria were subjected to Gram's reaction (Oladeji and Odelade, 2016), motility test and biochemical tests for identification. Isolates were screened for their ability to produce indole, oxidase, catalase, and urease, production and others following standard methods described and used by previous authors (Aborisade *et al.*, 2024). The isolates were also subjected to sugar fermentation test to determine their ability to utilize standard sugar to produce acid with or without gas (Aborisade *et al.*, 2024).

Production of Fruit-enriched Fermented Milk using Lactic Acid Bacteria Isolated Inoculum Preparation

Overnight (18-hours old) cultures of isolates were inoculated into MRS broth and incubated at 37 degree celsius for 24 hours to obtain actively growing cells. The cultures were adjusted to 0.5 McFarland standard using normal saline and used as single and mixed culture inocula (Barat and Ozcan, 2018).

Fermentation Procedure

In sterile fermentation flasks, powdered milk (80 g) was prepared using 200 mL of sterile distilled water and heated for 10 minutes at 70 °C in a water bath shaker. After completion of heating, flasks were allowed to cool to 40 °C before being inoculated. Flask A received an inoculation of 5 mL of standardised inoculum from isolate A. Flask B received the same 5 mL of standardised inoculum from isolates A and B to test the effect of both starting cultures together. Flask C was the control (uninoculated). The inoculated flasks were allowed to incubate at 28 °C for four hours without shaking (Barat and Ozcan, 2018). In this study, isolate B could not be tested as a sole pure starter culture, therefore the contribution of isolate B to fermentation processes could not be fully evaluated. Future studies need to incorporate each isolate separately (isolate A, isolate B), together (combined), and no-inoculation (uninoculated control) to provide better insight and comparison.

Fortification of Fermented Milk with Fruit Juices

Pasteurization of the juice was carried out at 70 °C at 10 minutes, after which the juice was cooled and filtered with a sterile muslin cloth in order to eliminate pulp. The fermented milk was inoculated by adding 10% (v/v) of the purified juice and then the LAB cultures were added. A control sample that was free of juice was also made.

Proximate Analysis of Fermented Products Moisture Content

Precisely 5 ml of fermented milk samples were added to pre-weighed aluminium dishes and kept in an oven preset at 80 °C for 2 hours, the dishes were cooled in desiccators and re-weighed until constant weight. The moisture was obtained in percentages of weight loss against starting sample weight (Nemo and Bacha, 2020).

Ash Content

Samples (20 ml) were burnt to ash in pre-weighed platinum crucibles at 550 °C. The residual ash weight was used as the ash content calculated as a percentage of the original sample weight (Nemo and Bacha, 2020).

Crude Fat Content

Soxhlet extraction of 15 ml of samples was carried using petroleum ether for 6 hours. The solvent evaporated in a rotary evaporator. The amount of lipid was presented as a percentage of dry matter (Nemo and Bacha, 2020).

Crude Protein Content

Samples were (1 g) digested using K_2SO_4 , $CuSO_4$ and concentrated H_2SO_4 by Kjeldahl digestion technique and subsequently distilled using NaOH and titrated with HCL. The total nitrogen was determined and expressed as crude protein multiplied by 6.38 N factor of milk (Nemo and Bacha, 2020).

Carbohydrate and Total Energy

Carbohydrate was determined using sum of other parameters and difference from 100 percent: 100 - sum of moisture, ash, fat, fibre and crude protein. Energy was estimated as (protein x 16.7) + (lipid x 37.7) + (Carbohydrate x 16.7) (Nemo and Bacha, 2020).

Total Titratable Acidity

Exactly 25 ml of sample was titrated with 0.01 M NaOH using phenolphthalein as indicator. Titratable acidity was calculated based on NaOH volume used (Nemo and Bacha, 2020).

Sensory Evaluation

Sensory evaluation was conducted after ethical approval was obtained from the Department of Microbiology, Kwara State University. The panel members were informed about the purpose of the study, the nature of the samples, and their right to withdraw from the evaluation at any stage. Only individuals who gave consent participated in the sensory assessment. The samples were served under hygienic conditions in coded containers to prevent bias.

A sensory evaluation was conducted using five trained assessors. The assessors evaluated the fermented milk samples for appearance, aroma, texture, aftertaste and overall acceptability using a five-point hedonic scale, where 1 represented "dislike extremely" and 5 represented "like extremely". The samples were chilled before serving and presented in coded containers. Water was provided to the assessors for mouth rinsing between samples. The sensory scores were recorded and expressed as mean $\pm$ standard deviation (Nemo and Bacha, 2020).

Bacteriocinogenic Assay of Crude Bacteriocin

Extraction and Partial Purification of Crude Bacteriocin

The LAB cultures were inoculated into 1 liter of MRS broth at 30 degree celsius and incubated for 72 hours. The culture obtained was filtered after centrifugation at 1000 rpm for 5 minutes, cell-free supernatants obtained were adjusted to a pH of 6.5. Ammonium sulfate was added to it to precipitate protein, centrifuged at 1000 rpm for 5 minutes, and dialysed using 0.1M phosphate buffer at pH 7.0. The resulting liquid obtained was taken as the crude bacteriocin, these were placed at -20 °C until used (Ahmad *et al.*, 2017).

Bacteriocinogenic Screening on Test Bacteria

The partially purified bacteriocins (100 uL) were added to 6 cm diameter wells on Mueller-Hinton agar pre impregnated with standardized suspensions of the test pathogens (*E. coli*, *S. aureus* and *B. subtilis* and not *S. enterica* and *P. aeruginosa*) (adjusted to 0.5 McFarland standard). The plates were pre-incubated at 4 degree celsius temperature condition for easy penetration of the bacteriocins into the agar matrices and subsequently incubated at 37 °C for 24 hours. Zone of growth inhibition was measured in mm (Ahmad *et al.*, 2017).

Statistical Analysis

Data obtained from proximate composition, titratable acidity, and sensory evaluation were recorded in triplicate where applicable and expressed as mean $\pm$ standard deviation. The data was analysed using one-way analysis of variance. Differences between means were considered statistically significant at $p < 0.05$. Where significant differences occurred, a post-hoc test should be used to identify the sample pairs responsible for the difference. The analysis was carried out using Microsoft Excel 2016.

Results and Discussion

Colonial Morphology and Biochemical Characteristics of Lactic Acid Bacteria Isolated from Cheese Whey

Two species of *Lactobacillus* were obtained from the cheese whey and identified as *Lactobacillus* sp. 1 and *Lactobacillus* sp. 2. The two isolates were Gram positive, rod shaped, non-motile bacteria, negative to oxidase, citrate, and indole production but *Lactobacillus* sp 2 was positive to urease production (Table 1). The characteristics were in tandem with previous submission on the identification features of *Lactobacillus* species (Mokoena *et al.*, 2021). The proficiency of *Lactobacillus* species in dairy whey has been noticed many times naturally in milk and milk environments, their species contribute greatly to

spontaneous acidic fermentation in dairy and other products (He *et al.*, 2022). The *Lactobacillus* species have fermentative potency with sugar production, an attribute that is highly pivotal to food fermentation especially in the dairy industry and

other food processing industries (Benitez *et al.*, 2021).

Table 1: Morphological and Biochemical Characteristics of the Lactic Acid Bacteria Isolated from Cheese Whey

Isolated from Cheese Whey								
Isolate s	Morphological	Characteristics	Biochemical Characteristics					
			Catalase	Citrate	Urease	Indole	Coagulase	Oxidase
Isolate A	Rod	White	+	-	-	-	-	-
Isolate B	Rod	Cream	+	-	+	-	-	-

Keys: + = Positive; - = Negative; Isolate A = *Lactobacillus* sp. 1; Isolate B = *Lactobacillus* sp. 2

Colonial Morphology and Biochemical Characteristics of the Test Foodborne Pathogens

The morphology and biochemical characteristics of the test bacteria are presented in Table 2. The observed characteristics were in consent with early submissions on these organisms. They are most often in association with food and are frequently isolated from food and food environments, especially under conditions of

inferior hygiene (Kivanc and Yapici, 2018). *Escherichia coli* and *S. aureus* are among the frequently reported isolates in Foodborne disease. They are major agents in spoilage and Foodborne infections and have been recovered from milk obtained from informal markets in Africa (Elmonir *et al.*, 2018). Their survival in processed food could be attributed to low hygiene quality and insufficient heat during the processing and distribution line (Machado *et al.*, 2017).

Characteristics	Organism 1	Organism 2	Organism 3	Organism 4	Organism 5
Shape	Rod	Rod	Cocci	Rod	Rod
Colour / Pigment	–	–	+	–	+
Gram Reaction	–	–	+	+	–
Motility	+	+	–	+	+
Oxidase	–	–	–	–	+
Catalase	+	+	+	+	+
Citrate	–	+	+	+	+
Coagulase	–	–	+	Variable	–
Indole Test	+	–	–	–	–
Urease	–	–	+	–	–
Methyl Red (MR)	+	+	+	–	–
Voges Proskauer (VP)	–	–	+	+	–
Glucose Fermentation	+	+	+	+	+
Lactose Fermentation	+	–	+	Variable	–
Sucrose Fermentation	+	–	+	+	+
Gas Production	+	–	–	–	–
Hydrogen Sulphide (H ₂ S)	–	+	–	–	–
Probable Organisms	<i>Escherichia coli</i>	<i>Salmonella enterica</i>	<i>Staphylococcus aureus</i>	<i>Bacillus subtilis</i>	<i>Pseudomonas aeruginosa</i>

Table 2: Colonial Morphology and Biochemical Characteristics of Tested Food borne Pathogens

Physicochemical Composition of Fermented Milk Products

The physicochemical composition of the samples showed slight variations, with moisture content ranging from 72.28 to 72.71% and ash content ranging from 0.99 to 1.09%, which are within the expected range for fermented dairy beverages (Managa *et al.*, 2021; Paula *et al.*, 2021). The fermented samples recorded higher carbohydrate and protein contents than the control, with Sample B having the highest protein value of 4.21%. This increase may be linked to the proteolytic activity of lactic acid bacteria, which can release soluble nitrogen compounds and improve the nutritional quality of fermented milk (Hamad *et al.*, 2019; Nasution *et al.*, 2025). However, this differs from

some studies where protein reduction was reported with increased buttermilk inclusion (Pereira *et al.*, 2021). The higher lipid content of the control sample may explain its higher calorific value. Titratable acidity was also higher in the fermented samples, with values of 7.21 to 7.25 mg/ml compared with 5.04 mg/ml in the control. This increase may be due to the conversion of available sugars into lactic acid by lactic acid bacteria during fermentation (Hamad *et al.*, 2019; Managa *et al.*, 2021; Nasution *et al.*, 2025). This agrees with previous findings on pineapple-enriched yoghurt, where fruit addition and lactic acid bacteria fermentation contributed to increased acidity (Islam *et al.*, 2021).

Table 3: Physicochemical Composition of Fermented Powdered Milk

Sample	Moisture (%)	Ash (%)	Carbohydrate (%)	Lipid (%)	Crude protein (%)	Calorific value (kJ/100 g)	Titrateable acidity (mg/ml)
Sample A	72.42 ± 0.08	0.99 ± 0.02	22.56 ± 0.10	0.09 ± 0.01	3.92 ± 0.05	445.86 ± 0.90	7.25 ± 0.02
Sample B	72.28 ± 0.07	1.09 ± 0.03	22.26 ± 0.09	0.12 ± 0.01	4.21 ± 0.06	447.13 ± 1.10	7.21 ± 0.01
Control	72.71 ± 0.09	0.99 ± 0.02	15.90 ± 0.12	7.41 ± 0.15	2.96 ± 0.04	594.80 ± 1.50	5.04 ± 0.02

Values are presented as mean ± standard deviation of triplicate determinations

Keys: Sample A = Milk fermented with *Lactobacillus* sp.1 only (Single Starter Culture); Sample B = Milk fermented with *Lactobacillus* sp.1 and 2 (Combined Starter Culture); Ctrl = Control (Uninoculated Milk)

Organoleptic Properties of the Fermented Milk

The sensory result showed that Sample B performed better than Sample A across all the evaluated sensory attributes, with a total acceptability score of 84.80% compared with 66.40% for Sample A. This suggests that the combined lactic acid bacteria culture improved the sensory quality of the pineapple-enriched fermented milk, possibly through better aroma, texture and flavour development during fermentation (Pereira *et al.*, 2021; Buvaneswaran *et al.*, 2023). This finding agrees with previous studies that reported high acceptability scores for pineapple-enriched fermented dairy products

(Pandey *et al.*, 2019; Pereira *et al.*, 2021; Nag and Goyal, 2023). However, it contrasts with Hamad *et al.* (2019), where the control sample was preferred, possibly because pineapple acidity affected coagulation and sensory quality. Since only five assessors were used in the present study, the sensory findings should be considered preliminary and should be confirmed using a larger sensory panel in future studies (Molero and Bríñez, 2018).

The control sample, an uninoculated milk sample, was not included in the organoleptic evaluation because it was not representative of the lactic acid bacteria (LAB) fermented fruit mix product we were evaluating. The sensory evaluation was restricted to Samples A and B which were both actual products created throughout the course of this project.

Table 4: Organoleptic Properties of the Fermented Milk Samples

Sample	Appearance	Aroma	Texture	Aftertaste	Overall acceptability	Total acceptability score (%)
Sample A	3.40 ± 0.55	3.20 ± 0.45	3.40 ± 0.55	3.20 ± 0.45	3.40 ± 0.55	66.40
Sample B	4.20 ± 0.45	4.40 ± 0.55	4.20 ± 0.45	4.20 ± 0.45	4.20 ± 0.45	84.80

Values are presented as mean ± standard deviation based on the scores of five assessors

Keys: Sample A = Milk fermented with *Lactobacillus* sp. 1 only; Sample B = Milk fermented with *Lactobacillus* sp. 1 and fruit blend

Note: Since the uninoculated control was not a fermented product or had LAB starter cultures added, organoleptic evaluation was not conducted. The sensory evaluation was only designed to compare the acceptability of the LAB fermented milk samples, so only Samples A and B were organoleptically evaluated.

Antimicrobial Activity of Bacteriocin Produced by LAB Isolates

The crude bacteria of strain B produced a bacteriocin that inhibited the growth of *Escherichia coli* and *Staphylococcus aureus*, while the crude bacteria of strain A produced a bacteriocin that inhibited the growth of the same organisms plus *Bacillus subtilis* (Kanagaraj *et al.*, 2012; Yang *et al.*, 2012; Afrin *et al.*, 2021). The results of these studies suggest that lactic acid bacteria from the cheese whey can produce bacteriocins that possess antibacterial activity against some pathogenic microorganisms that are important causes of foodborne disease and potentially have the potential to be used as biopreservatives in fermented milk with pineapple (Kanagaraj *et al.*, 2012; Yang *et al.*, 2012; Silva *et al.*, 2018; Volpane *et al.*, 2021). The mode of action of bacteriocins is

hypothesized to involve the disruption of the structural integrity of bacterial cell membranes through pore formation, increased membrane permeability, and the loss of proton motive force (Ibrahim *et al.*, 2021; Imade *et al.*, 2021; Sidhu and Nehra, 2021). The differences in biochemical activity of isolate A as opposed to isolate B could be attributed to differences in bacteriocin production between the two isolates, to differences in their target cell wall structure, or differences in their membrane permeability (Ibrahim *et al.*, 2021; Imade *et al.*, 2021). This supports the findings of previous studies showing that bacteriocins produced by lactic acid bacteria from cheese and fermented dairy products are inhibitory to *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and certain strains that have resistance to antibiotics (Kanagaraj *et al.*, 2012; Afrin *et al.*, 2021; Bashir and Ali, 2021; Imade and Omonigho, 2021; Thuy *et al.*, 2024). However, resistance to these bacteriocins has been reported among some Gram-negative bacteria such as *Escherichia coli*, suggesting that the bacteriocin activity is influenced by both the strain of bacteria and the type of crude extract used (Afrin *et al.*, 2021). In general, these findings support the idea that bacteriocin-producing LAB could be used as bioprotective cultures in dairy product formulation and processing, particularly when there is a need for improved microbial safety and shelf-life of the finished product (Silva *et al.*, 2018; Ahansaz *et al.*, 2023; Kotsaki *et al.*, 2025).

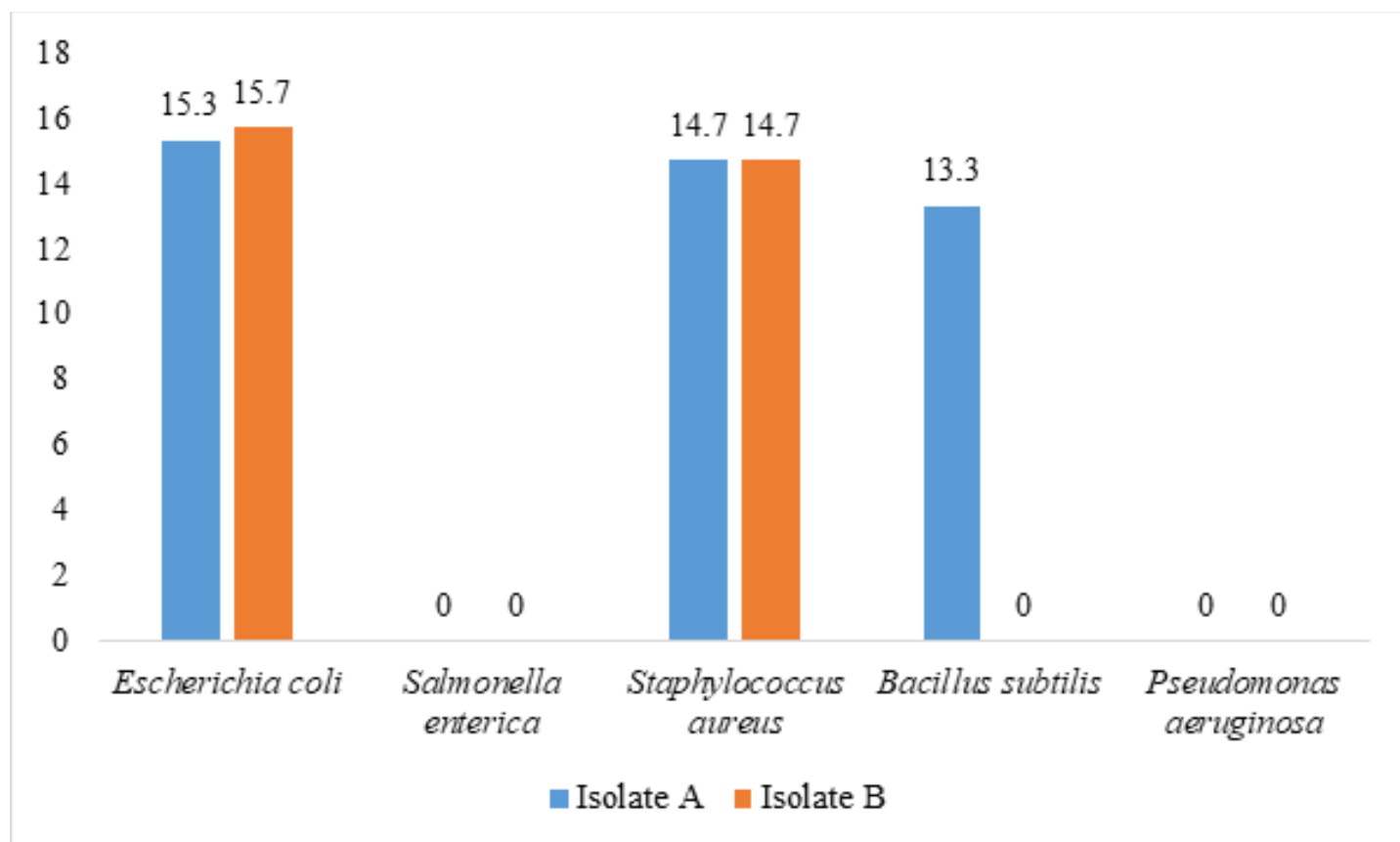


Figure 1: Zones of Inhibition Exhibited by Bacteriocins from Lactic Acid Bacteria Isolates Against Selected Foodborne Pathogens.



Plate 1: Clear zones of inhibition indicated by arrowheads, confirming antimicrobial activity of certain lactic acid bacteria isolates against test pathogens. The plates with no arrow heads indicate no zone of inhibition.

Conclusion

This study evaluated the fermentative and antibacterial activities of lactic acid bacteria isolated from cheese whey. The findings showed that the isolates were able to ferment milk and contribute to acidity development in the fermented product. The crude bacteriocin produced by the isolates also showed antibacterial activity against selected foodborne pathogens, including *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*. The observed zones of inhibition suggest that the isolates possess bioprotective potential and may help improve the microbial safety of fermented dairy products. Overall, the study confirms that cheese whey-derived lactic acid bacteria have promising dual functions as starter cultures and antibacterial agents in pineapple-enriched fermented milk production. However, further validation is required before industrial application.

Recommendation

Further studies should be carried out to identify the isolates using molecular techniques such as 16S rRNA gene sequencing. The bacteriocins produced by the isolates should also be purified and characterised to determine their stability, safety and spectrum of antibacterial activity. Future studies should test each isolate separately and in combination to better understand their individual and combined fermentative effects. Larger sensory panels, shelf-life studies, probiotic screening and safety assessment are also recommended to confirm the suitability of the isolates for possible use in the dairy industry.

References

- Aborisade, W. T., Ajao, A. T., Ntagbu, F. G., & Bale, S. I. (2024). Isolation and screening for potential endosulfan-degrading bacteria from soil. *Dutse Journal of Pure and Applied Sciences*, 10(2), 1–10. <https://doi.org/10.4314/dujopas.v10i2a.7>
- Afrin, S., Hoque, M. A., Sarker, A. K., Satter, M. A., & Bhuiyan, M. N. I. (2021). Characterization and profiling of bacteriocin-like substances produced by lactic acid bacteria from cheese samples. *Access Microbiology*, 3(6), Article 234. <https://doi.org/10.1099/acmi.0.000234>
- Ahansaz, N., Tarrah, A., Pakroo, S., Corich, V., & Giacomini, A. (2023). Lactic acid bacteria in dairy foods: Prime sources of antimicrobial compounds. *Fermentation*, 9(11), 964. <https://doi.org/10.3390/fermentation9110964>
- Ahmad, V., Khan, M. S., Jamal, Q. M. S., Alzohairy, M. A., Al Karaawi, M. A., & Kamal, M. A. (2017). Methods of screening, purification and antimicrobial potentialities of bacteriocin in health care. *Current Drug Metabolism*, 18(9), 814–830. <https://doi.org/10.2174/1389200218666170116111309>
- Ajibola, W. G., Adeyemo, S. M., & Omafuvbe, B. O. (2021). Effect of starter culture fermentation of milk on the production, sensory attributes and storage of wara, a Nigerian unripened soft cheese. *Ife Journal of Science*, 22(3), 107–117. <https://doi.org/10.4314/ijfs.v22i3.9>
- Anyogu, A., Olukorede, A., Anumudu, C., Onyeaka, H., Areo, E., Adewale, O., Odimba, J. N., & Nwaiwu, O. (2021). Microorganisms and food safety risks associated with indigenous fermented foods from Africa. *Food Control*, 129, 108227. <https://doi.org/10.1016/j.foodcont.2021.108227>
- Aworh, M. K., Nilsson, P., Egyir, B., Owusu, F., & Hendriksen, R. S. (2024). Rare serovars of nontyphoidal *Salmonella enterica* isolated from humans, beef cattle and abattoir environments in Nigeria. *PLoS ONE*, 19(1), e0296971. <https://doi.org/10.1371/journal.pone.0296971>
- Ayeni, F. A., Sánchez, B., Adeniyi, B. A., de los Reyes-Gavilán, C. G., Margollés, A., & Ruas-Madiedo, P. (2011). Evaluation of the functional potential of *Weissella* and *Lactobacillus* isolates obtained from Nigerian traditional fermented foods and cow's intestine. *International Journal of Food Microbiology*, 147(2), 97–104. <https://doi.org/10.1016/j.ijfoodmicro.2011.03.014>
- Baba, W. N., Din, S., Punoo, H. A., Wani, T. A., Ahmad, M., & Masoodi, F. A. (2016). Comparison of cheese and paneer whey for production of a functional pineapple beverage: Nutraceutical properties and shelf life. *Journal of Food Science and Technology*, 53(6), 2558–2568. <https://doi.org/10.1007/s13197-016-2218-8>
- Bakar, S. A. A., Muhamad, N., Nordin, M. F. M., & Zaidel, D. N. A. (2024). Physicochemical properties and sensory evaluation of yogurt formulated with pineapple and roselle fibres. *Journal of Advanced Research in Micro and Nano Engineering*, 18(1), 1–8. <https://doi.org/10.37934/armne.18.1.18>
- Barat, A., & Özcan, T. (2017). Growth of probiotic bacteria and characteristics of fermented milk containing fruit matrices. *International Journal of Dairy Technology*, 71, 120–129. <https://doi.org/10.1111/1471-0307.12391>
- Bashir, A., & Ali, K. (2021). Antagonistic activity of bacteriocins produced by *Lactobacillus* isolates against multidrug resistant pathogens. *Journal of Pharmaceutical Research International*, 33(59B), 76–87. <https://doi.org/10.9734/jpri/2021/v33i59b34354>
- Benítez, S. F., Sadañoski, M. A., Velázquez, J. E., Zapata, P. D., & Fonseca, M. I. (2021). Comparative study of single cultures and a consortium of white rot fungi for polychlorinated biphenyls treatment. *Journal of Applied Microbiology*, 131(4), 1775–1786. <https://doi.org/10.1111/jam.15073>

- Borgonovi, T. F., Casarotti, S. N., & Penna, A. L. B. (2021). Lacticaseibacillus casei SJRP38 and buriti pulp increased bioactive compounds and probiotic potential of fermented milk. *LWT*, 143, 111124. <https://doi.org/10.1016/j.lwt.2021.111124>
- Borgonovi, T. F., Fugaban, J. I. I., Bucheli, J. E. V., Casarotti, S. N., Holzapfel, W. H., Todorov, S. D., & Penna, A. L. B. (2024). Dual role of probiotic lactic acid bacteria cultures for fermentation and control pathogenic bacteria in fruit-enriched fermented milk. *Probiotics and Antimicrobial Proteins*, 16(5), 1801–1816. <https://doi.org/10.1007/s12602-023-10135-w>
- Buveneswaran, M., Sunil, C. K., Rawson, A., Vidyalakshmi, R., & Natarajan, V. (2023). Effect of pineapple core powder on white finger millet vegan probiotic beverage: Nutrition, sensory and storage. *Food Chemistry Advances*, 4, 100593. <https://doi.org/10.1016/j.focha.2023.100593>
- Byresh, T. S., Buveneswaran, M., Meena, L., Sunil, C. K., Chidanand, D. V., Vidyalakshmi, R., & Natarajan, V. (2022). Effect of addition of pineapple peel powder on white finger millet vegan probiotic beverage. *Journal of Food Processing and Preservation*, 46(10), e16905. <https://doi.org/10.1111/jfpp.16905>
- Chukwu, O. S., Uzoh, C. V., Yusuf, I., Aroh, K. E., Owolabi, O. J., Kalu, A. C., Egwu-Ikechukwu, M. M., Nnabugwu, C. C., Azuama, O. C., Ilang, D. C., Ugwu, B., Okata-Nwali, D. O., & Ugwuocha, C. S. (2023). Isolation, identification and antibiotic resistance profile of public health threat enteric bacteria from milk and dairy products retail in Abakaliki, South-East, Nigeria. *Journal of Pure and Applied Microbiology*, 17(3), 1620–1627. <https://doi.org/10.22207/jpam.17.3.23>
- Elmonir, W., Abo-Remela, E., & Sobeih, A. (2018). Public health risks of *Escherichia coli* and *Staphylococcus aureus* in raw bovine milk sold in informal markets in Egypt. *The Journal of Infection in Developing Countries*, 12(7), 533–541. <https://doi.org/10.3855/jidc.9509>
- Fayyaz, K., Nawaz, A., Olaimat, A. N., Akram, K., Farooq, U., Fatima, M., Siddiqui, S. A., Rana, I. S., Mahnoor, M., & Shahbaz, H. M. (2022). Microbial toxins in fermented foods: Health implications and analytical techniques for detection. *Journal of Food and Drug Analysis*, 30(4), 523–537. <https://doi.org/10.38212/2224-6614.3431>
- Focardi, S. (2022). The microbiology of cheese and dairy products is a critical step in ensuring health, quality and typicity. *Clinical Journal of Dairy and Veterinary Sciences*, 3(3), 1–9. <https://doi.org/10.54026/cjdvs1043>
- Garcia, S. L. A., Silva, G. M. da, Medeiros, J. M. S., Queiroga, A. P. R. de, Queiroz, B. B. de, Farias, D. R. B. de, Correia, J. O., Florentino, E. R., & Buriti, F. C. A. (2020). Influence of co-cultures of *Streptococcus thermophilus* and probiotic lactobacilli on quality and antioxidant capacity parameters of lactose-free fermented dairy beverages containing *Syzygium cumini* (L.) Skeels pulp. *RSC Advances*, 10(17), 10297–10308. <https://doi.org/10.1039/c9ra08311a>
- Hamad, M., El-Sayed, S., & Anees, R. (2019). Technology and microbiological studies on some probiotic dairy beverages fortified with pineapple pulp. *Journal of Food and Dairy Sciences*, 10(4), 125–129. <https://doi.org/10.21608/jfds.2019.36187>
- He, J., Guo, K., Chen, Q., & Wang, Y. (2022). Camel milk modulates the gut microbiota and has anti-inflammatory effects in a mouse model of colitis. *Journal of Dairy Science*, 105(5), 3782–3793. <https://doi.org/10.3168/jds.2021-21345>
- Ibrahim, S. A., Ayivi, R. D., Zimmerman, T., Siddiqui, S. A., Altemimi, A. B., Fidan, H., Esatbeyoglu, T., & Bakhshayesh, R. V. (2021). Lactic acid bacteria as antimicrobial agents: Food safety and microbial food spoilage prevention. *Foods*, 10(12), 3131. <https://doi.org/10.3390/foods10123131>
- Imade, E. E., Omonigho, S. E., Babalola, O. O., & Enagbonma, B. J. (2021). Lactic acid bacterial bacteriocins and their bioactive properties against food-associated antibiotic-resistant bacteria.

Annals of Microbiology, 71(1), Article 44. <https://doi.org/10.1186/s13213-021-01652-6>

- Islam, Z., Tabassum, S., Harun-ur-Rashid, M., Vegarud, G. E., Alam, M., & Islam, M. A. (2021). Development of probiotic beverage using whey and pineapple (*Ananas comosus*) juice: Sensory and physico-chemical properties and probiotic survivability during in vitro gastrointestinal digestion. *Journal of Agriculture and Food Research*, 4, 100144. <https://doi.org/10.1016/j.jafr.2021.100144>
- Kanagaraj, N., Duraisamy, S., Balakrishnan, S., Narayanapillai, U., & Ramasamy, G. (2012). Characterization of bacteriocin producing lactic acid bacteria and its application as a food preservative. *African Journal of Microbiology Research*, 6(6), 1138–1146. <https://doi.org/10.5897/AJMR11.1214>
- Kivanc, M., & Yapici, E. (2018). Survival of *Escherichia coli* O157:H7 and *Staphylococcus aureus* during kefir fermentation and storage. *Food Science and Technology*, 39(1), 225–230. <https://doi.org/10.1590/fst.39517>
- Kotsaki, P., Aspri, M., & Papademas, P. (2025). Novel whey fermented beverage enriched with a mixture of juice concentrates: Evaluation of antimicrobial, antioxidant, and angiotensin I converting enzyme inhibitory activities before and after simulated gastrointestinal digestion. *Microorganisms*, 13(7), 1490. <https://doi.org/10.3390/microorganisms13071490>
- Machado, S. G., Baglinière, F., Marchand, S., Van Coillie, E., Vanetti, M. C., De Block, J., & Heyndrickx, M. (2017). The biodiversity of the microbiota producing heat-resistant enzymes responsible for spoilage in processed bovine milk and dairy products. *Frontiers in Microbiology*, 8, 302. <https://doi.org/10.3389/fmicb.2017.00302>
- Managa, G. M., Akinola, S. A., Remize, F., Garcia, C., & Sivakumar, D. (2021). Physicochemical parameters and bioaccessibility of lactic acid bacteria fermented chayote leaf (*Sechium edule*) and pineapple (*Ananas comosus*) smoothies. *Frontiers in Nutrition*, 8, 649189. <https://doi.org/10.3389/fnut.2021.649189>
- Melia, S., Juliyarsi, I., Kurnia, Y. F., Rossi, E., & Alzahra, H. (2022). Selection of potential lactic acid bacteria as a candidate probiotic on cheese making by-product whey. *Advances in Animal and Veterinary Sciences*, 10(7), 1633–1640. <https://doi.org/10.17582/journal.aavs/2022/10.7.1633.1640>
- Misganaw, W., & Teketay, W. (2016). Isolation and identification of lactic acid bacteria from raw cow milk. *International Journal of Advanced Research in Biological Sciences*, 3(8), 44–49.
- Mokoena, M. P., Mutanda, T., & Olaniran, A. O. (2016). Probiotic potential of lactic acid bacteria from African fermented foods. *Food & Nutrition Research*, 60, 29630. <https://doi.org/10.3402/fnr.v60.29630>
- Nag, I., & Goyal, C. (2023). Optimization and characterization of a value-added fermented ready-to-serve whey-based beverage with high antioxidant potential. *Indian Journal of Dairy Science*, 76(6), 522–533. <https://doi.org/10.33785/ijds.2023.v76i06.002>
- Nasution, S., Kurniawati, M. Y., Lianti, L., & Rahmadi, I. (2025). Lactic acid bacteria, acidity, pH and vitamin C in yogurt from skim milk, whole milk and soybean juice powder fermented with pineapple. *IOP Conference Series: Earth and Environmental Science*, 1485(1), 012010. <https://doi.org/10.1088/1755-1315/1485/1/012010>
- Nemo, R., & Bacha, K. (2020). Microbial, physicochemical and proximate analysis of selected Ethiopian traditional fermented beverages. *LWT*, 131, 109713. <https://doi.org/10.1016/j.lwt.2020.109713>
- Noguerol, A. T., Igual, M., & Pagán, M. J. (2021). Comparison of biopreservatives obtained from a starter

- culture of *Pediococcus acidilactici* by different techniques. *Food Bioscience*, 42, 101114. <https://doi.org/10.1016/j.fbio.2021.101114>
- Olaniran, A. F., Osemwegie, O., Taiwo, E. A., Okonkwo, C. E., Ojo, O. A., Abalaka, M., Michael, T., et al. (2023). Application and acceptability of microbiomes in the production process of Nigerian indigenous foods: Drive towards responsible production and consumption. *Preventive Nutrition and Food Science*, 28(2), 108–117. <https://doi.org/10.3746/pnf.2023.28.2.108>
- Oladeji, S. O., & Odelade, K. A. (2016). Screening, isolation and identification of microorganisms from petrochemical contaminated environment. *Brazilian Journal of Biological Sciences*, 3(5), e226. <https://doi.org/10.21472/bjbs.030518>
- Osuntoki, A., & Korie, I. (2010). Antioxidant activity of whey from milk fermented with *Lactobacillus* species isolated from Nigerian fermented foods. *Food Technology and Biotechnology*, 48(4), 505–511.
- Oyedokun, N. O., Oyeleke, S. B., Abioye, O. P., & Bala, J. D. (2023). Isolation, biochemical and molecular characterization of lactic acid bacteria from whey of a Nigerian traditionally fermented yoghurt drink. *Science World Journal*, 18(3), 452–458. <https://doi.org/10.4314/swj.v18i3.19>
- Pandey, A., Mishra, A. A., Shukla, R. N., Dubey, P. K., & Vasant, R. K. (2019). Development of the process for whey based pineapple beverage. *International Journal of Current Microbiology and Applied Sciences*, 8(6), 3212–3228. <https://doi.org/10.20546/ijcmas.2019.806.383>
- Paula, N. R. F. de, Araújo, É. de O., Martins, C. R., & Martins, V. R. (2021). Processing of fermented milk drink with different whey concentrations and addition of fruit pulps. *Australian Journal of Crop Science*, 15(7), 1013–1019. <https://doi.org/10.21475/ajcs.21.15.07.p3022>
- Pereira, A. C. S., Jardim, F. B. B., & Jerônimo, M. (2021). Development of pineapple and wine flavoured fermented dairy product. *Research, Society and Development*, 10(7), e16522. <https://doi.org/10.33448/rsd-v10i7.16522>
- Priyashantha, H., Madushan, R., Pelpolage, S., Wijesekara, A., & Jayarathna, S. (2025). Incorporation of fruits or fruit pulp into yoghurts: Recent developments, challenges, and opportunities. *Frontiers in Food Science and Technology*, 5, 1581877. <https://doi.org/10.3389/frfst.2025.1581877>
- Ravyts, F., De Vuyst, L., & Leroy, F. (2012). Bacterial diversity and functionalities in food fermentations. *Engineering in Life Sciences*, 12(4), 356–367. <https://doi.org/10.1002/elsc.201100119>
- Sangoyomi, T. E., Owoseni, A. A., & Okerokun, O. (2010). Prevalence of enteropathogenic and lactic acid bacteria species in wara: A local cheese from Nigeria. *African Journal of Microbiology Research*, 4(15), 1624–1630. <https://doi.org/10.5897/AJMR.9000580>
- Sessou, P., Keisam, S., Gagara, M., Komagbe, G. S., Farougou, S., Mahillon, J., & Jeyaram, K. (2023). Comparative analyses of the bacterial communities present in the spontaneously fermented milk products of Northeast India and West Africa. *Frontiers in Microbiology*, 14, 1166518. <https://doi.org/10.3389/fmicb.2023.1166518>
- Sharma, H., Fidan, H., Özoğul, F., & Rocha, J. M. (2022). Recent development in the preservation effect of lactic acid bacteria and essential oils on chicken and seafood products. *Frontiers in Microbiology*, 13, 1092248. <https://doi.org/10.3389/fmicb.2022.1092248>
- Shukla, M. (2012). Development of probiotic beverage from whey and pineapple juice. *Journal of Food Processing and Technology*, 4(2), 206. <https://doi.org/10.4172/2157-7110.1000206>
- Sidhu, P. K., & Nehra, K. (2021). Bacteriocins of lactic acid bacteria as potent antimicrobial peptides against food pathogens. In *Biomimetics*. IntechOpen. <https://doi.org/10.5772/intechopen.95747>

- Silva, C. C. G., Silva, S. P. M., & Ribeiro, S. C. (2018). Application of bacteriocins and protective cultures in dairy food preservation. *Frontiers in Microbiology*, 9, 594. <https://doi.org/10.3389/fmicb.2018.00594>
- Sušković, J., Kos, B., Beganović, J., Pavunc, A. L., Habjanič, K., & Matošević, S. (2010). Antimicrobial activity: The most important property of probiotic and starter lactic acid bacteria. *Food Technology and Biotechnology*, 48(3), 296–307.
- Terzioğlu, M. E., Küçük, N. Y., & Bakırcı, İ. (2022). The effect of pineapple addition at different rates to sheep yoghurt on antioxidant activity, 5-hydroxymethylfurfural content, and ABT-2 probiotic culture growth. *Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 15, 84–97. <https://doi.org/10.18185/erzifbed.1199636>
- Thuy, T. T. D., Lu, H., Bregente, C. J. B., Huang, F.-C. A., Tu, P.-C., & Kao, C. (2024). Characterization of the broad-spectrum antibacterial activity of bacteriocin-like inhibitory substance-producing probiotics isolated from fermented foods. *BMC Microbiology*, 24(1), Article 94. <https://doi.org/10.1186/s12866-024-03245-0>
- Uzeh, R. E., & Imafidon, S. (2022). The occurrence of antibiotic-resistant enteric bacteria in selected Nigerian traditional dairy products. *African Health Sciences*, 22(4), 619–626. <https://doi.org/10.4314/ahs.v22i4.67>
- Volpane, V. V. A., Vergoza, D. M. M., Alderite, K. L., Bilang, J. S. D., Villa, N. M. P. D., Domingo, B. M., Foronda, P. T., & Lirio, M. R. F. (2021). Applications of bacteriocin and protective cultures in dairy products: A mini-review. *Asian Journal of Biological and Life Sciences*, 10(2), 251–257. <https://doi.org/10.5530/ajbls.2021.10.36>
- Yakubu, A., Isa, O. A., Clement, Z. W., Busayo, O. O., & Reuben, R. C. (2020). Occurrence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in fresh milk and milk products in Nasarawa State, North-Central Nigeria. *Journal of Microbiology and Antimicrobials*, 12(1), 32–41. <https://doi.org/10.5897/JMA2020.0424>
- Yang, E., Fan, L., Jiang, Y., Doucette, C., & Fillmore, S. (2012). Antimicrobial activity of bacteriocin-producing lactic acid bacteria isolated from cheeses and yogurts. *AMB Express*, 2(1), Article 48. <https://doi.org/10.1186/2191-0855-2-48>
- Yusuf, M. S., Kabir, J., Bellò, M., Aliyu, M., Esonu, D. O., Yusuf, F. L., & Babashani, M. (2023). Occurrence of tet gene-bearing antimicrobial-resistant *Escherichia coli* from dairy farms in Nigeria. *Folia Veterinaria*, 67(3), 25–32. <https://doi.org/10.2478/fv-2023-0024>