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Characterization and molecular identification of lactic acid bacteria isolated from kefir and evaluation of their antibacterial activity against food-borne pathogens in Saudi Arabia

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Abstract. – OBJECTIVE: Pathogenic bacteria are usually present in raw milk. In order to prevent pathogens from growing, milk should be fermented. The present research work aimed to isolate lactic acid bacteria (LAB) from kefir, a fermented milk beverage, and assess their antibacterial activity against chosen pathogenic strains of bacteria.

MATERIALS AND METHODS: An experimental investigation was carried out in the laboratory. Samples of kefir were collected from a local organic food store in Jeddah City, Saudi Arabia. LAB isolates were identified phenotypically and biochemically. The agar well diffusion technique evaluated the antimicrobial activities (AMAs) of LAB isolates against *Escherichia coli, Listeria monocytogenes,* and *Salmonella Typhimurium*. The isolates were molecularly identified through polymerase chain reaction PCR amplification of the 16S rRNA gene.

RESULTS: Six LAB isolates were identified and given the following numbers: SK9, SK17, SK23, SK24, SK28, and SK33. The isolates belong to Enterococcus durans and Leuconostoc mesenteroides. After screening for antibacterial activity against food-borne bacteria, SK28 showed the strongest AMA against *E. coli* and *S. Typhimurium*. SK23 showed the highest AMA against *L. monocytogenes*, while SK33 showed no AMA against *L. monocytogenes*.

CONCLUSIONS: LAB isolates exhibited AMA against the selected strains of bacteria. Enterococcus isolates showed the highest antibacterial activities against the tested bacterial strains. Therefore, in the era of antimicrobial resistance, they might serve as antibiotic alternatives. *L. monocytogenes* was the least sensitive to the antimicrobial activities of LAB isolates. Further experiments are required to isolate and identify the molecules responsible for the antimicrobial activities.

Key Words:
Lactic acid bacteria, Bacteriocin, Kefir, Probiotics, Antibacterial activity, Inhibition of food-borne pathogens, Dairy products, Fermented food, Phenotypic, 16S rRNA.

Introduction

Lactic acid bacteria (LAB) are essential for food fermentation. They are called lactic acid bacteria because they produce lactic acid as their primary metabolite[1]. LAB is a group of Gram-positive, catalase-negative, and non-spore-forming aerotolerant bacteria that involves many genera[2]. LAB are used as probiotics and have been isolated from various sources[3]. Probiotics can be defined as safe, live, normal flora that, when administered adequately, provide the host's health benefits[4,5]. Probiotics are essential for maintaining the microbial community's function and composition, preventing pathogens' growth, and improving the digestive system's function. For this reason, we can say that probiotic organisms play a very important role in the food industry, and they can be used to preserve the consumers' health as a complementary medicine instead of drugs[6].

Probiotic bacteria may have been originally obtained from the natural environment, gut microbiota, and foods. They are added to a variety of foods, including cheese, yogurt, bars of many kinds, ice cream, morning cereals, and baby formula[7,8]. Probiotics can compete with pathogens for binding sites and nutrients, inhibiting their growth by stimulating the host's immune system and producing antimicrobials like bacteriocin[9]. *Enterococci* are essential among LAB because they produce high amounts of bacteriocin[10,11]. In the food industry, bacteriocins are mainly used to prevent food-borne diseases. Probiotic bacteria can interact successfully with pathogenic bacteria using their bacteriocins by making pores in the target cell membrane. Thus causing leakage of nutrients and adenosine triphosphate

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ATP depletion, which consequently results in cell death or damage[12,13]. Because bacteriocins are nontoxic, inactivated by proteases, and genetically engineered, they are considered safe natural food preservatives[14-16]. Modern food processing technology is challenged by the adverse impacts of chemical preservers, which inhibit the growth of pathogens and prolong food shelf-life. Therefore, bacteriocins can be utilized as an alternative to chemical preservatives and antibiotics, which have been the focus of several investigations for decades[17].

For centuries, fermented foods and beverages have played a crucial role in the evolution of food, offering high nutritional content and a variety of advantages. Fermentation preserves food and increases its shelf life[18] as it has been connected to preventing illness and improving behavioral disorders, including stress and anxiety[19]. It has long been known that the probiotic bacteria in fermented milk products, such as kefir, can positively impact health either directly from the live probiotic culture or indirectly through metabolites[20-22].

Since kefir includes a wide range of beneficial microbes and their bioactive components, it is regarded as a product with significant potential for food development. It offers a variety of significant health advantages, such as prophylactic, therapeutic, and physiological properties[23]. The ability of kefir to modulate the gut flora by increasing the number of probiotic bacterial strains is the cause of its inhibitory activities against food-borne pathogens[24-27]. Kefir consumption can benefit the consumer's health since it contains probiotics such as acetic acid, lactic acid, and antibacterial proteins that have bacteriostatic activity on certain pathogenic microorganisms[25-27].

A fascinating outcome has been obtained globally by applying antibacterial peptides and metabolites to prolong the shelf-life of dairy products by destroying the pathogens[28]. Therefore, the aim of the present work was to identify and isolate LAB from kefir and assess its antimicrobial activities against bacterial pathogens responsible for certain foodborne diseases.

Materials and Methods

Samples Collection

Samples of kefir were collected from a local organic food store in Jeddah City, Saudi Arabia. The samples were transferred in sterilized containers directly to the laboratory of the Microbiology Department at King Abdulaziz University and stored in a refrigerator till further analysis.

Isolation of Lactic Acid Bacteria

LAB were isolated by the serial dilution method explained by Ismail et al[29] with few modifications. In brief, 1 g from the sample was vortexed with 9 ml of sterilized distilled water, and aliquots of 0.1 ml of each dilution were spread on de Man, Rogosa, and Sharpe (MRS) agar (Scharlau (Barcelona, Spain), incubated under anaerobic conditions at 37°C for 24-48 hrs. Next, single colonies with distinct morphological features were selected and sub-cultured by streaking on new MRS agar at least three times to obtain a pure culture.

Phenotypic and Biochemical Identification

Preliminary identification of the isolates was performed depending on phenotypic (cell morphology and Gram stain) and biochemical characteristics (catalase test, carbohydrates fermentation test, and methyl red test). Catalase-negative and Gram-positive isolates were chosen as probable LAB[30,31]. Stock cultures of the selected isolates were then stored at −80°C in MRS broth supplemented with 30% glycerol (v/v).

Screening for Antibacterial Activities Against Food-Borne Pathogens

The primary screening for potential antagonistic activities of LAB was conducted by an agar well-diffusion assay. Pathogenic strains were obtained from the Microbiology Department of King Fahd Medical Research Center (KFMRC), Jeddah, Saudi Arabia. They include *Escherichia coli* ATCC 11775, *Listeria monocytogenes* ATCC 13932, and *Salmonella Typhimurium* ATCC 14028. These pathogenic indicator bacteria were grown in nutrient broth (HIMEDIA, Mumbai, Maharashtra, India) and incubated at 37°C for 24 h. Muller Hinton agar plates [OXOID (Basingstoke, Hampshire, England)] were inoculated with overnight cultures of indicator bacteria using sterilized cotton swabs. Then, four wells of 6 mm diameter were made and inoculated with 100 µl of overnight cultures of LAB, which were grown in MRS broth anaerobically for 24 h at 37°C. Plates were placed for two h, before incubation to ensure the diffusion of LAB broth

into the medium and further incubated for 24 h at 37°C under anaerobic conditions. Following incubation, the zones of inhibition around the spots were observed and measured[32,33].

Preparation of Cell-Free Supernatant (CFS), Neutralized Cell-Free Supernatant (NCFS), and Evaluation of their Inhibitory Effects (Screening for Bacteriocin activity)

Using agar well diffusion assay, LAB isolates that showed inhibitory action against indicator strains were selected for further bacteriocin activity conformation. The selected LAB were inoculated in MRS broth at 37°C for 24 h under anaerobic conditions. Subsequently, overnight cultures were centrifuged at 4,500 rpm for 30 minutes at 4°C to collect the cell-free supernatant (CFS)[34]. CFS of each LAB were adjusted to pH 7 using 1 M of NaOH to eliminate the organic acids effects and then filter-sterilized through a 0.22 µm pore filter. The hydrogen peroxide (H_2O_2) effect was eliminated by anaerobic incubation. Overnight cultures of indicator strains were transferred to Muller Hinton agar plates, and four wells of 6 mm diameter were made. 100 µl of both non-treated CFS and treated CFS (NCFS), was added into the wells, and plates were placed until the supernatant spread onto the agar. Plates were then incubated under anaerobic conditions for 24 h at 37°C. Inhibition zones were observed and compared[35].

Molecular Identification of Bacteriocin Like Inhibitory Substance (BLIS)-Producing Strains

The total genomic DNA of LAB waereobtained according to Azcárate-Peril and Raya[36] (2001) with few modifications. Bacterial cells were harvested from an overnight culture of the strains, and the pellets were mixed with 200 µl of TES buffer and 20 µl of lysozyme (10 mg/ml). The mixture was transferred to a water bath for 20 min at 37°C. Then, 20 µl of proteinase K (10 mg/ml) was added to each sample and transferred to a water bath for an additional 20 min at 37°C. Subsequently, the mixture was transferred to an ice bath for 5 min. After that, 250 µl of 4M sodium acetate was added, followed by 250 µl of chloroform: isoamyl (24:1). The mixture was stirred gently and centrifuged at 13,000 rpm for 2 min. The top layer was transferred to the new Eppendorf, and 1 v/v of isopropanol was added. The mixture was preserved at –20°C. The next day, the mixture was centrifuged at 13,000 for 2 min, the liquid layer was removed, and the remaining DNA was dried at room temperature and then resuspended with 50 µl of distilled water. Gel electrophoresis was performed on isolated DNA. The amplification of the 16S rRNA gene was done by using a forward primer 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and reverse primer 1492R (5′-AAGGAGGTGATCCAGCCGCA-3′). Meanwhile, a 1 kb DNA ladder (Gene ruler) was used as the marker. DNA amplification was achieved by using a PCR master mix according to the manufacturer's procedures (Thermo Fisher Scientific®, Waltham Middlesex County, Massachusetts, USA). Amplification was performed in a thermocycler (Mastercycler® Gradient, Eppendorf, Hamburg, Germany) for 5 min at 94°C, followed by 32 cycles of 45s at 94°C, 45s at 60°C and 90s at 72°C, with a final extension for 10 min at 72°C. A suitable aliquot of each PCR amplicon was electrophoresed and visualized under a UV transilluminator (BioDoc-IT system, Japan). The PCR products were sent to Macrogen (Seoul, South Korea) for sequencing.

Analysis of Phylogenetic Tree

A phylogenetic tree was assembled for the data by the Maximum Likelihood method. Firstly, the initial tree was constructed by a rapid method such as Neighbor-Joining. Its branch length is accustomed to maximizing the likelihood of the data set for tree topology under the preferred evolution model. Then, topology variants were made using the Nearest Neighbor Interchange (NNI) method for searching topologies that best fit the data. Maximum-likelihood branch lengths were computed for those variant topologies, and the greatest likelihood was retained as the best choice. The search continued till no greatest likelihoods were observed. Alignment, phylogenetic, and molecular evolutionary analysis were conducted using (MEGA-X software, developed by Pennsylvania State University, USA). to create a Neighbor-Joining tree. To identify the bacterial strains, sequences of LAB isolates were matched with the bacteria that are stored at National Center for Biotechnology Information (NCBI) through basic local alignment search tool (BLAST) databases. After sequence alignment, the phylogenetic relationship of the identified strains was confirmed. A phylogenetic tree has been generated by a thousand replicates after the use of MEGA-X software[37].

Statistical Analysis

To conduct the statistical analysis, SPSS 21 (IBM Corp., Armonk, NY, USA) and Microsoft Excel spreadsheet have been used. The findings are represented as means ± standard deviations. The results are statistically analyzed by one-way ANOVA, which was followed by Tukey's test. Results with $p < 0.05$ are considered "significant".

Results

Isolation of LAB from Kefir

LAB were isolated from kefir samples. Isolation was achieved by a conventional microbiological technique and inoculation onto solid media. After inoculating appropriate dilutions of kefir samples on MRS agar, the cultivated LAB isolates have undergone both morphological and biochemical assays for phenotypic identification.

Identification of LAB Isolates

After the LAB had grown, several distinct colonies were selected through a macroscopic examination of the colonies on the MRS agar Petri plate surface. Following repurification, the colonies have had a creamy appearance. The LAB isolates' shape, size, and coloration have been assessed after Gram-staining of bacteria. A total of six isolates have been obtained and preliminarily identified by their morphology and biochemical characteristics (Table I). The isolates grew at 37°C within 24-48 hr under anaerobic conditions. All isolates were Gram-positive, catalase-negative, and methyl red-positive. All isolates were cocci, occurring singularly or in pairs (Figure 1). According to the above findings, the isolated strains were identified as lactic acid bacteria.

Molecular Identification of Bacteriocin Like Inhibitory Substance (BLIS)-Producing Strains

By using 16S rRNA gene analysis, the isolates were identified. The 16S rRNA gene was amplified by universal primers as stated before. A 1,500 bp fragment was generated through PCR amplification (Figure 2). Figure 3 shows a phylogenetic tree constructed from the evolutionary analysis by MEGA-X software. It displays the inferred phylogenetic relationship based on neighbor-joining analysis of 16S rRNA gene sequence of SK9, SK17, SK24, and SK28 isolates with closely related members of the genus *Enterococcus durans*. SK23 and SK33 are closely related to the genus *Leuconostoc mesenteroides* (Figure 3). Names of identified isolates are shown in Table I.

Antimicrobial Activity of the Isolates against Indicator Bacteria

Agar well diffusion antimicrobial assay was used to determine the antimicrobial activity of six lactic acid bacteria isolates according to their ability to prevent growing of the test bacteria (*E. coli*, *L. monocytogenes*, and *S. Typhimurium*). The antimicrobial activities of LAB were shown by the clear zone that formed around the wells. This clear zone is also called the inhibition zone. The isolates exhibited antagonizing activity against the test bacteria of the present work. According to Table II and Figures 4 and 5, the SK17 isolate did not show any antimicrobial activity. However, the SK23 and SK28 isolates had high antimicrobial activity against *E. coli* (16.8 ± 0.28 and 18.8 ± 0.76, respectively), *L. monocytogenes* (9.3 ± 0.76 and 9 ± 0.5, respectively) and *S. Typhimurium* (15.83 ± 0.28 and 23.83 ± 0.28, respectively). CFS of SK24 is effective against *E. coli* only. SK9 was only effective against *E. coli* and *S. Typhimurium* and did not show any antimicrobial activity against *L. monocytogenes*. SK28 showed the most potent antimicrobial activities against *E. coli* and *S. Typhimurium*. SK23 showed the highest antimicrobial activity against *L. monocytogenes*, while SK33 showed no antimicrobial activity against *L. monocytogenes*. The diameter of the inhibition zone produced by cell culture, CFS, and NCFS of the six isolates against *L. monocytogenes*, *E. coli*, and *S. Typhimurium* are shown in Table II. Based on the inhibition zone, the antimicrobial activity of isolates against Gram-negative was greater than Gram-positive bacteria. Moreover, CFS's antimicrobial activities were more significant than NCFS's for all isolates with AMAs. In contrast, cell culture's antimicrobial activities were comparable with CFS's (Table II and Figure 4).

The means of inhibition zone done by LAB strains against indicator bacteria were statistically compared. Table III shows non-significant differences between groups that were compared using one-way ANOVA. A Tukey's test was then run (Table IV) to determine precisely the variables that do not significantly differ.

Table I. Morphological and biochemical characteristics of LAB isolates.

Isolates	Strain name	Morphology examination		Biochemical assays		Carbohydrates fermentation		
		Gram staining	Cell morphology	Catalase	MR	Glucose	Lactose	Sucrose
Sk9	*Enterococcus durans*	+	Cocci	-	+	+	+	+
Sk17	*Enterococcus durans*	+	Cocci	-	+	+	+	+
Sk23	*Leuconostoc mesenteroides*	+	Cocci	-	+	+	+	+
Sk24	*Enterococcus durans*	+	Cocci	-	+	+	+	+
Sk28	*Enterococcus durans*	+	Cocci	-	+	+	+	+
Sk33	*Leuconostoc mesenteroides*	+	Cocci	-	+	+	+	+

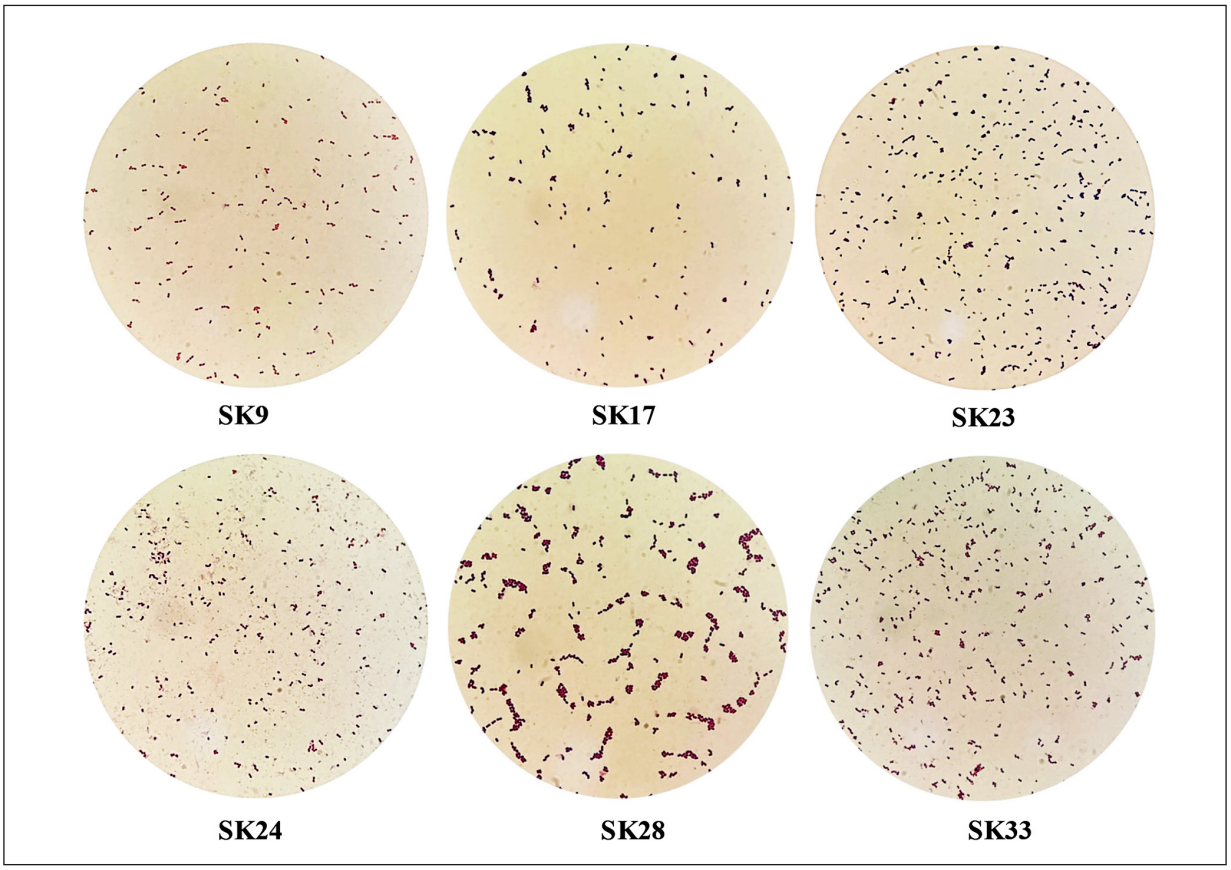

Figure 1. Gram staining from purified lactic acid bacteria. Isolates under microscope ×100.

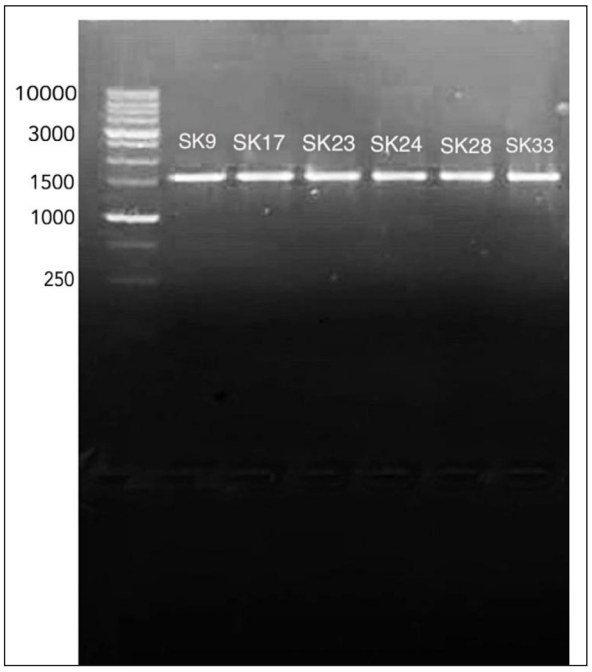

Figure 2. Agarose Gel Electrophoresis of 16SrRNA gene PCR products of the six LAB isolates and DNA size marker.

Discussion

The main goal of the fermentation of milk is to preserve its nutrient value and extend its shelf life. LAB are necessary for the underlying characteristics of fermented foodstuffs such as kefir[38]. For scientific and commercial objectives, the isolation and identification of LAB from the natural environment is essential. It is believed that lactic acid bacteria isolates are a unique source of novel molecules with antimicrobial activities[39]. The present work focused on isolating and identifying different strains from kefir and testing their antimicrobial activities.

Six lactic acid bacteria were isolated from kefir, identified, and tested for their antimicrobial activities against indicator bacteria. All LAB isolates showed AMA with variable inhibition zone diameters on pathogenic clinical isolates of *E. coli*, *L. monocytogenes*, and *S. Typhimurium*.

The isolates' metabolite is what gives them their antibacterial effects. The metabolites include ethanol, lactic acid, and other secondary

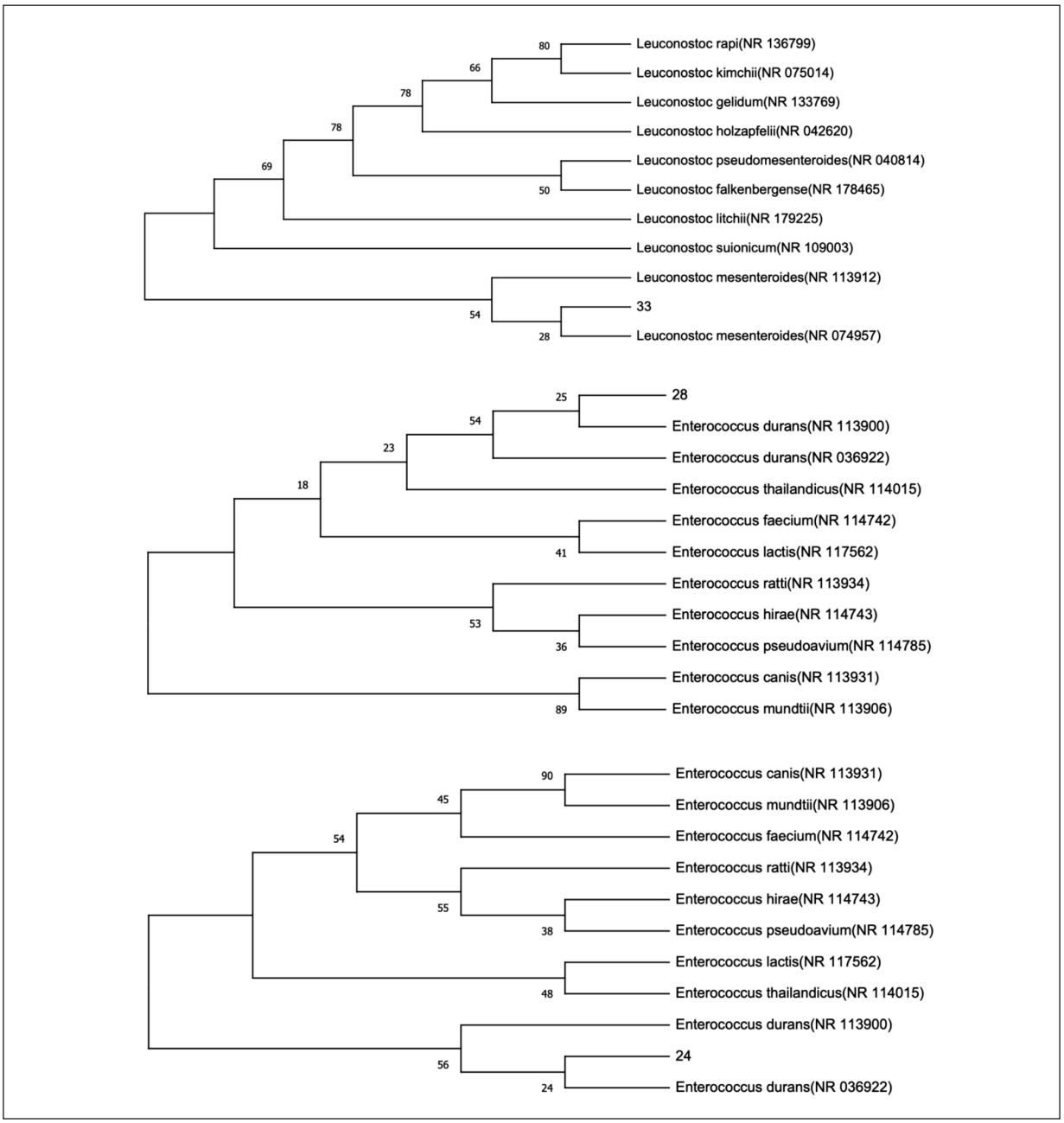

Figure 3. Phylogenetic analysis of LAB strains. Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences showing the position of LAB strains.

metabolites such as hydrogen peroxide and bacteriocin. Lactic acid has the potential to enter the pathogen's environment, which will subsequently impair the integrity of the cell membrane and interfere with the pathogen's capacity to absorb nutrients. Additionally, the growth and metabolism of the bacteria can be diminished[40]. Conversely, the existence of bacteriocin, which is produced by lactic acid bacteria, also contributes to the antibacterial action. It functions as a single hit inactivation, which means that a bacteriocin molecule attacks the cell of bacteria[41].

In the current study, based on the phylogenetic analysis, *Enterococcus* and *Leuconostoc* genera have been identified from kefir, consistent with those of earlier investigations on kefir in which LAB genera were isolated[42,43]. According to Kim et al[44], lactic acid bacteria from many genera,

Table II. Antimicrobial activities of LAB isolates against indicator strains. Comparison between the diameter of inhibition zones produced by Cell culture, CFS, and NCFS.

	E. coli			*L. monocytogenes*			*S. Typhimurium*		
Isolates No.	**Cell culture**	**CFS**	**NCFS**	**Cell culture**	**CFS**	**NCFS**	**Cell culture**	**CFS**	**NCFS**
SK9	14.33 ± 0.58	13.30 ± 1.15	8.16 ± 1.04	-	-	-	12.50 ± 0.50	11.83 ± 0.76	8.8 ± 0.76
SK17	-	-	-	-	-	-	-	-	-
SK23	17.33 ± 0.57	16.80 ± 0.28	12.6 ± 0.57	9.83 ± 0.29	9.3 ± 0.76	7.5 ± 0.5	16.33 ± 0.76	15.83 ± 0.28	12.76 ± 0.68
SK24	9.00 ± 0.01	8.80 ± 0.28	-	-	-	-	-	-	-
SK28	18.50 ± 1.32	18.80 ± 0.76	9.5 ± 0.5	9.33 ± 0.58	9 ± 0.5	7.6 ± 1.5	25.83 ± 0.76	23.83 ± 0.28	14.3 ± 0.28
SK33	7.83 ± 0.29	7.50 ± 0.50	-	-	-	-	12.17 ± 0.29	12.3 ± 1.15	-

Cell-free supernatant (CFS), non-treated CFS (NCFS).

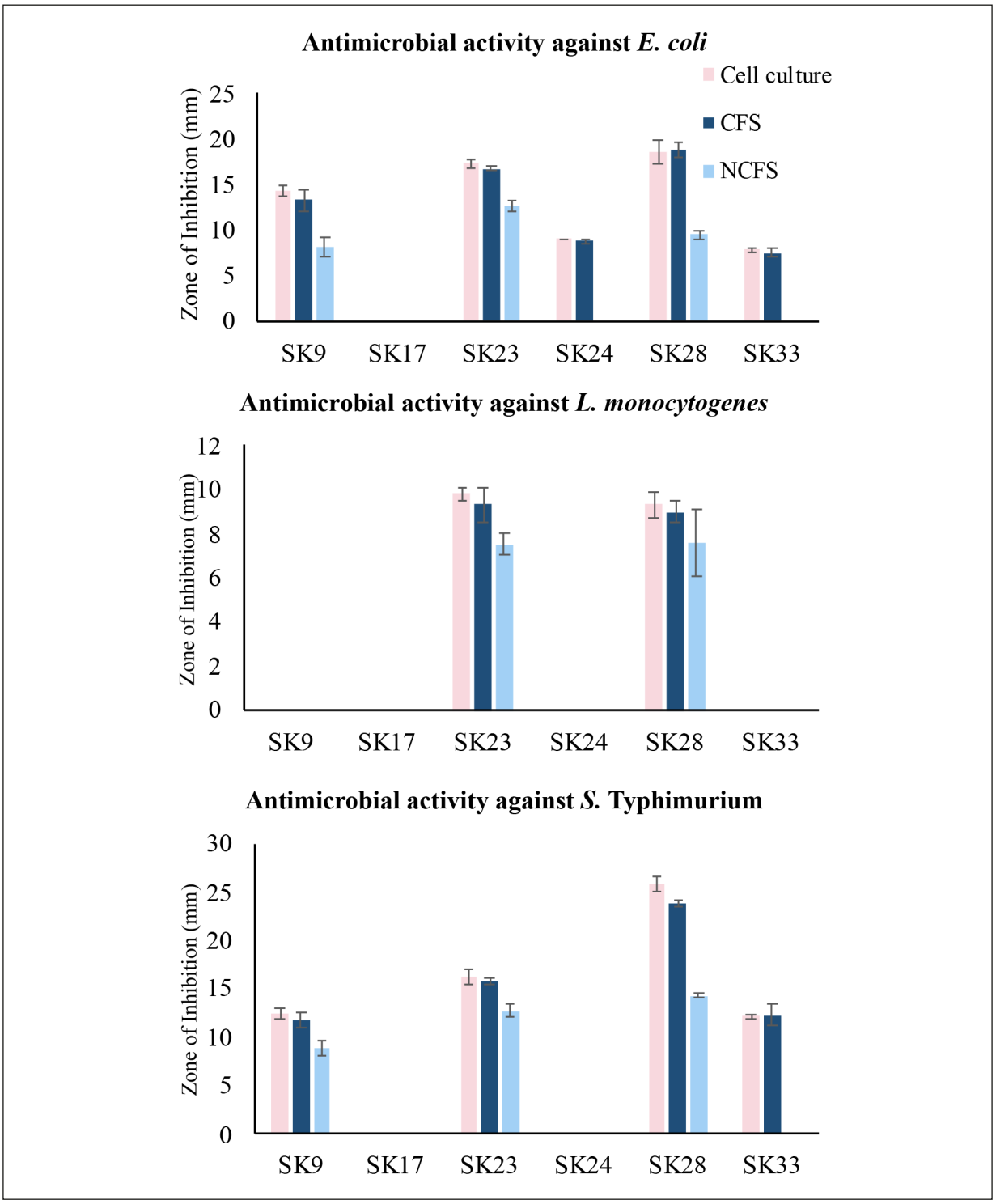

Figure 4. Antimicrobial activities of the six isolates (Cell culture, CFS, and NCFS) against the selected pathogens. Data are presented as mean ± SD.

including Leuconostoc, are responsible for fermentation. According to LAB identification, the identified LAB species include *Enterococcus durans* (4 isolates) and *Leuconostoc mesenteroides* (2 isolates). The present result is consistent with a work of identification of related strains[43].

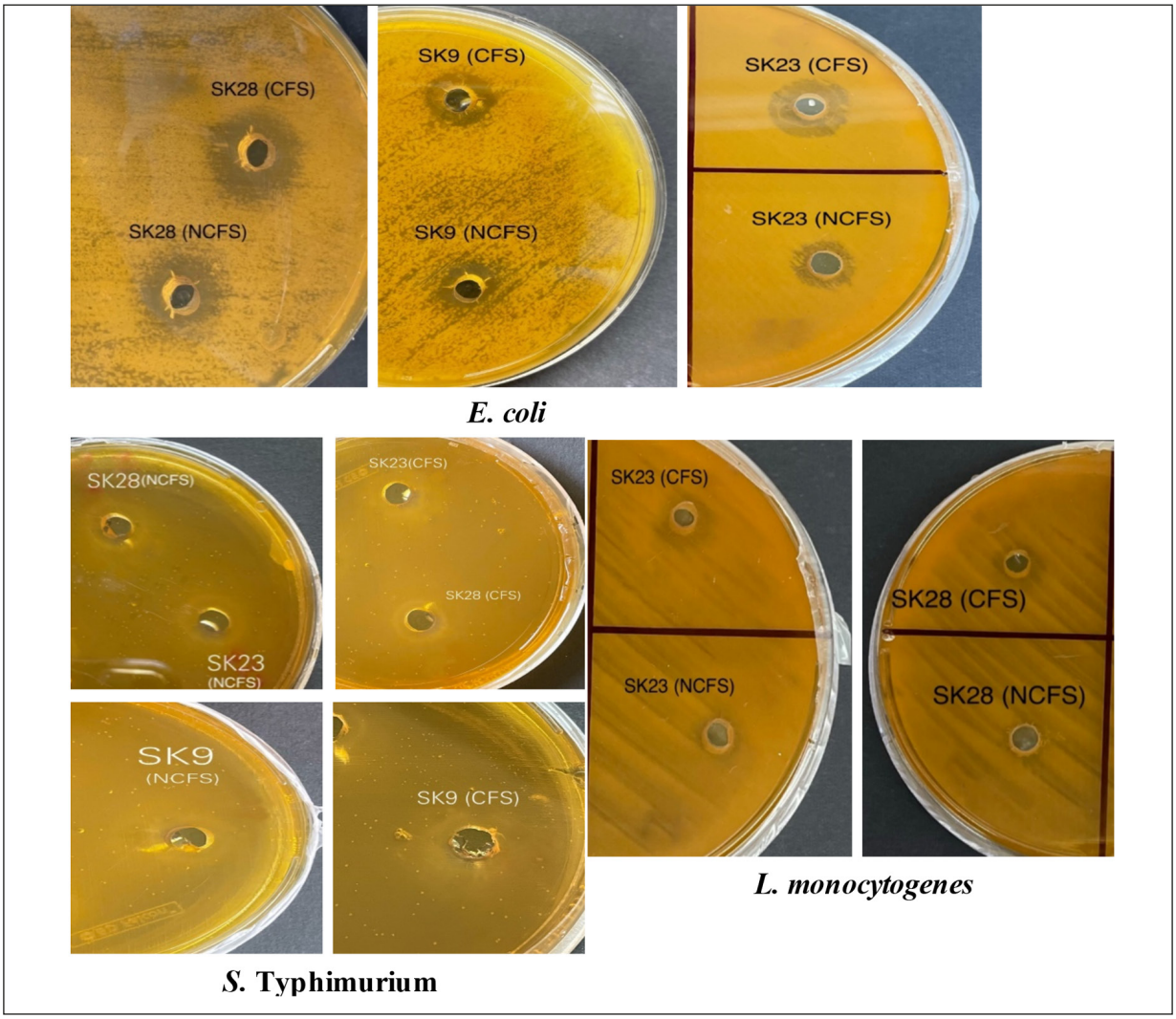

Figure 5. Evaluation of AMAs of CFS and NCFS of LAB isolates against *E. coli*, *L. monocytogenes* and *S. Typhimurium*. Clear areas around the spots reflecting zones of inhibition.

LABs are significantly assessed in the manufacturing of food because of their valuable effects. The antibacterial activity of LAB as probiotics is one of their most crucial characteristics[45,46]. On LAB screening, SK28 isolate showed the most potent antagonizing activities against *E. coli* and *S. Typhimurium* with a zone of inhibition 18.8 ± 0.76 mm and 23.83 ± 0.28, respectively, where-

Table III. ANOVA output of antimicrobial activities of LAB isolated from kefir against test/indicator bacteria.

ANOVA		Sum of squares	Df	Mean square	F	*p*-value
E. coli	Between groups	142.57	2	71.28	1.66	0.223
	Within groups	642.67	15	42.84		
	Total	785.24	17			
L. monocytogenes	Between groups	1.53	2	0.76	0.04	0.964
	Within groups	310.26	15	20.68		
	Total	311.79	17			
S. Typhimurium	Between groups	96.79	2	48.40	0.63	0.547
	Within groups	1,156.96	15	77.13		
	Total	1,253.76	17			

Table IV. ANOVA output of antimicrobial activities of LAB isolated from kefir against test/indicator bacteria.

Multiple comparison among groups - Tukey's test			
	Comparisons	**Mean difference**	***p*-value**
E. coli	Cell culture *vs.* CFS	0.283	0.9969
	Cell culture *vs.* NCFS	6.107	0.2696
	CFS *vs.* NCFS	5.823	0.3008
L. monocytogenes	Cell culture *vs.* CFS	0.133	0.9986
	Cell culture *vs.* NCFS	0.667	0.9651
	CFS *vs.* NCFS	0.533	0.9775
S. Typhimurium	Cell culture *vs.* CFS	0.500	0.9947
	Cell culture *vs.* NCFS	5.150	0.5787
	CFS *vs.* NCFS	4.650	0.6382

Cell-free supernatant (CFS), non-treated CFS (NCFS).

as SK23 and Sk9 showed weaker antimicrobial activity against *E. coli* and *S. Typhimurium*. On the other hand, SK 17 exhibited no antimicrobial activity against any tested bacterial species. *L. monocytogenes* was the least sensitive species to the antimicrobial activity of LAB isolates. These results are consistent with research by Prihanto et al[47] on isolates from fermented fish with inhibition zone diameters between 10-20 mm.

The results of the present investigation also manifest a good zone of inhibition against *E. coli* in comparison with research achieved in Cameron[48] and in Malaysia[49], which reported 4.5 ± 0.1 mm and 1.3 ± 0.5 mm as the highest zone of inhibition produced by LAB against *E. coli*. The present results exhibited the maximum antimicrobial activity of LAB isolates CFS against *Salmonella* species was 23.839 ± 0.28 mm, which is superior to results achieved by Sari et al[50] and Al-Allaf et al[51]. They stated that the maximal inhibition zones of LAB against *Salmonella* species were 7.5 mm and 0.75 mm, respectively. Our findings denoted higher antimicrobial activities when compared with results obtained in Indonesia, as stated by Prihanto et al[47], where the maximal LAB zone of inhibition against *Salmonella* species was 10.3 mm. According to the inhibition zones produced by LAB isolates, *Enterococcus* isolates exhibited a slightly higher inhibition of the tested bacteria, subsequently, *Leuconostoc* isolates. This result resembles research findings in Ethiopia[52], where *Enterococcus* isolates demonstrated a high inhibition of the tested bacteria.

The Phylogenetic tree confirmed that four isolates are located in *Enterococci durans* species. A large group of bacteriocins are created by *Enterococci*[53]. Bacteriocins delivered by *Enterococci* are commonly considered safe bacterial products[54,55]. They prevent various food-borne pathogens, such as *L. monocytogenes*, from growing[56-58]. Bactericidal activities of bacteriocins against Gram-negative bacteria were considered very low, possibly due to their outer membrane lipopolysaccharides[59-61].

Nevertheless, in this study, we found that LAB isolates might also prevent the growth of Gram-negative indicator strains, including *E. coli* and *S. Typhimurium*, in addition to Gram-positive strains. According to similar publications[62], class II bacteriocin can only block a few Gram-negative bacteria. Moreover, they reported that bacteriocin generated by *Enterococci* isolated from the newborn human infant's faces might stop the growth of *E. coli* and *S. Typhimurium*. Bacteriocins produced by *Enterococcus* are categorized as class II bacteriocin. In Gram-negative bacteria, bacteriocins may interact with the receptor, resulting in conformational changes. Moreover, the receptor may serve as an anchor, inserting bacteriocin and inducing the membrane to rupture[63]. In recent years, interest in BLIS-producing LAB has grown because of their potential to create natural antibacterial agents that improve food safety. In the current study, different LABs exhibited numerous inhibitory activities (ranging from 7.5 mm to 23.83 mm) against the tested pathogenic bacteria. BLIS from LAB is reported to have a narrow spectrum of antimicrobial activity that is more powerful against similar Gram-positive bacteria[64].

Nonetheless, BLIS action might extend to Gram-negative because it may destroy nucleic acids, break bacterial cell membranes, and interfere with internal enzymatic systems. The activities of BLIS against *E. coli* have been recorded by Pei et al[65]. Other research studies[66,67] have also

revealed a broad spectrum of BLIS activity from LAB against Gram-positive and Gram-negative bacteria.

Amongst the examined pathogens, *S. Typhimurium* was more sensitive to LAB isolates than *E. coli* and *L. monocytogenes*. Based on the primary screening, the antimicrobial activity of cell culture was comparable with that of CFS. While the antimicrobial activity of the LAB isolates CFS in the current investigation was higher than that of the NCFS of the identical isolates. The antibacterial activity was dramatically reduced when the pH was adjusted for CFS. This proved that lactic and acetic acids made by lactic acid bacteria made an extensive contribution to their antibacterial activities. The undissociated forms of lactic and acetic acids might pass through the membrane of the pathogenic microorganism. Thus, the cytoplasm becomes more acidic, and the glycolysis pathway's enzymes become inhibited. These acids dissociate at increased intracellular pH to form hydrogen ions, interfering with crucial metabolic processes, including oxidative phosphorylation and substrate translocation[68,69].

Recommendations

As LAB isolates from *Enterococcus* genera showed high antagonistic activities against test bacteria, further experiments are required to isolate and identify the molecules responsible for the antimicrobial activities. Since the increased use of antibiotics causes incidental damage to the human body by disrupting the normal microbiota of the intestine, kefir consumption is highly recommended to prevent the growth of pathogenic microorganisms and to promote the development of healthy gut microbiota.

Limitations

The present study has a limitation regarding the isolation and identification of the molecule responsible for the antimicrobial activity. It is only conducted for isolation, characterization, and molecular identification of lactic acid bacteria from kefir and evaluate their antibacterial activity against some food-borne pathogens in Saudi Arabia by using different methods.

Conclusions

LABs were isolated and identified from different samples of kefir. The predominant isolates of these bacteria belong to *Enterococcus* and *Leuconostoc* genera. These isolates displayed antibacterial activities against test bacteria, including *E. coli*, *L. monocytogenes*, and *S. Typhimurium*. Amongst indicator bacteria, *S. Typhimurium* was extremely sensitive to LAB's antibacterial activities. *E. coli* was moderately sensitive and had a smaller zone of inhibition. On the other hand, *L. monocytogenes* showed the most minor sensitivity to the antimicrobial activities of LAB isolates of kefir. Moreover, the antimicrobial activity of the CFS of LAB isolates was more potent than that of the NCFS of the isolates. LAB in kefir confirms that it is considered safe to consume, demonstrating a favorable product soon as a natural antibacterial for bio-preservation in food production.

Conflict of Interest

The authors declare that they have no conflict of interests.

Informed Consent

Not applicable.

Ethics Approval

This research does not contain any experiments with human or animal subjects. Thus, ethics approval was not required.

Data Availability

The author confirms that the data supporting the findings of this study are available within the article, including tables and figures.

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