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Effectiveness of Bacteriocins in Yoghurt on Inhibiting the Growth of Escherichia Coli ATCC 25922

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ABSTRACT

Background: Escherichia coli is an important indicator of food contamination and a foodborne pathogen capable of causing serious infections. Naturally produced antimicrobial metabolites in yoghurt, including bacteriocins, have attracted increasing attention as potential alternatives to synthetic food preservatives. This study aimed to evaluate the antibacterial activity of yoghurt against E. coli ATCC 25922 at different concentrations. Methods: A true experimental study with a posttest-only control group design was conducted. Antibacterial activity was evaluated using the agar well diffusion method against E. coli ATCC 25922. Yoghurt was tested at concentrations ranging from 5% to 50% with 5% intervals. Data were analyzed using the Shapiro–Wilk normality test, followed by the Kruskal–Wallis test and Mann–Whitney post hoc analysis. Results: Chemical analysis estimated bacteriocin, lactic acid, and hydrogen peroxide levels in yoghurt at 148.00 mg/L, 35.12 mg/L, and 11.75 mg/L, respectively. No antibacterial activity was observed at yoghurt concentrations below 25%. Inhibition zones first appeared at a concentration of 25% and increased progressively with increasing yoghurt concentration, reaching the largest inhibition zone at 50%. Significant differences in inhibition zone diameters were observed among treatment groups (p < 0.05), indicating a concentration-dependent antibacterial effect. Conclusions: Yoghurt exhibited concentration-dependent antibacterial activity against E. coli ATCC 25922, with 25% representing the minimum concentration capable of inhibiting bacterial growth. The observed antibacterial activity is likely attributable to the synergistic effects of bacteriocins, lactic acid, and hydrogen peroxide produced during yoghurt fermentation. These findings support the potential application of yoghurt-derived antimicrobial metabolites as natural biopreservatives for improving food safety.

1. INTRODUCTION

Food contamination is a serious global threat to public health, causing approximately 600 million cases of illness and 420,000 deaths annually due to the consumption of contaminated food (WHO, 2024). In Indonesia, food poisoning has become a serious issue in the Free Nutritious Meal (MBG) program in 2025, with 5,914 cases reported up to September 2025, predominantly caused by Escherichia coli (Pakpahan, 2025), as well as 17 outbreaks across 10 provinces during January–May 2025 (Rahmawati, 2025). As one of the most widely accepted indicators of fecal contamination and food hygiene, E. coli is frequently used to assess microbiological food safety because its presence reflects inadequate sanitation during food processing and handling. Therefore, strategies capable of inhibiting the growth of E. coli have the potential to reduce the risk of foodborne diseases and contribute to improving food safety, including in large-scale public nutrition programs such as MBG. Therefore, food safety is a crucial aspect of the food and beverage industry, requiring attention to critical factors such as water quality, pH, pathogenic bacteria, viruses, parasites, and toxic metabolites (Tenggana et al., 2020). Among various approaches to improving food safety, the utilization of natural antimicrobial compounds produced during food

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fermentation has attracted increasing attention because of their potential to replace synthetic preservatives. One approach to improving food safety is the utilization of fermented products such as yoghurt produced by lactic acid bacteria (LAB), which play a role in forming sensory characteristics and producing probiotics as well as antimicrobial compounds in the form of lactic acid, hydrogen peroxide, and bacteriocins that are capable of inhibiting the growth of pathogenic bacteria (Le Roy, 2022).

These antimicrobial metabolites produced by LAB are considered promising natural biopreservatives because they not only prolong food shelf life but also suppress the growth of foodborne pathogens (Inggas et al., 2025). LAB are gram-positive anaerobic bacteria capable of adapting to extreme conditions and synthesizing various antimicrobial metabolites, including organic acids, H_2O_2, and bacteriocins, which function by lowering pH, damaging cell membranes, and inhibiting the growth of pathogenic bacteria (Fugaban et al., 2022). Several studies have demonstrated the effectiveness of LAB bacteriocins in inhibiting E. coli and Staphylococcus aureus, although their inhibitory activity against gram-negative bacteria is relatively lower due to the complexity of their outer membrane (Raningsih et al., 2021). The outer membrane of Gram-negative bacteria acts as a permeability barrier that limits bacteriocin penetration. Nevertheless, previous studies have suggested that membrane destabilization induced by organic acids or chelating compounds may facilitate bacteriocin entry, thereby enhancing antibacterial activity against E. coli (Hernández-Gómez et al., 2021; Soltani et al., 2021; Kumar et al., 2022).

Antibacterial testing commonly uses E. coli ATCC 25922 as a standard strain because it is non-pathogenic, genetically stable, and internationally recognized as a quality-control strain for antimicrobial susceptibility testing (Minogue et al., 2014). Several previous studies have reported that bacteriocins isolated from LAB or yoghurt exhibit antibacterial activity against E. coli. However, most investigations evaluated crude bacteriocin preparations at a single concentration or focused on purified bacteriocins without assessing antibacterial activity in whole yoghurt matrices (Moghaddam et al., 2006; Tufail et al., 2019; Hassan et al., 2020). Furthermore, comparative data describing antibacterial activity across a wide concentration range of yoghurt against E. coli ATCC 25922 remain scarce. This limitation indicates that the concentration-dependent antibacterial effectiveness of yoghurt-derived bacteriocins has not been fully elucidated. Consequently, the minimum effective concentration and the concentration producing the greatest inhibitory activity against E. coli ATCC 25922 remain unclear. This represents the main scientific problem addressed in the present study.

The novelty of this study lies in evaluating the antibacterial activity of yoghurt containing naturally produced bacteriocins using a broad concentration range (5–50%), combined with a negative control and assessment by the agar well diffusion method. The concentration range was selected based on previous antibacterial studies employing serial concentration gradients while allowing identification of the minimum effective inhibitory concentration under laboratory conditions (Tufail et al., 2019). In addition, the agar well diffusion method was chosen because it provides better diffusion of liquid samples into agar media and generally produces greater sensitivity and reproducibility than the conventional disk diffusion method for testing liquid antimicrobial substances (Nurhayati et al., 2020; Bonev et al., 2008).

The findings of this study are expected to provide experimental evidence supporting the utilization of yoghurt-derived antimicrobial metabolites as natural biopreservatives for improving food safety and reducing contamination by indicator bacteria such as E. coli. Such evidence may also contribute to the development of safer fermented food products and support preventive strategies against foodborne contamination in public food service programs, including the MBG program. Therefore, this study aimed to evaluate the antibacterial effectiveness of various yoghurt concentrations (5–50%) against Escherichia coli ATCC 25922 using the agar well diffusion method and to determine the minimum concentration capable of producing effective bacterial growth inhibition.

2. METHOD

This study employed a true experimental design with a posttest-only control group design to evaluate the antibacterial activity of yoghurt-derived bacteriocins against Escherichia coli ATCC 25922. The experiment consisted of ten treatment groups containing yoghurt at concentrations of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, and 50%, and one negative control group containing sterile distilled water (aquadest). Distilled water was selected as the negative control because it has no intrinsic antibacterial activity and allows differentiation between inhibition caused by yoghurt metabolites and the diffusion medium itself. Each treatment and control group was performed in triplicate, and the placement of samples during antibacterial testing was randomized to minimize experimental bias.

The yoghurt used in this study was produced from pasteurized cow's milk fermented with Lactobacillus bulgaricus and Streptococcus thermophilus, both recognized as bacteriocin-producing lactic acid bacteria. The antibacterial activity evaluated in this study originated from the yoghurt matrix containing naturally produced bacteriocins together with other antimicrobial metabolites, including lactic acid and hydrogen peroxide. No bacteriocin purification procedure was performed prior to antibacterial testing.

The antibacterial assay was carried out using the agar well diffusion method. A suspension of E. coli ATCC 25922 was prepared and adjusted to the 0.5 McFarland standard before inoculation onto Mueller-Hinton Agar (MHA). Wells of uniform diameter were prepared on the agar surface, and each well was filled with the designated yoghurt concentration. Plates were incubated at 37°C for 24 hours, after which the diameter of the inhibition zone surrounding each well was measured in millimetres using a calibrated digital caliper.

The independent variable was yoghurt concentration (5–50%), whereas the dependent variable was the inhibition zone diameter against E. coli ATCC 25922. Controlled variables included the bacterial strain, inoculum density, culture medium, incubation temperature and duration, well diameter, sample volume, and measurement procedure to ensure experimental consistency. Prior to data collection, all measuring instruments were calibrated according to laboratory procedures, and all experiments were conducted under identical laboratory conditions.

Data analysis was conducted using descriptive and inferential statistical analyses. Descriptive statistics were used to summarize inhibition zone diameters and the levels of bacteriocins, lactic acid, and hydrogen peroxide at each yoghurt concentration. Normality of the data was evaluated using the Shapiro–Wilk test, whereas homogeneity of variance was assessed before selecting the appropriate comparative statistical test. Since the data were not normally distributed, the nonparametric Kruskal–Wallis test was used to compare inhibition zone diameters among treatment groups, followed by the Mann–Whitney post hoc test for pairwise comparisons. Linear regression analysis was performed to evaluate the relationship between yoghurt concentration and the estimated levels of bacteriocins, lactic acid, and hydrogen peroxide, as well as to predict metabolite concentrations at 100% yoghurt concentration. Regression analysis was conducted after confirming that the assumptions required for linear regression were adequately satisfied. Statistical analyses were performed using SPSS version 25 with a significance level of 95% ($p < 0.05$) (Dahlan, 2014).

3. RESULT AND DISCUSSION

Result

The test bacterium E. coli ATCC 25922 on MacConkey in figure 1, Agar media showed round pink colonies with smooth edges and convex elevation, indicating lactose-fermenting properties, and exhibited rod-shaped cell morphology, arranged singly, and classified as a Gram-negative bacterium. The bacteria used in this study were L. bulgaricus and S. thermophilus grown on MRS medium, forming opaque white colonies with a round shape, smooth edges, and convex elevation. Microscopically, both are Gram-positive bacteria, in which L. bulgaricus is rod-shaped or coccoid and does not form spores, while S. thermophilus is spherical in shape and non-motile.

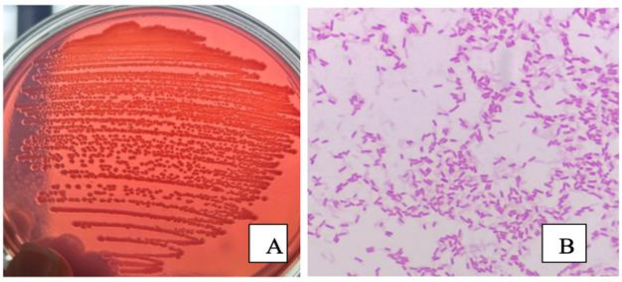

Figure 1. Colony Morphology of E. coli ATCC 25922 on MacConkey Agar (A) and Cell Morphology of E. coli ATCC 25922 with Gram Staining at 100× Magnification (B)

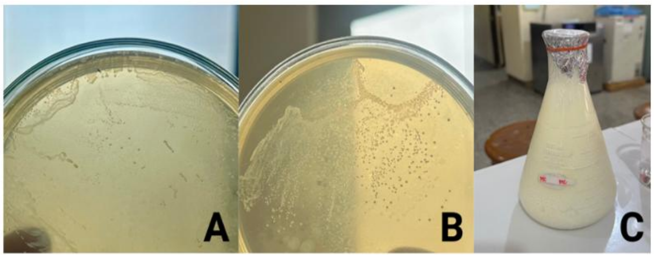

Figure 2. Identification of LAB Colony Morphology: Colony Morphology of L. bulgaricus (A) and S. thermophilus (B) Used in Yoghurt Production (C)

In figure 2, the yoghurt starter was prepared from skim milk inoculated with L. bulgaricus and S. thermophilus, pasteurized at 63 °C for 30 minutes, and then incubated at 37 °C for 24 hours. The yoghurt was subsequently produced from cow's milk pasteurized at 90 °C for 30 minutes, inoculated with 2% starter (1:1), and incubated at 37 °C for 24 hours until a white yoghurt with a characteristic sour aroma and a final pH of 4.0 was obtained.

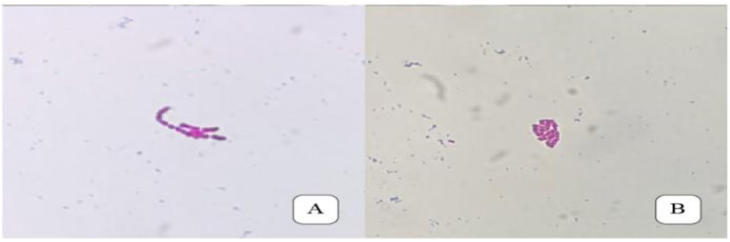

Figure 3. Identification of LAB Cell Morphology: Cell Morphology of L. bulgaricus (A) and S. thermophilus (B). The starter bacteria used were consistent with the expected microscopic characteristics.

Bacteriocin levels were measured using UV/Vis spectrophotometry at a wavelength of 600 nm after reaction with $FeCl_3$ (figure 3), with fetal bovine serum (FBS) as a reference. The normality of bacteriocin, lactic acid, and peroxide levels was tested using the Shapiro–Wilk test and showed an increase with increasing yoghurt concentration.

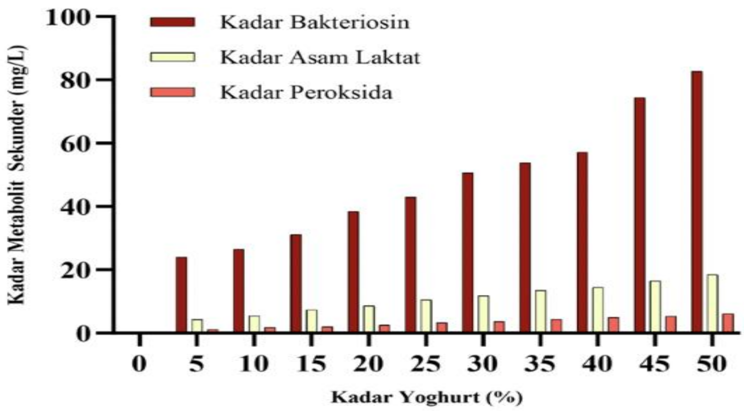

Figure 4. Graph of Secondary Metabolite Levels

In figure 4, the data on bacteriocin, lactic acid, and peroxide levels showed a normal distribution based on the Shapiro–Wilk test ($\alpha = 0.05$). Subsequently, linear regression analysis was used to estimate the levels of each secondary metabolite in yoghurt at a 100% concentration.

Table 1. Secondary Metabolites in Yoghurt

Yoghurt Levels	Bacteriocin			Lactat Acid			Peroxide		
	Levels (mg/L)	P-value	Equation	Levels (mg/L)	P-value	Equation	Levels (mg/L)	P-value	Equation
0%	0	< 0.0001	Y = 1.389X + 9.099	0	< 0.0001	Y = 0.3338X + 1.736	0	< 0.0001	Y = 0.1139X + 0.3573
5%	24,08			4,3			1,15		
10%	26,55			5,44			1,78		
15%	31,21			7,34			1,99		
20%	38,47			8,61			2,62		
25%	42,99			10,51			3,25		
30%	50,66			11,77			3,67		
35%	53,81			13,54			4,41		
40%	57,1			14,43			4,94		
45%	74,36			16,46			5,36		
50%	82,71			18,48			6,09		
100%*	148,00			35,12			11,75		

*= tested using linear regression

The antibacterial assay was performed using the well diffusion method. The objective was to evaluate the antibacterial activity of yoghurt at various concentrations against the growth of E. coli ATCC 25922.

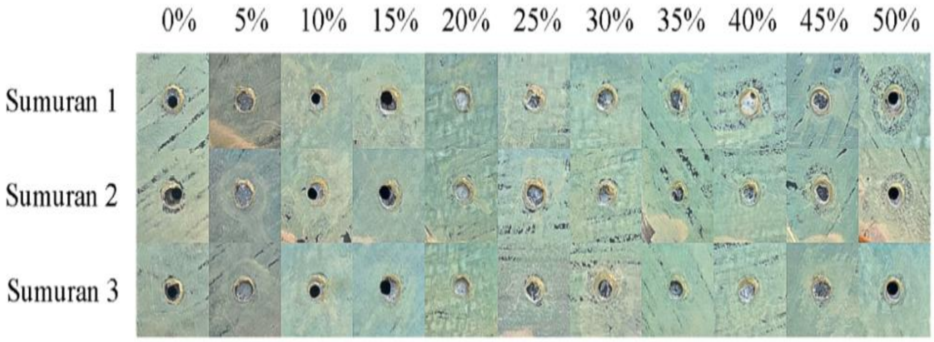

Figure 5. Results of the Antibacterial Test of Yoghurt against E. coli ATCC 25922

As expected, bacteriocins in yoghurt exhibited antibacterial activity, with the smallest inhibition zone observed at a concentration of 25%.

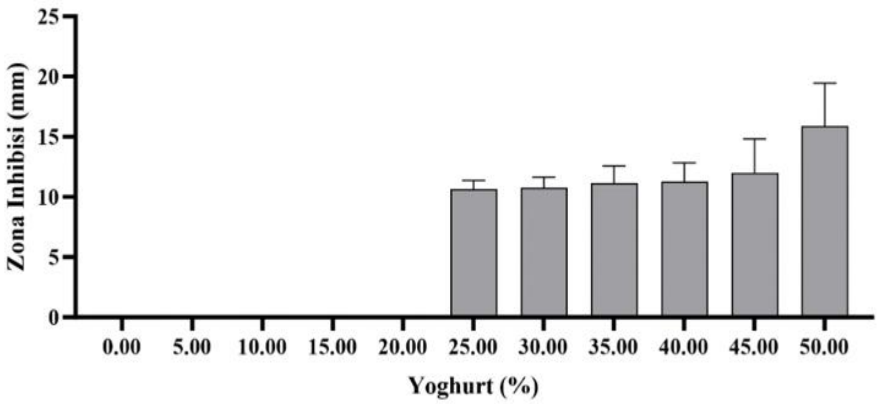

Figure 6. Inhibition Zone Size of Yoghurt against E. coli ATCC

Based on this study, no inhibition zones were formed at yoghurt concentrations of 0–20%. At concentrations of 25–50%, inhibition zones began to form, with mean diameters at 25% (10.63 mm), 30% (10.75 mm), 35% (11.13 mm), 40% (11.25 mm), 45% (12.00 mm), and 50% (15.88 mm). Thus, based on these results, descriptively, the higher the concentration used, the larger the inhibition zone formed (Figure 6).

Table 2. Inhibition Zone Diameter of Yoghurt against E. coli ATCC 25922

Yoghurt Levels (%)	Bacteriocin (mg/L)	Inhibition Zone Diameter (mm)	P-value
0%	0[abcdef]	0,00 ± 0,00	
5%	24,08[ghijkl]	0,00 ± 0,00	
10%	26,55[mnopqr]	0,00 ± 0,00	
15%	31,21[stuvwx]	0,00 ± 0,00	
20%	38,47[abcdyz]	0,00 ± 0,00	
25%	42,99[agmsy]	10,63 ± 0,74	< 0.001
30%	50,66[bhntz]	10,75 ± 0,89	
35%	53,81[aciou]	11,13 ± 1,46	
40%	57,1[abdjpv]	11,25 ± 1,58	
45%	74,36[acekqw]	12,00 ± 2,83	
50%	82,71[adflrx]	15,88 ± 3,56	

Normality analysis using the Shapiro-Wilk test indicated that the data were not normally distributed. Therefore, the analysis was continued using the non-parametric Kruskal-Wallis test. A significant difference was observed, although the significance occurred only at certain test concentrations.

Table 3. Kruskall-Wallis Test Result

Konsentrasi yoghurt	Sampel	*Kruskal-Wallis* H	Signifikansi (Nilai-P)
5%	11	72,023	<0.001
10%	11		
15%	11		
20%	11		
25%	11		
30%	11		
35%	11		
40%	11		
45%	11		
50%	11		

The tested concentrations significantly inhibited the growth of E. coli ATCC 25922, with a significance value of <0.05.

The results showed in figure 7, a significant difference between the 0–20% and 25–50% concentrations. Increasing the concentration led to a significant increase in the diameter of the inhibition zone ($p < 0.05$).

	0%	5%	10%	15%	20%	25%	30%	35%	40%	45%	50%
0%	-	1	1	1	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
5%	1	-	1	1	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
10%	1	1	-	1	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
15%	1	1	1	-	1	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
20%	1	1	1	1	-	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
25%	<0.001	<0.001	<0.001	<0.001	<0.001	-	0.904	0.787	0.764	0.353	0.004
30%	<0.001	<0.001	<0.001	<0.001	<0.001	0.904	-	0.857	0.81	0.385	0.006
35%	<0.001	<0.001	<0.001	<0.001	<0.001	0.787	0.857	-	0.952	0.457	0.01
40%	<0.001	<0.001	<0.001	<0.001	<0.001	0.764	0.81	0.952	-	0.496	0.011
45%	<0.001	<0.001	<0.001	<0.001	<0.001	0.353	0.385	0.457	0.496	-	0.015
50%	<0.001	<0.001	<0.001	<0.001	<0.001	0.004	0.006	0.01	0.011	0.015	-

Figure 7. Hasil Uji Mann-Whitney

Discussion

In this study, the yoghurt was produced by fermenting cow's milk using Lactobacillus bulgaricus and Streptococcus thermophilus starters, in accordance with international yoghurt production standards (FAO & WHO, 2024). These two bacteria act synergistically, with S. thermophilus supporting the initial growth of L. bulgaricus, while the proteolytic activity of L. bulgaricus provides nutrients for S. thermophilus, thereby accelerating pH reduction, texture formation, and fermentation stability (Nguyen et al., 2018; Ge et al., 2024). Milk treatment through solids standardization and high-temperature heating contributed to a firmer yoghurt texture due to whey and casein protein interactions (Sodini et al., 2002; Asaduzzaman et al., 2021). Organoleptically, the resulting yoghurt exhibited a characteristic acidic aroma, a clean white color, and a final pH of 4.0, indicating optimal fermentation (Ragab et al., 2023).

During fermentation, L. bulgaricus and S. thermophilus produced secondary metabolites, including bacteriocins, lactic acid, and hydrogen peroxide, with concentrations increasing alongside yoghurt concentration. Bacteriocins are ribosomally synthesized and post-translationally modified antibacterial peptides that inhibit target bacteria by disrupting cell membrane integrity (Héchard & Sahl, 2002; Sharma et al., 2022; Singh et al., 2024). In this study, the bacteriocin level was estimated to reach 148 mg/L at 100% yoghurt concentration based on regression analysis, demonstrating a positive correlation between bacteriocin concentration and antibacterial activity. Lactic acid, produced via homolactic fermentation, reduced environmental pH to 4.0–4.5, thereby inhibiting the growth of Gram-negative bacteria such as E. coli by destabilizing cell membranes and impairing enzymatic function (Abedi & Hashemi, 2020; Angelova et al., 2018). Additionally, hydrogen peroxide acted as an oxidative agent that damages bacterial DNA and membranes, further enhancing the antibacterial effect of yoghurt (Hertzberger et al., 2014; Zhang et al., 2019). Antibacterial testing showed that yoghurt began to form inhibition zones against E. coli ATCC 25922 at concentrations ≥25%, with the inhibition zone diameter increasing proportionally to yoghurt concentration. This increase reflects the accumulation of antibacterial compounds diffusing into the agar medium, exceeding the effective inhibitory threshold (Chandrasekar et al., 2015).

Mechanistically, bacteriocins form pores in bacterial cell membranes, leading to ion leakage and impaired cellular energy production (Kumariya et al., 2019; Pérez-Ramos et al., 2021). Statistical analysis revealed significant differences between yoghurt concentrations, particularly between 0–20% and 25–50% ($p < 0.05$), as well as a significant positive linear relationship between bacteriocin levels and inhibition zone diameter ($R^2 = 0.8254$). These findings confirm that the antibacterial activity of yoghurt against E. coli ATCC 25922 is influenced by the synergistic effects of bacteriocins, lactic acid, and hydrogen peroxide, which increase with yoghurt concentration (Bonev et al., 2008; Flanagan & Steck, 2017). This study has several limitations, including the use of a single bacterial test strain (E. coli ATCC 25922), a maximum yoghurt concentration of only 50%, the use of a single cow milk-based yoghurt type, and bacteriocins that were not purified, allowing for the potential contribution of other compounds to the observed antibacterial activity.

Future studies should focus on isolating and purifying bacteriocins produced by yoghurt starter cultures, evaluating their antibacterial activity against a broader range of foodborne pathogens, determining MIC and MBC values, and assessing their effectiveness in real food systems. Such investigations will provide a more comprehensive understanding of the potential application of bacteriocins as natural antimicrobial agents in the food industry..

4. CONCLUSION

This study demonstrated that increasing yoghurt concentration was associated with enhanced antibacterial activity against Escherichia coli ATCC 25922, as reflected by the progressive enlargement of the inhibition zone. The observed antibacterial activity is likely attributable to the synergistic effects of antimicrobial metabolites naturally produced during yoghurt fermentation, including bacteriocins, lactic acid, and hydrogen peroxide, rather than to bacteriocins alone. These findings provide preliminary evidence supporting the potential of yoghurt as a natural source of antimicrobial compounds capable of inhibiting foodborne indicator bacteria such as E. coli. Therefore, yoghurt-derived antimicrobial metabolites may have promising applications as natural biopreservatives to improve food safety and reduce dependence on synthetic preservatives in the food industry. Further studies are recommended to isolate and purify bacteriocins, determine their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), perform molecular characterization of bacteriocins, and evaluate their antibacterial efficacy against a broader range of foodborne pathogens as well as in food model systems to support practical applications..

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