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Antibacterial Activity of Lactiplantibacillus Strains Isolated from Commercial Yogurt against Foodborne Pathogenic Bacteria

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Abstract

Background and Objective: Lactic acid bacteria are well known as beneficial microorganisms and most of them are probiotic distributed widely, especially in fermented dairy products e.g. yogurt. This study aimed to isolate, characterize and assess antibacterial effects of lactic acid bacteria that produce bacteriocin-like inhibitory substances against foodborne pathogenic bacteria.

Material and Methods: In the present study, 17 lactic acid bacteria strains were isolated from 10 commercial yogurt samples and the antibacterial effects of lactic acid bacterial cell culture, cell-free supernatant and neutralized cell-free supernatant were assessed against standard foodborne pathogenic bacteria of *Escherichia coli*, *Listeria monocytogenes*, *Klebsiella pneumonia* and *Salmonella typhimurium* using agar well diffusion assay. Although various treatments were used, most of the lactic acid bacterial isolates showed antibacterial activity against the foodborne pathogenic bacteria. Moreover, *Lactiplantibacillus pentosus* (SY1), *Lacticaseibacillus rhamnosus* (SY5), *Lactiplantibacillus plantarum* (SY8) and *Lactiplantibacillus plantarum* (SY9) showed significantly the best antibacterial activity against the foodborne pathogens and thus were further identified using 16S rRNA gene molecular method.

Results and Conclusion: Results showed that four isolates could produce bacteriocin-like inhibitory substances, which were significantly effective to inhibit growth of the pathogens. Primary screening for antibacterial activity showed that 10 lactic acid bacterial strains inhibited *Escherichia coli*. The results revealed that *Listeria monocytogenes* was inhibited by six lactic acid bacterial isolates, while *Salmonella typhimurium* was inhibited by one lactic acid bacterial isolate. Moreover, results showed that *Klebsiella pneumoniae* was not affected by the isolates or treatment methods. It is concluded that the bacteriocin-like inhibitory substance of lactic acid bacterial isolates was effective; hence, it could be used as a natural food additive to prevent foodborne infections and improve the food quality.

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1. Introduction

Lactic acid bacteria (LAB) are a broadly well-known group of bacteria that have been used for centuries in food fermentation. These bacteria are involved in the production of various foods, including yogurt, which produced by inoculation of starter cultures of *Lactobacillus* (*L.*) *delbrueckii* subsp. *bulgaricus* and *Streptococcus* (*S.*) *thermophilus* strains to milk [1]. The LAB are Gram-positive, catalase-negative, non-spore forming and aerotolerant anaerobic bacteria that produce lactic acid as the final product during sugar fermentation [2]. They are generally recognized as safe (GRAS) for human consumption according to the United States Food and Drug Administration and the European Food Safety Authority [3,4]. A large number of LAB strains are characterized and marketed as probiotics due to their beneficial effects, including *Lactobacillus*, *Enterococcus* and *Streptococcus* spp. [5]. Probiotics are defined as live microorganisms, which confer health benefits on the hosts when administered in adequate quantities [6]. Nowadays, there are increasing interests in probiotics, which began after years of safe uses in fermented dairy products due to their beneficial effects on human gut health. These effects generally include preventing pathogen growth in the

gastrointestinal tract by modifying their immunogenicity, producing antimicrobial substances, improving barrier protection, improving degradation of enteric antigens, preventing mucosal adherence and decreasing cancer risk [7-9]. The LAB can be incorporated in foods as starter cultures or natural microflora and play role in food preservation due to production of various antimicrobial compounds e.g. organic acids, hydrogen peroxide and bacteriocins [10-12].

The LAB may contain bacteriocins, as proteinaceous compounds that inhibit or kill other related or unrelated microorganisms [13]. Bacteriocin producing LAB have been classified also as GRAS and can be used as safe additives for food preservation. Several studies documented antibacterial effects of bacteriocins from LAB against foodborne pathogens e.g. *Escherichia coli*, *Listeria* (*L.*) *monocytogenes*, *Pseudomonas aeruginosa*, *Klebsiella* (*K.*) *pneumonia*, *Staphylococcus aureus*, *Salmonella* (*S.*) *paratyphi* and *Acinetobacter baumannii* [14-16]. Nowadays, there is a growing awareness towards food safety, which encourages food scientists to introduce natural compounds in food processing and preservation instead of chemicals to suppress growth of foodborne pathogens and extend shelf life of the food products. Thus, isolating and screening for useful strains contribute to the development of food industries. Moreover, LAB not only affect foodborne pathogens but include clear effects on multidrug-resistant bacteria [15].

The objectives of this study were to isolate and characterize LAB from local yogurt samples and assess their ability to produce bacteriocins or bacteriocin-like inhibitory substances (BLIS) against common foodborne pathogens.

2. Materials and Methods

2.1 Collection of the samples

Ten samples of fermented milk and yogurt products were collected from local markets in Jeddah, Saudi Arabia. Samples were transferred directly to the laboratory of Microbiology Department, King Abdulaziz University, Jeddah, Saudi Arabia, in sterilized containers and stored in refrigerators until further analysis.

2.2 Isolation of lactic acid bacteria

Isolation of LAB was carried out using serial dilution method described by Ismail et al. with some modifications [17]. Briefly, 1 g from each sample was vortexed with 9 ml of sterilized distilled water (DW) and aliquots of 0.1 ml of each dilution were spread-plated on de Man, Rogosa and Sharpe (MRS) agar plate media (Scharlau, Spain). All plates were incubated under anaerobic conditions at 37 °C for 24-48 h. Then, single colonies with distinct morphological characteristics were selected and subcultured by streaking on fresh MRS agar plate media for at least three times.

2.3 Phenotypic and biochemical identifications

A preliminary identification of the isolates was carried out depending on phenotypic (cell morphology and Gram stain) and biochemical characteristics (catalase test, carbohydrate fermentation test and methyl red test). Gram-positive and catalase-negative isolates were selected as presumptive LAB for further identifications [17,18]. Stock cultures of the selected isolates were stored at -80 ◦C in MRS broth (Biolab, Hungary) supplemented with 30% (v v^{-1}) glycerol.

2.4 Assessment of the antibacterial activity of lactic acid bacteria against foodborne pathogens

2.4.1 Foodborne pathogen collection

Four common foodborne pathogens (*Escherichia coli* ATCC 11775, *Listeria monocytogenes* ATCC 13932, *Klebsiella pneumonia* ATCC 700613 and *Salmonella typhimurium* ATCC 14028) were used in this study, which were previously collected from King Fahd Medical Research Center, Department of Microbiology, and Jeddah, Saudi Arabia.

2.4.2 The antibacterial activity using lactic acid bacteria cell culture as primary Screening.

The primary screening for potential antagonistic activity was carried out using agar well-diffusion assay against the four foodborne pathogens (*E. coli*, *L. monocytogenes*, *K. pneumonia* and *S. typhimurium*). The pathogenic bacteria were cultured in nutrient broth (HIMEDIA, India) and incubated at 37 °C for 24 h. Muller-Hinton (MH) agar plates (Oxoid, USA) were inoculated with overnight cultures of the indicator bacteria using sterilized cotton swabs. Then, four wells of 6-mm diameter were prepared and inoculated with 100 µl of the overnight cultures of LAB, which were anaerobically cultured in MRS broth at 37 °C for 24 h. Uncultured MRS broth was used as control. Plates were set for 2 h before incubation to ensure diffusion of LAB broth into the media and then incubated at 37 °C for 24 h under anaerobic conditions. After incubation, inhibition zones around the wells were measured [19,20].

2.4.3 Antibacterial activities of the cell-free supernatant and neutralized cell-free supernatant

2.4.3.1 Preparation of cell-free supernatant

The cell-free supernatant was prepared to exclude the competitive exclusion effects of live cells. Prepration was carried out based on a method described by Rzepkowska et al. [21] with some modifications. The LAB were inoculated into MRS broth under anaerobic condition and incubated at 37 °C for 24 h. Then, overnight cultures were centrifuged at 4500 rpm for 30 min (G-force = 3629) for 30 min at 4 °C to collect cell-free supernatant (CFS). The CFS of each LAB was filter-sterilized using 0.22-µm filters.

2.4.3.2 Preparation of neutralized cell-free supernatant

Preparation of neutralized cell-free supernatant (NCFS) was carried out to detect BLIS and to exclude other inhibitors such as organic acids and hydrogen peroxide. Then the CFS was neutralized to pH 6.5 using 1 M of NaOH to eliminate inhibitory effects of organic acids and then filter-sterilized through 0.22-µm filters. The hydrogen peroxide effect was eliminated using anaerobic incubation.

2.4.3.3 Assessment of the antibacterial activity of cell-free supernatant and neutralized cell-free supernatant

Antibacterial effects of the two treatments were assessed using agar well diffusion assay [22]. Overnight culture of the pathogens were transferred to MH agar plates and four wells of 6-mm diameter were prepared in the agars. Totally, 100 µl of CFS were added into each well and plates were set until the supernatant diffused into the agar. Then, plates were anaerobically incubated at 37 °C for 24 h. A similar method was used on NCFS and inhibition zones were reported.

2.5 Molecular identification of bacteriocin-like inhibitory substance producing strains

The total genomic DNA of LAB was collected according to Azcarate-Peril and Raya [23] with some modifications. Bacterial cells were harvested from an overnight culture of the strains and pellets were mixed with 200 µl of TES buffer and 20 µl of lysozyme (10 $mg.ml^{-1}$). Mixture was transferred to water bath for 20 min at 37 °C. Then, 20 µl of proteinase K (10 $mg.ml^{-1}$) were added to each sample and transferred to water bath for additional 20 min at 37 °C. Then, mixture was transferred to ice bath for 5 min and 250 µl of 4 M sodium acetate were added to the mixture followed by 250 µl of chloroform:isoamyl (24:1). Mixture was stirred gently and centrifuged at 13,000 rpm for 2 min (G-force = 30285). The top layer was transferred to a fresh microtube and 1 v v^{-1} of isopropanol was added to the microtube. This was stored at -20 °C overnight. Then, mixture was centrifuged at 13,000 rpm (G-force = 30285) for 2 min, the liquid layer was removed and the remaining DNA was dried at room temperature (RT). This was resuspended with 50 µl of DW. Gel electrophoresis was carried out on the isolated DNA. Amplification of 16S rRNA gene was carried out using forward 27F (5′-AGAGTTTGA-TCCTGGCTCAG-3′) and reverse 1492R (5′-AAGGAGGT-GATCCAGCCGCA-3′) primers. The DNA amplification was achieved using PCR master mix (Thermo Fisher Scientific, USA) based on the manufacture's guidelines. Amplification was carried at 94 °C for 5 min using thermo-cycler (Mastercycler Gradient, Eppendorf, Germany). This was followed by 32 cycles of 45 s at 94 °C, 45 s at 60 °C and 90 s at 72 °C with a final extension at 72 °C for 10 min. Appropriate aliquot of each PCR amplicon was electrophoresed and visualized under UV using transilluminator (BioDoc-IT System, Japan). The PCR products were sequenced by Macrogen (Seoul, Korea). Sequences were analysed using BLAST of NCBI. Phylogenetic tree was constructed using maximum likelihood method and MEGA-X software.

2.6 Statistical analysis

Data were analyzed using GraphPad Prism 9.5.1(528) software. Results were displayed as the mean ±SD (standard deviation) of three replicates. Statistical analysis was carried out using one-way ANOVA followed by multiple mean comparison Tukey's test and *p*-values less than 0.05 were reported as statistically significant.

3. Results and discussion

3.1 Isolation of lactic acid bacteria

Seven various brands of yogurts were collected from local markets in Jeddah, Saudi Arabia. A total of 17 isolates were detected and preliminary identified using their morphology and biochemical characteristics (Table 1). The isolates were cultured in MRS agar plate media at 37 °C within 24-48 h under anaerobic conditions.

3.2 Phenotypic and biochemical identifications

All isolates were Gram positive, catalase negative and methyl red positive. Of these isolates, 14 isolates were rods in single or pair form. The other three isolates were cocci, appearing single or paired (Table 1). Based on the findings, the isolated strains were identified as LAB [24].

3.3 Assessment of the antibacterial activity of lactic acid bacteria against foodborne pathogens

Antagonistic activity of 17 isolated LAB strains were assessed against *E. coli*, *L. monocytogenes*, *K. pneumonia* and *S. typhimurium* using agar well diffusion assay. Antibacterial effects were assessed based on the zones of inhibition around the wells, which ranged from strong (18-26 mm) to moderate (10-17 mm) and weak (6-9 mm). Cell culture and CFS of 11 out of 17 strains showed significant inhibitory effects against at least one indicator bacterium and degrees of antagonism varied (Figure 1A, 1B and 1C). In contrast, NCFS of the studied LAB showed significantly lower or no inhibitory effects, compared to cell cultures and CFS. Effects of organic acids were excluded by neutralizing the pH to 6.5 with NaOH while the hydrogen peroxide activity was suppressed through anaerobic incubation. Thus, the inhibitory activity of NCFS was mostly linked to BLIS. Results showed that *E. coli* was inhibited significantly by the cell cultures and CFS of ten LAB isolates. After neutralizing the CFS, only four isolates showed clear zones around the wells. The *L. monocytogenes* was affected by the cell culture and CFS of six LAB isolates and NCFS of three isolates.

Moreover, *S. typhimurium* was suppressed by the cell culture and CFS of three isolates and NCFS of one isolate. In contrast, findings revealed that *K. pneumonia* was resistant to all the LAB isolates (Figure 2A, 2B and 2C). Further identifications on species level were used on the isolates that showed antagonistic activities through their NCFS. Based on the best antibacterial activity, selected isolates of *Lactiplantibacillus pentosus* (SY1), *Lacticasei-bacillus rhamnosus* (SY5), *Lactiplantibacillus plantarum* (SY8) and *Lactiplantibacillus plantarum* (SY9) were cultured on agar plate and assessed using light microscope after Gram staining (Figure 3A and 3B). These isolates were identified and characterized as SY1, SY5, SY8 and SY9 using 16S rRNA gene molecular method (Figure 4).

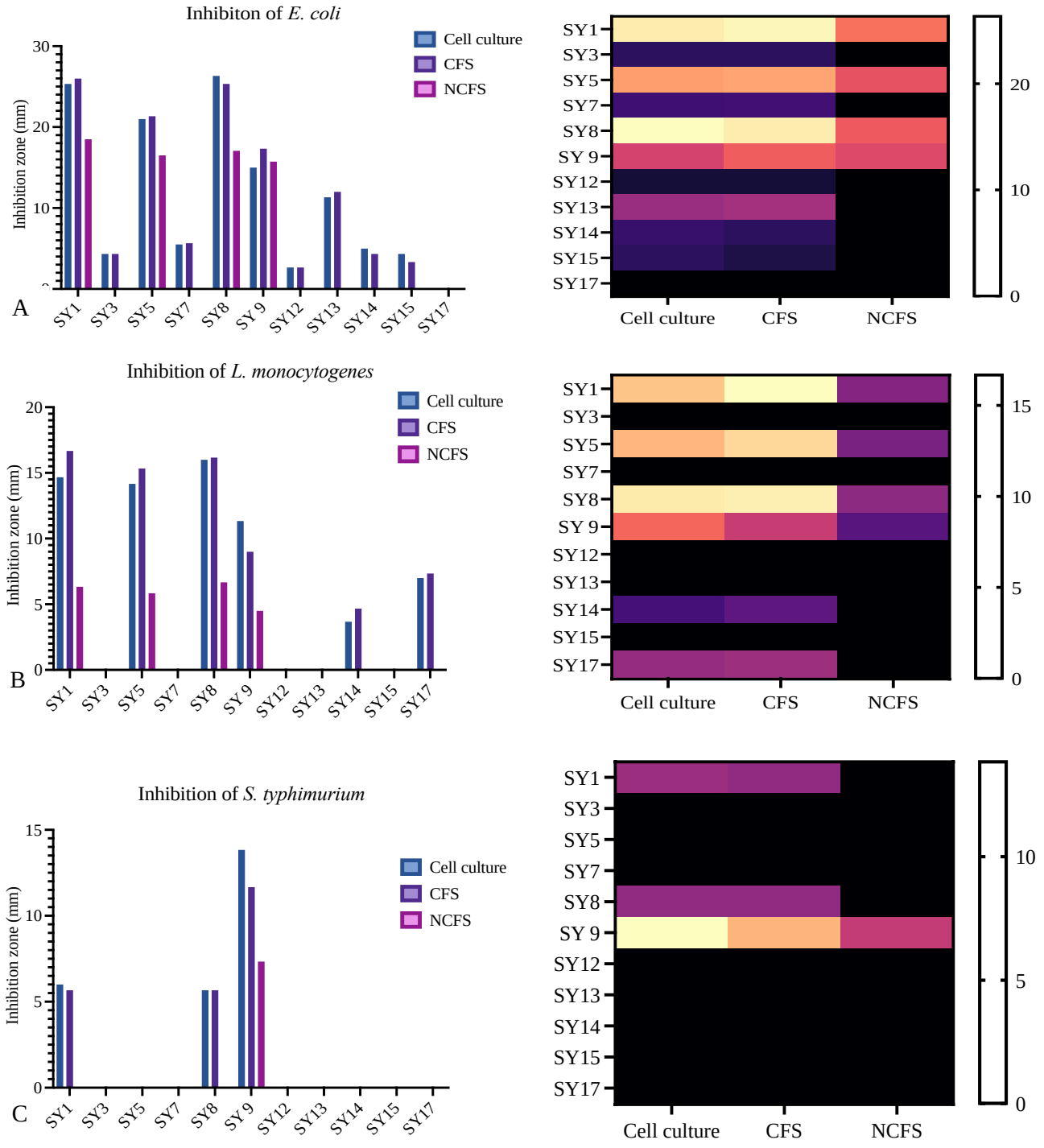
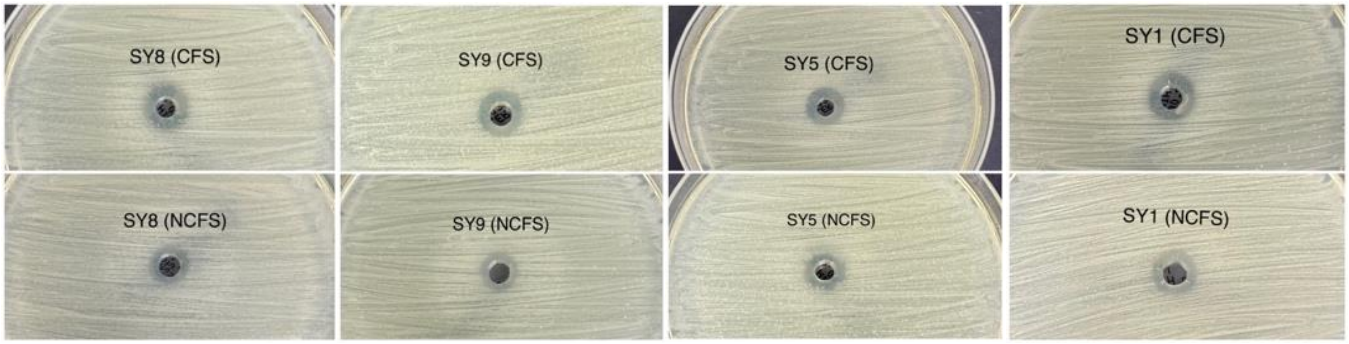
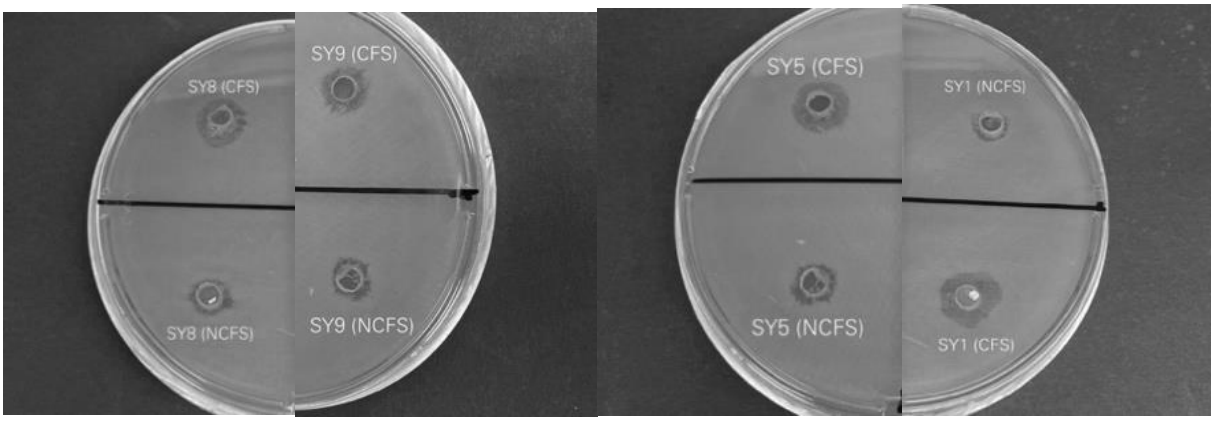


Figure 1. Inhibition zone diameters produced by the lactic acid bacterial cell culture, cell-free supernatant and neutralized cell-free supernatant against *Escherichia coli* (A), *Listeria monocytogenes* (B), *Salmonella typhimurium* (C)

A



B



C

Figure 2: Assessment of the antibacterial activity of cell-free supernatant and neutralized cell-free supernatant from the lactic acid bacterial isolates against *Escherichia coli* (A), *Listeria monocytogenes* (B) and *Salmonella typhimurium* (C) with clear inhibition zones around the wells

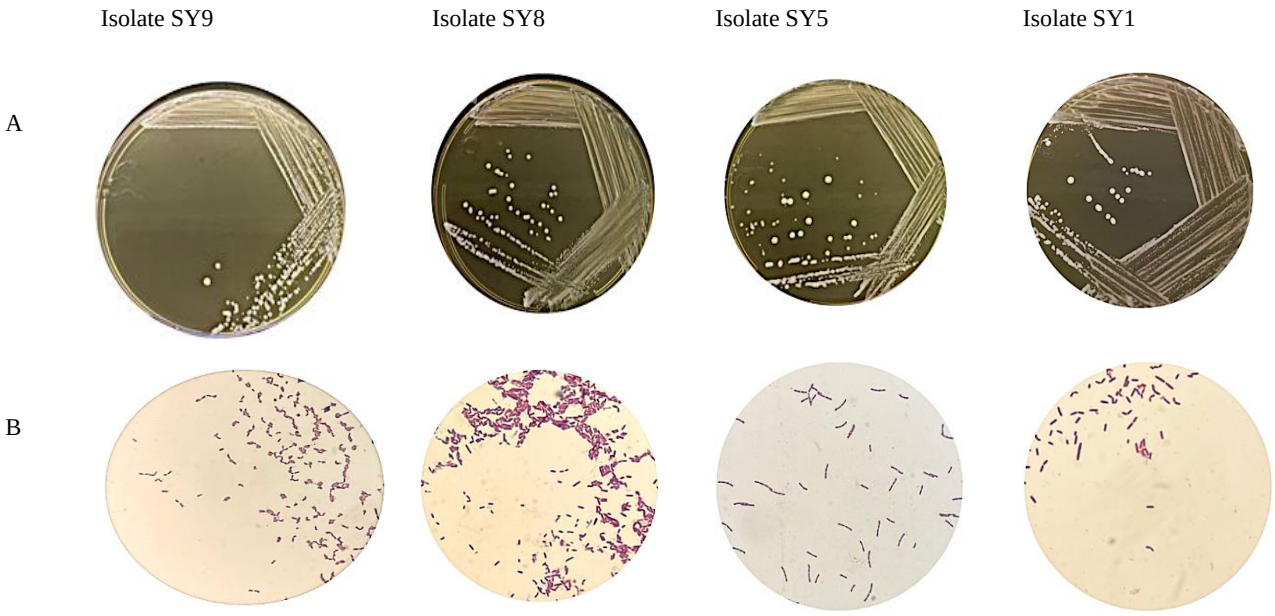


Figure 3. Purified lactic acid bacterial isolates under microscope with magnification of 100 using Gram staining (A). The bacterial growth after 24 h at 37 °C using streak plate method (B)

Table 1. Morphological and biochemical characteristics of the lactic acid bacterial isolates

Isolate No.	Morphology examination		Biochemical assays				
	Gram staining	Cell morphology	Catalase	MR	Carbohydrates fermentation		
					Glucose	Lactose	Sucrose
SY1	+	Rod	-	+	+	+	+
SY2	+	Cocci	-	+	+	-	+
SY3	+	Rod	-	+	+	+	+
SY4	+	Rod	-	+	+	+	+
SY5	+	Rod	-	+	+	+	+
SY6	+	Cocci	-	+	+	+	-
SY7	+	Rod	-	+	+	+	+
SY8	+	Rod	-	+	+	+	+
SY9	+	Rod	-	+	+	+	+
SY10	+	Rod	-	+	+	-	+
SY11	+	Rod	-	+	+	+	+
SY12	+	Rod	-	+	+	+	-
SY13	+	Rod	-	+	+	+	+
SY14	+	Rod	-	+	+	+	+
SY15	+	Cocci	-	+	+	-	+
SY16	+	Rod	-	+	+	+	-
SY17	+	Rod	-	+	+	+	+

Several studies have detected diversity of LAB strains in yogurts, including *Enterococcus* (*E.*) *faecium*, *L. pentosus* [25], *S. thermophilus*, *L. bulgaricus* [26], *L. helveticus* and *L. plantarum* [27]. Naturally, LAB can produce several antimicrobial compounds such as organic acids, hydrogen peroxide and bacteriocins, enabling them to antagonize various bacterial pathogens [28]. Bacteriocins are proteinaceous compounds that produced by the bacteria, inhibiting or killing other related or unrelated microorganisms [13,29]. In the present study, antibacterial activities of 17 LAB isolates were analyzed against *E. coli*, *L. monocytogenes*, *K. pneumonia* and *S. typhimurium* using agar well diffusion assay. Cell culture, CFS and NCFS of the LAB isolates were used to detect their antagonism [21].

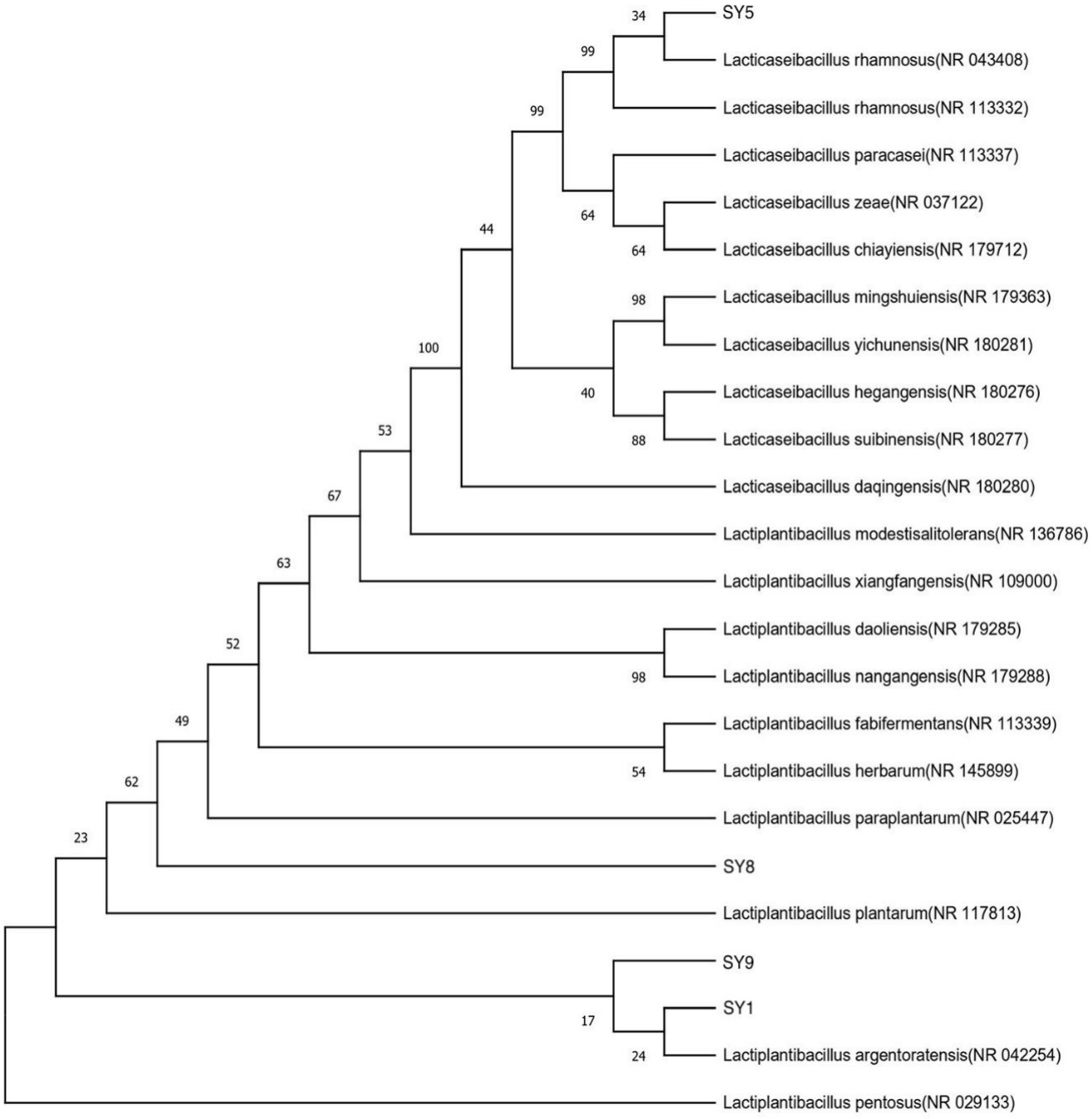


Figure 4: Phylogenetic analysis of the lactic acid bacterial strains. Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing position of the identified lactic acid bacterial strains

As seen in Figure 1A, 1B, and 1C , the cell culture and CFS of ten LAB isolates inhibited growth of *E. coli*, while six isolates inhibited growth of *L. monocytogenes* and three isolates inhibited growth of *S. typhimurium*. For *K. pneumoniae*, none of the isolates nor treatments showed inhibitory activities. This can be described by studies that verified that probiotics include lower effects than antibiotics on *K. pneumoniae* originally reported resistant to most antibiotics. This also explains lack of the effects of probiotics on this bacterium [30]. Several studies reported antibacterial activities of yogurt-derived LAB against various foodborne pathogens [16,31-33]. During the primary screening for antibacterial activities of the LAB, it was seen that *E. coli* was the most affected bacterium within all the pathogenic strains. Cell culture of SY8 included the highest inhibitory effect against *E. coli* (26.33±0.57 mm). Moreover, cell

cultures of SY1, SY5 and SY9 showed significant inhibitory effects against *E. coli* (25.33 ±1.15 mm, 21 ±0.00 mm and 15 ±0.00 mm, respectively). These results were similar to those by Rzepkowska et al., who isolated LAB from fermented meats and detected that the highest inhibitory effect of LAB cell culture against *E. coli* included 10.5 mm [21]; lower than that from the current study. The current results are similar to those by Goa et al. [34], who documented 12 ±1.80 mm as the highest inhibition zone against *E. coli*. However, the results of the present study show higher inhibition zones. These effects might be due to the competitive exclusion, production of metabolic compounds or bacteriocins [35]. Findings illustrated that CFS of SY1 exhibited the highest inhibitory effect against *E. coli* (26.00 ±1.00 mm). Furthermore, CFS of SY8, SY5, SY9 and SY13 showed antagonistic effects (25.3 ±0.57 mm, 21.3 ±1.15 mm, 17.3 ±0.5 mm and 12 ±1 mm, respectively). These results were similar to those of Fadare et al. [36], who reported that the CFS of LAB isolated from Sauerkraut showed inhibitory effects against *E. coli* with the highest inhibition zone of 14 ±0.05 mm. In contrast, the present results were different from those by Jose et al. [37], who reported that CFS of *Lactobacilli* isolates did not affect *E. coli*. Moreover, data showed that NCFS of SY1, SY5, SY8 and SY9 have inhibitory effects against *E. coli*. According to Bahri et al., NCFS of LAB did not inhibit growth of *E. coli* [38]. In contrast, the present results supported results of Voidarou et al., who reported that BLIS produced by LAB included inhibitory effects against various pathogenic strains such as *E. coli* [39].

The *L. monocytogenes* was highly inhibited by the cell culture of SY8 (16.00 ±0.00 mm), followed by SY1, SY5 and SY9 (14.6 ±0.57 mm, 14.16 ±0.28 mm and 11.33 ±0.57 mm, respectively). These results supported those of Rzepkowska et al., who reported that the cell culture of LAB showed inhibitory effects against *L. monocytogenes* and the highest inhibition zone was 22.5 mm [21]. Data illustrated that CFS of SY1 was the most effective component against *L. monocytogenes* (16.6 ±0.5 mm), followed by SY8, SY5 and SY9 (16.10 ±1.04 mm, 15.30 ±0.57 mm and 9.00 ±1.00 mm, respectively). According to Yazgan et al., [40] CFS of LAB strains isolated from various fermented food products showed inhibitory effects against various pathogenic bacteria, including *L. monocytogenes*. The present results illustrated that NCFS of SY1, SY5, SY8 and SY9 showed significant inhibitions against *L. monocytogenes*. Bahri et al. reported that NCFS of LAB inhibited growth of *L. monocytogenes* as well as other pathogenic strains [38]. Moreover, Voidarou et al., detected that BLIS produced by LAB showed antagonistic effects against *L. monocytogenes* [39]. The results of the present study were similar to those by these studies. The *S. typhimurium* was inhibited by the cell culture of SY9 (13.83 ±0.28 mm), SY1 (5.30 ±4.72 mm) and SY8 (5.60 ±5.10 mm). These results were similar to results by Sirichokchatchawan et al., who reported that the live cells of LAB showed antagonistic effects against *S. typhimurium* [41]. Furthermore, CFSs of SY9, SY8 and SY1 were able to antagonize *S. typhimurium* (11.6 ±2.51 mm, 6.33 ±0.57 mm and 5.00±4.35 mm, respectively). In contrast, only NCFS of SY9 was able to antagonize *S. typhimurium*. Bahri et al. reported that *S. typhimurium* was not affected by the NCFS of LAB strains [38]. However, the present results are similar to those by Voidarou et al., who reported that NCFS of LAB inhibited growth of various pathogenic bacteria, including *Salmonella* spp. [39]. The antibacterial activities were previously reported as species and strain-specific [42].

Lactiplantibacillus pentosus (SY1) (formerly known as *Lactobacillus pentosus*) produced BLIS that was active against *E. coli* and *L. monocytogenes* with inhibition zone between 18.5 ±0.5 mm and 6.63 ±0.35 mm, respectively. According to Heredia-Castro et al. [43], bacteriocin-like extracts from *L. pentosus* strains did not suppress growth of pathogenic strains, including *E. coli* and *L. monocytogenes*. This finding is in contrast with the present finding, which indicated a significant antibacterial activity against the two Gram-positive and Gram-negative foodborne pathogens. Findings from the present study were similar to those by Wayah and Philip, [44] who reported that *L. pentosus* produced a bacteriocin called Pentocin MQ1, which was able to inhibit growth of various pathogenic bacteria, including *L. monocytogenes* and *E. coli*. This result is similar to that of Yi et al., who isolated a bacteriocin-producing LAB strains from Chinese homemade pickles and dry-cured meats and detected that *L. pentosus* bacteriocin was effective against all indicator pathogens, including *L. monocytogenes* and *E. coli* [45]. In the present study, *Lacticaseibacillus rhamnosus* (SY5) (formerly known as *Lactobacillus rhamnosus*) produced BLIS that was effective against *E. coli* and *L. monocytogenes* with inhibition zones of 16.5 ±0.5 mm and 7.03 ±0.83 mm, respectively. This result is similar to a study by Chen et al. [14] on a novel bacteriocin isolated from *L. rhamnosus* against various foodborne pathogens. Results revealed that the bacteriocin CLK_01 showed a broad antibacterial spectrum against Gram-positive and negative pathogens, e.g. *E. coli*. Nespolo and Brandelli [46] reported that *L. rhamnosus* and *L. plantarum* isolated from ovine cheese produced BLIS against *L. monocytogenes*. Simova et al. [47] isolated LAB strains from Bulgarian dairy products and assessed their antibacterial activities against pathogens. Results indicated that seven LAB strains were bacteriocin producers and antagonized a wide range of pathogens. The *L. rhamnosus* inhibited *E. coli* and *L. monocytogenes.*

Moreover, our findings showed that *Lactiplantibacillus plantarum* (SY9) and (SY8) (formerly known as *Lactobacillus plantarum*) produced BLIS that was active against *E. coli* (15.7 ±0.2 mm by strain SY9 and 17.06 ±0.8 mm by strain

SY8), *L. monocytogenes* (7.46 ±0.68 mm by strain SY9 and 8.66 ±0.57 mm by SY8) and *S. typhimurium* (7.93 ±1.00 mm by strain SY9 only). A study published that the bacteriocin B391 of *L. plantarum* isolated from artisanal French cheese included antagonistic activities against *L. monocytogenes* strains [48]. Another study documented antibacterial effects of a novel bacteriocin produced by *L. plantarum* against various bacterial pathogens. Findings verified that bacteriocin plantaricin W3-2 showed broad-spectrum antibacterial activities against indicator strains, including *E. coli* and *L. monocytogenes* [49]. Man and Xiang [50] demonstrated that *L. plantarum* from koumiss produced a bacteriocin that was active against *E. coli*, *L. monocytogenes* and *S. typhimurium*.

5. Conclusion

In this study, various LAB strains were isolated from samples of yogurts. The dominant isolates belonged to *Lactiplantibacillus* spp. These isolates have a significant antibacterial activities against *E. coli*, followed by *L. monocytogenes* and *S. Typhimurium* respectively, compared to the indicator bacteria. However, *K. pneumoniae* was not affected by the isolates. Antibacterial activity of the LAB isolates with CFS was more than that of NCFS in all isolates. Yogurt includes LAB strains that are safe to consume by humans and addressed as natural antibacterials and biopreservatives in food industries. It is important to carry out further studies to assess other types of probiotics and compare their effects with those of antibiotics. Moreover, studies on use of probiotics in management of other pathogenic bacteria are important.

6. Conflict of Interest

The authors report no conflict of interest.

7. Acknowledgements

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8. Authors Contributions

All the authors have significantly contributed to the conceptualization and design of the study; collection, analysis and interpretation of data; writing of the article or its critical revision for significant intellectual content and final approval of the version.

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فعالیت ضدباکتریایی سویه‌های لاکتوباسیلوس جداشده از ماست‌های تجارتی در برابر باکتری‌های بیماریزای غذازاد

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چکیده

سابقه و هدف: باکتری‌های لاکتیک اسید به‌عنوان میکروارگانیسم‌های مفید شناخته شده‌اند و بیشتر آن‌ها زیست‌یارهایی[1] هستند که در محصولات لبنی تخمیر شده مانند ماست توزیع گسترده‌ای دارند. هدف از این مطالعه جداسازی، شناسایی و ارزیابی اثرات ضدمیکروبی باکتری‌های لاکتیک اسیدی است که تولیدکننده مواد بازدارنده شبه‌باکتریوسین در برابر باکتری‌های بیماری‌زای غذازاد[2] هستند.

مواد و روش ها: در مطالعه حاضر، ۱۷ سویه باکتری لاکتیک اسید از ۱۰ نمونه ماست تجاری جدا و اثرات ضدباکتریایی کشت سلول باکتریایی لاکتیک اسید، مایع رویی بدون سلول و مایع رویی بدون سلول خنثی شده در برابر باکتری های بیماری‌زای غذازاد استاندارد *اشرشیاکلی*، *لیستریا مونوسیتوژنز*، *کلبسیلا پنومونیا* و *سالمونلا تیفی‌موریوم* با استفاده از روش انتشار چاهکی آگار بررسی شد. اگرچه از تیمارهای گوناگونی استفاده شد، اما اکثر جدایه‌های باکتری لاکتیک اسید درجات مختلفی از فعالیت ضدمیکروبی را در برابر باکتری‌های بیماری‌زای غذازاد نشان دادند. به علاوه، *لاکتی‌پلانتی‌باسیلوس پنتوسوس* (SY1)، *لاکتی‌کازیی باسیلوس رامنوسوس* (SY5)، *لاکتی‌پلانتی‌باسیلوس پلانتاروم* (SY8) و *لاکتی‌پلانتی‌باسیلوس پلانتاروم* (SY9) به طور قابل‌توجهی بهترین فعالیت ضدمیکروبی را در برابر باکتری‌های بیماری‌زای غذازاد نشان دادند و بعدا با استفاده از روش‌های مولکولی rRNA شناسایی شدند.

یافته‌ها و نتیجه‌گیری: نتایج نشان داد که چهار جدایه می‌توانند مواد بازدارنده شبه باکتریوسین را در تمام باکتری‌های لاکتیک اسید تولید کنند. این ماده به طور قابل توجهی موثر و قادر به مهار رشد باکتری‌های بیماری‌زایی بود که غذاها را آلوده و مشکلات سلامتی ایجاد می‌کرد. بر اساس غربالگری اولیه برای فعالیت ضد میکروبی، ۱۰ سویه باکتریایی لاکتیک اسید دارای اثرات بازدارنده علیه *اشرشیا کلی* هستند. نتایج فعلی نشان داد که *لیستریا مونوسیتوژنز* توسط شش جدایه باکتری لاکتیک اسید مهار شد، در حالی‌که *سالمونلا تیفی‌موریوم* توسط یک جدایه باکتری لاکتیک اسید مهار شد. علاوه بر این، نتایج نشان داد که *کلبسیلا پنومونیا* تحت تأثیر جدایه‌ها یا روش‌های درمانی قرار نگرفت. نتیجه‌گیری می‌شود که ترکیب بازدارنده شبه باکتریوسین حاصل از جدایه‌های باکتریاایی اسید لاکتیک موثر بود. از این رو، می‌توان از آن به عنوان یک افزودنی غذایی طبیعی برای جلوگیری از عفونت‌های غذازاد و بهبود کیفیت غذا استفاده کرد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.

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- *کلبسیلا پنومونیا*
- باکتری‌های لاکتیک اسید
- *سالمونلا تیفی موریوم*

***نویسنده مسئول**

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[1] probiotic
[2] foodborne pathogenic bacteria