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Antibacterial Activity of Probiotics Isolated from Commercialized Yogurt Against Pathogenic Bacteria

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ABSTRACT

Probiotics have garnered considerable attention as potential agents for inhibiting pathogenic bacteria due to their beneficial effects on gut health and immune modulation. The study aimed to identify probiotic strains with significant antibacterial potential that could be used as natural alternatives to traditional antibiotics. The antibacterial activity of cell-free supernatant (CFS) and cell lysate (CL) of both LAB isolates against skin and food-borne bacteria was observed via disc diffusion assay, where the inhibition zone sizes ranged from 8.5 ± 0.15 mm to 12.0 ± 0.82 mm in diameter. The standard broth dilution was performed to estimate the MIC and MBC values for both isolates, which exhibited 25-50 g/mL values against Gram-positive bacteria, while against Gram-negative bacteria, the values ranged between 12.5-100 µg/mL. Only the CL of both isolates exhibited bactericidal effects on the tested bacteria with a ratio ≤ 4. This study's inhibitory effects and results have proven the presence of antibacterial composition in commercial yoghurt as a natural agent that can inhibit the growth of common skin and food-borne pathogenic bacteria. As the issue of antibiotic resistance continues to grow, this study offers promising implications for developing alternative, sustainable antibacterial interventions to safeguard public health.

INTRODUCTION

Probiotics, which have the potential to improve health in several ways, have quickly become a standard component of many fields, including biology, medicine, nutrition, and food science. These products contain live non-pathogenic bacteria and can be used as dietary supplements, preventative measures, or treatments (Raghuwanshi, 2015). According to the current definition provided by the Food and Agriculture Organization of the United Nations World Health Organization, probiotics are defined as live microorganisms that confer a health benefit on the host when administered in appropriate quantities (Hill

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et al., 2014). Probiotics can improve the health of the digestive tract and the immune system (Garrigues et al., 2013). Fermented foods naturally contain probiotic bacteria from the genus Lactobacillus. Many illnesses from pathogens invading the digestive tract can be prevented by keeping the gut healthy with probiotics or fermented foods (Mokoena et al., 2016). Probiotics can be yeast, bacteria, or moulds, with bacterial species being the most prevalent. Two genera that account for many of the most popular probiotics are species of Lactobacillus and Bifidobacterium (Alok et al., 2017). Because of their beneficial effects on the host, lactic acid bacteria and bifidobacteria are purposefully introduced into the food chain as starter cultures and probiotics (Huang et al., 2020).

Foodborne sickness can be caused by various pathogens, with harmful bacteria being the most common. These bacteria and their toxins induce foodborne illness by entering the body via several routes. During growing, harvesting, preparing, preservation, processing, and distribution, contamination is possible during growing, harvesting, preparing, preservation, processing, and distribution (Sharif et al., 2018). Typically, many skin pathogens reside on the skin as commensals, but microbial dysbiosis or imbalance, host genetic variation, and immunological state may drive the change from commensal to pathogen (Findley & Grice, 2014). Antibiotic resistance in bacterial pathogens has also become a major concern for public health, with drug-resistant microorganisms accounting for 25,000 fatalities each year in Europe alone (Colomb-Cotinat et al., 2016). Therefore, discovering new antimicrobial agents from probiotics is crucial to combat pathogenic microorganisms.

EXPERIMENTAL

Test Microorganisms

Clinical samples of skin and food-borne pathogenic bacteria, namely *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus hominis*, and *Salmonella typhi* were obtained from the Microbiology Laboratory, Faculty of Applied Science, Universiti Teknologi Mara (UiTM), Shah Alam.

Preparation of Lactic Acid Bacteria (LAB) Samples

The yoghurt samples of the Farm Fresh brand were purchased from Giant Hypermarket. Afterwards, 1.0 mL of yoghurt sample was aseptically inoculated into 9.0 mL de Man Rogosa Sharpe (MRS) broth, and a ten-fold serial dilution was conducted. All the samples were then incubated anaerobically at 37°C for 48 hours. After that, 10µL of the enriched samples in MRS broth were cultured onto an agar plate medium of MRS by conducting the spread plate method. The plates were incubated anaerobically for 24-48 hours at 37°C. Then, the targeted colonies were tested using catalase test, oxidase test, and Gram staining to be observed under the microscope. Oxidase-negative, catalase-negative, and gram-positive bacilli were purified by streaking on MRS agar and incubated anaerobically.

Culture and Maintenance of Test Microorganisms

Five selected bacterial strains were used to perform an antibacterial activity test of bacteria isolated from yoghurt. The selected bacteria consist of two Gram-positive (*S. aureus* and *S. hominis*) and three Gram-negative (*S. typhi*, *E. coli*, and *P. aeruginosa*). For stock culture, the acquired bacterial cultures were subcultured on Nutrient Agar and incubated at 37°C for 24 hours. The stock cultures were stored at 4°C until further use. Pure colonies were inoculated in a single loopful and suspended in 10.0 mL of glycerol to prepare the inoculum suspension and kept at -20°C until further use.

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Preparation of Cell-Free Supernatant (CFS) and Cell Lysate (CL)

In this study, the preparation of LAB samples for CFS and CL was utilized for antibacterial testing. Reuben et al. (2019) reported that the CFS was prepared by inoculating one pure colony into 10mL MRS broth and incubating for 48 hours at 37°C under anaerobic conditions. After that, the bacterial culture was centrifuged at 4°C for 15 minutes at 10,000 × g. After centrifugation, the cell pellets generated from filtering the CFS at 0.22µM were utilized to make cell lysates. The CFS was stored at -40°C. The CL was prepared following the methods described by Ahmed et al. (2013). A 3.0 mL sterile distilled water was used to wash the cell pellets three times. Finally, three microcentrifuge tubes were filled with 1.0 mL cell suspension and centrifuged at 10,000 x g for 10 minutes. The supernatant was then discarded. Next, 0.5 mL of 0.01M lysis buffer was added to the pellets, and the mixture was heated at 100°C for 8 minutes. The CL was re-centrifuged at 10,000 × g for 10 minutes, and the resulting cell supernatant was transferred to a separate microcentrifuge tube. The CL was stored at -40°C.

Antibacterial Assay

The disc diffusion method was used to test the antibacterial activity of the CFS and CL of the yoghurt sample against the pathogenic bacteria (Nnachetam et al. 2020). Using a spectrophotometer, the final OD625nm of the microorganisms cultivated overnight was adjusted with Mueller-Hinton broth (MHB) to a standard of approximately 0.08 – 0.10 to match the 0.5 McFarland standard (10^8 CFU/mL). Optimally, a sterile cotton swab was dipped into the adjusted suspension after 15 minutes, after adjusting the turbidity of the inoculum suspension. The swab was rotated multiple times and pressed firmly against the interior bottle wall above the fluid level to remove the excess inoculum from the swab. The entire agar surface was swabbed to ensure an even distribution of pathogenic bacteria, and the procedure was repeated while the plate was rotated. The sterilized 8 mm blank discs were impregnated with 100µL of the sample. Chloramphenicol, and sterile distilled water as positive and negative controls, respectively. The plates were incubated for 24 hours at 37°C in an incubator. The diameter of the inhibition zone formed on the plate was measured and recorded in millimeters (mm) using a ruler. The assay was conducted in triplicate.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The standard broth dilution method was used to study the antibacterial efficacy of CFS and CL of yoghurt samples by evaluating the visible growth of microorganisms in the broth. Serial ten-fold dilutions of CFS and CL in concentrations ranging from 100 µg/mL to 3.125 µg/mL with adjusted bacterial concentration (0.5 McFarland standard) were used to determine MIC in MHB. The control contained only inoculated broth and was incubated for 24 hours at 37°C. The MIC value was determined by the lowest concentration of CFS and CL, which successfully inhibits bacterial growth of bacteria after 24 hours of incubation. After the MIC determination, aliquots of tubes that showed no visible bacterial growth were inoculated on MHA plates and incubated for 24 h at 37°C. MBC endpoint is reached when 99.9% of the bacterial population is eliminated at the lowest concentration of an antibacterial agent. This was accomplished by observing agar plates before and after incubation for the presence or absence of pathogens (Parvekar et al., 2020).

Bacterial growth reduction

With a few minor adjustments, the Jalil et al., (2022) approach was used to test the crude extracts' efficacy. This study made every crude extract at MIC and 2 × MIC values. A 50 mL Erlenmeyer flask was filled with 8.9 mL of MHB for treatment, and 1.0 mL of crude extracts at a final concentration of MIC was added thereafter. After adding 0.1 mL of bacterial suspension to the flask with a final bacterial concentration of 5

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× 10^8 CFU/mL, it was shaken for 24 hours at 37°C at 150 rpm. A volume of 1.0 mL of the mixture was removed after the incubation period of 12 and 24 hours (Norhisham et al., 2025). Using the spread plate count method, the bacterial colony was measured as a colony-forming unit (CFU).

Statistical Analysis

All analyses were performed in triplicate (n=3), and the findings were analyzed using a one-way Analysis of Variance (ANOVA) test and reported as the mean ± standard deviation. The Statistical Package for the Social Sciences (SPSS) Version 26.0 (t-test) was used to compare the effect of extracts against various bacterial strains and the control.

RESULTS AND DISCUSSION

Isolation and Identification of *Lactobacillus* spp.

Antibiotics are becoming less effective at controlling the development of pathogens, resulting in the global spread of drug resistance, which makes it more difficult to treat infections and death. Increases in antimicrobial resistance have garnered a great deal of focus on the development of novel and alternative treatments for infectious diseases. Sharma et al. (2017) stated that LABs are currently regarded as the most effective probiotics with robust antimicrobial agents. In this study, probiotic Lactobacilli were isolated from the commercial probiotic product "Farm Fresh" yoghurt (curd) by serially diluting in MRS broth [Dilutions (10^{-1} – 10^{-6})], which is generally sufficient to decrease the number of viable cells. After 48 hours of incubation on MRS medium, colonies of LAB (*Lactobacillus* spp.) appeared. Devi et al. (2013) reported that thirty colonies were isolated from MRS medium plates of various dilutions and then sub-cultured by streaking on MRS medium plates. Two strains were selected based on their colour differences, which are creamy and less creamy (Figure 1). The confirmed isolates were named LAB1 and LAB2. The colonies formed from the probiotics were mostly cream-coloured or off-white, small, circular, convex, and moist with smooth edges. Microscopically, all of the isolates stained purple; therefore, they were all Gram-positive bacteria (Figure 2). Both bacterial isolates were rod-shaped with rounded ends and oxidase- and catalase-negative. According to Amin et al. (2016), *Lactobacillus* sp. primarily appeared as chains or as single cells. The morphological and biochemical characteristics of isolated bacteria are illustrated in Table 1. Based on the data from the biochemical test and Gram-staining, it was presumed that the strains belonged to *Lactobacillus rhamnosus* for the creamy one (LAB1) and *Lactobacillus acidophilus* for the less creamy one (LAB2).

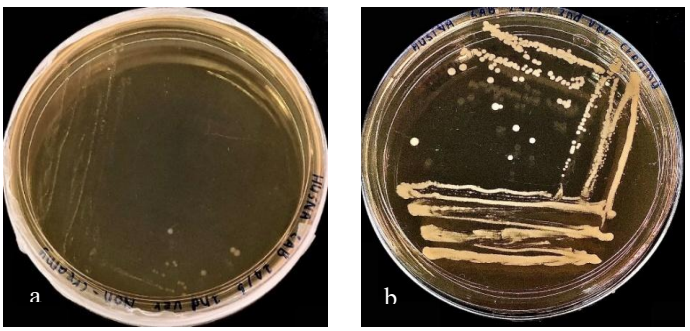

Fig. 1 Single-screened colonies on MRS media. (a) LAB – presumed as *Lactobacillus rhamnosus* (b) LAB2 – presumed as *Lactobacillus acidophilus.*

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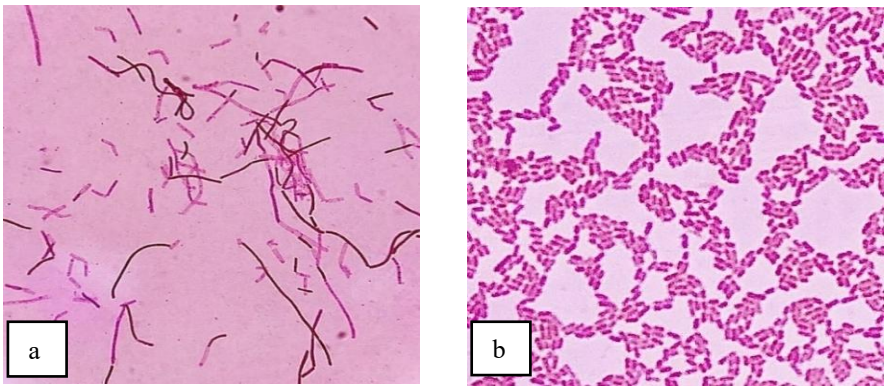

Fig. 2 Light microscopy observation of LAB isolates at 100 × magnification. (a) LAB1 – presumed as *Lactobacillus acidophilus*, (b) LAB2 – presumed as *Lactobacillus rhamnosus*

Table 1 Morphological and biochemical characteristics of isolated *Lactobacilli* sp.

Characteristics	Isolate-1 (LAB1)	Isolate-2 (LAB2)
Colonies morphology	White-creamy in colours, smooth and round	White but less creamy in colours, smooth and round
Gram stain	+	+
Oxidase test	-	-
Catalase test	-	-

+: positive, -: negative

Preparation of Cell-Free Supernatant (CFS) and Cell Lysates (CL)

The supernatant is typically a clear liquid deposited by settling, precipitation, or centrifugation that resides above the underlying material (Mani-López *et al*., 2022). In this experiment, CFS refers to the clear liquid medium acquired after *Lactobacillus* growth in MRS broth, which is centrifuged and filtered through a 0.22µm membrane to retain all bacterial cells. A cell-free supernatant, or CFS, is a liquid that contains the metabolites produced by microbial growth and the remaining nutrients from the medium used. Among other things, the organic acids, fatty acids, and proteinaceous chemicals found in CFS from LAB can have antibacterial potential (Mani-López et al., 2022). Meanwhile, CL refers to the fraction remaining in the tube resuspended in the lysis buffer. Figure 3 shows the centrifuged LAB grown in the MRS broth of the commercial yoghurt. According to Islam *et al*. (2017), cell lysis or cellular disruption is a process in which a cell's outer boundary or cell membrane is broken down or destroyed to liberate intercellular materials such as DNA, RNA, or organelles. The lysate method has several benefits. It is, first of all, easy to understand and possibly cheap. Secondly, it's possible that the cytoplasmic contents already include enough cofactors, which would save on the expense of adding more cofactors (Khattak et al., 2014). A cell lysate method is very successful in complicated systems, whereby using endogenous processes to produce the desired product may be desirable. Indeed, the

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preferred method for producing commercially available cell-free proteins is the lysate approach (Jewett & Swartz, 2004).

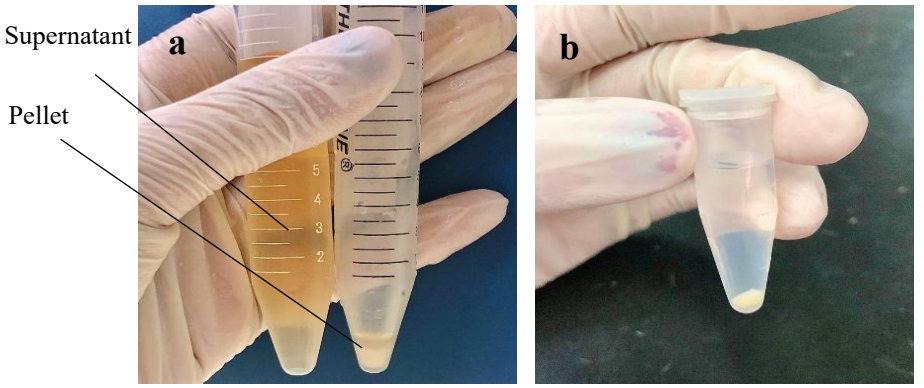

Fig. 3 Centrifugation of LAB. **(a)** Supernatant and pellet of LAB **(b)** Cell lysate washed with distilled water.

Evaluation of Antibacterial Activity

The antibacterial activity of CFS and CL from isolated strains was determined against pathogenic bacteria using a disc diffusion assay. The present study revealed that both isolates from (CL) showed antibacterial activity against most test pathogens used in this study (Table 2). However, no inhibitory effect of CFS against test bacteria was observed. CL of LAB1 and LAB2 exhibited significant antibacterial activity against *S. aureus*, with the CL of LAB2 showing the highest inhibition zone of 9.0 ± 0.05 mm (Figure 4). For *S. hominis*, CL of LAB2 showed the highest average inhibition zone of 11.0 ± 0.04 mm. The highest inhibition zone was the LAB1 strain against *S. typhi* with 12.0 ± 0.82 mm as shown in Figure 4. *P. aeruginosa* was the least susceptible to CL of LAB2, this may be due to the outer layer membrane that they have as a Gram-negative bacterium. Several factors make Gram-negative bacteria less sensitive to antibiotics including resistance to antimicrobials, particularly in nosocomial infections (Breijyeh et al., 2020), manufacturing of antibiotic-inactivating/modifying enzymes (Gauba & Rahman, 2023), alteration target site, penicillin-binding proteins, permeability-based resistance, and efflux pumps (Singh et al., 2022). Compared to Gram-positive bacteria, they are more resistant due to their unique structure such as outer layer membrane (Epand et al., 2016). Hasali et al. (2018) reported that *L. brevis* strain NJ42 was reported to have a significant inhibitory effect against *P. aeruginosa* with a diameter of 24 mm. The inhibitory effect of LAB1 against *S. aureus* showed the lowest inhibition zone with 8.5 ± 0.15 mm. The present finding also revealed that CL has a significant inhibitory effect compared to the cell-free supernatant (CFS) which had no inhibition zone. Similarly, Chowdhury et al., (2013) reported a similar observation, revealing that CL's antibacterial activity from *Lactobacillus* sp. was higher than cell culture supernatant against *Bacillus cereus* and *E. coli*. Orji et al. (2019) found that the cell lysate has bacteriocin, which has higher antibacterial action. Hasali et al. (2015) discovered that Lactobacillus brevis exhibited antibacterial activity against *S. epidermidis*, *P. aeruginosa,* and *L. monocytogenes* with inhibition zones of 32 mm, 16 mm, and 18 mm. In addition, Kenfack et al. (2018) stated that *L. plantarum* reacted to antimicrobial activity against *S. aureus* and *S. enterica* with an inhibition zone from 6.1 to 14.0 mm. In short, compared to CFS, cell lysates have better antibacterial action. This is because LAB is a natural antibiotic agent that can make metabolites, like bacteriocins, hydrogen peroxide, and organic acid which stop bacteria from growing. The ability of good probiotics to make antibacterial substances is the most important function used to describe them Hasali et al. (2018). Inglin et al. (2015) reported a similar observation that Lactobacilli's antimicrobial activities are

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directly linked to the production of organic acids such as lactic acid, acetic acid, and propionic acid, as well as other components such as hydrogen peroxide, bacteriocins, and peptides. These results proved that *L. acidophilus* and *L. rhamnosus* strains contain antibacterial properties that make them suitable for use as a natural preservative, particularly in dairy food products. Contradictory, Keeratikunakorn et al., (2023) reported that certain substances found in probiotics' cell-free supernatants (CFS), such as bacteriocins and antimicrobial peptides, can stop the growth of other bacteria. CFS exhibits inhibitory activity against various pathogenic bacteria thanks to its antimicrobial peptides and/or bacteriocin component obtained from probiotics, particularly lactic acid bacteria (LAB). Prior research on the antibacterial properties of CFS has examined a range of pathogens, such as *Salmonella* Typhimurium and Typhi, *Listeria monocytogenes*, and *Staphylococcus aureus*.

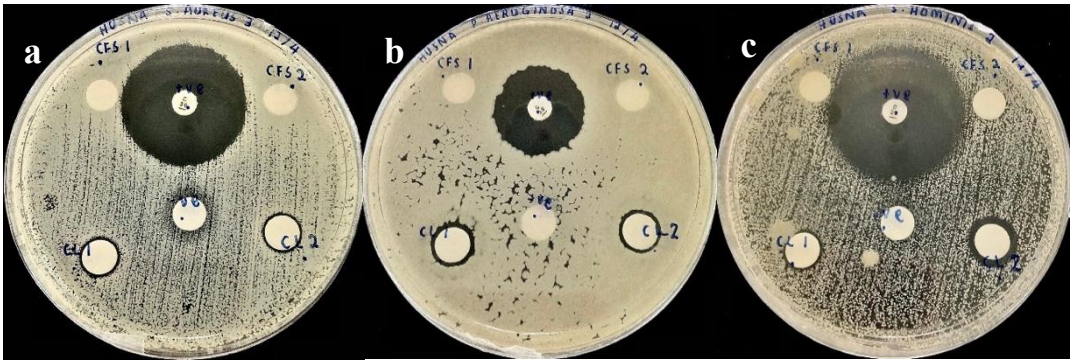

Figure 4 Disc diffusion assay for *Lactobacillus* isolates from Farm Fresh yoghurt against (**a**) *Staphylococcus aureus* (**b**) *Pseudomonas aeruginosa* (**c**) *Staphylococcus hominis.*

Table 2 Antibacterial activity of probiotic cell lysate (CL) against test pathogens

Isolates	Zone of inhibition (in mm) (Cell Lysates)				
	S. aureus	***S. hominis***	***P. aeruginosa***	***E. coli***	***S. typhi***
LAB1	8.5±0.15	9.0 ± 0.08	10.0 ± 0.06	9.0 ± 0.62	12.0 ± 0.82
LAB2	9.0 ± 0.05	11.0 ± 0.04	9.0 ± 0.08	10.0 ± 0.65	10.0 ± 0.65
Chloramphenicol	31.0 ± 0.04	32.0 ± 0.09	22.0 ± 0.16	17.0 ± 1.39	20.0 ± 1.41
Distilled water	No inhibition	No inhibition	No inhibition	No inhibition	No inhibition

Notes: Data were means of triplicate (n=3) ± standard deviation, Chloramphenicol = positive control, Distilled water = negative control

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined by using the dilution method. Akbar et al. (2019) reported strong antibacterial properties of *Lactobacillus lactis* against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhimurium*, and *E. faecalis*. *S. typhi* showed turbidity after incubation in nutrient broth with a concentration of CL of LAB1 at 6.25µg/mL, but not at higher concentrations, while *S. hominis* showed turbidity at 12.5µg/mL, but not at 25µg/mL or higher CL of LAB2 concentrations. Moreover, Owuama (2017) reported that *E. coli* exhibited turbidity at 5

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µg/mL, but not at 7.0 µg/mL, indicating that 7.0 µg/mL was the MIC, whereas *S. aureus* exhibited turbidity at 9.0 and 9.5 µg/mL, but not at 9.8 µg/mL, indicating that 9.8 µg/mL was the MIC for *S. aureus*. In this study, the MIC range for LAB1 was 12.5 to 50 µg/mL and for LAB2 was 25 to 100 µg/mL against test pathogens. The MBC for *S. typhi* and *S. hominis* was at the concentration of 50 µg/mL, as there was no growth of the bacteria on the nutrient agar plate. All MBC values for LAB1 were 100 µg/mL, and for LAB2 ranged from 50 to 100 µg/mL.

The MIC and MBC were varied between LAB1 and LAB2 against test pathogens (Table 3). Another study showed that the MBC of *E. coli* species is 7.5 µg/mL because no growth was observed after 24 hours of incubation at 37°C (Owuama, 2017). According to a study conducted by Ilavenil et al. (2021), the MIC range for *Lactococcus lactis* was 6.25 to 12.5 mg/mL, and the MBC range was from 12.5 to 25 mg/mL against *E. coli*, *P. aeruginosa*, and *S. aureus*. Based on the data obtained, it is suggested that the pathogens' growths were inhibited with increased concentration of CL, which is concurrent with the data obtained from the disc diffusion assay method. The finding revealed that the MIC and MBC of both types of LABs against different pathogens were different. LAB1 showed strong MIC for *S. typhi,* and LAB2 showed potent MIC for *S. hominis* and *P. aeruginosa*. However, an equal MBC was noted for LAB1 against all pathogens, whereas LAB2 displayed equal MBC against all pathogens except *E. coli*. Another study showed that *Lactococcus lactis* strain RWP-3 exhibited a high MIC against *E. faecalis*, while strain RWP-7 exhibited an effective MIC against P. aeruginosa. RWP-3 exhibited a strong MBC against *E. faecalis*, whereas RWP-7 demonstrated a potent MBC against *E. coli* and *P. aeruginosa* (Ilavenil et al., 2021).

The present finding also revealed that CLs of LAB1 and LAB2 possess bactericidal effects against all test bacteria. According to Jalil et al. (2022), antimicrobial compounds are considered bacteriostatic agents when the MBC/MIC ratio is greater than 4, and bactericidal agents when it is 4 or below. However, both bacteriostatic and bactericidal agents have advantages as antibiotics against pathogenic bacteria, in which bacteriostatic antibiotics do not reduce the number of bacteria; instead, they stop their growth, whereas bactericidal agents reduce their numbers. Bacteriostatic antibiotics allow the immune system to combat infections even when the bacteria are still alive, in contrast to bactericidal antibiotics. For instance, some bacteriostatic drugs work well in streptococcal and clostridial gangrene cases. This is because they stop the toxins from being produced, which are the main contributors to morbidity (Zulkamal et al., 2023)

Table 3 Minimum Inhibitory concentration and Minimum bactericidal Concentration (MIC/MBC) of cell lysates of *L. rhamnosus* (LAB1) and *L. acidophilus* (LAB2) against *S. aureus, S. hominis, P. aeruginosa, E. coli*, and *S. typhi.*

Pathogenic Bacteria	CL of LAB1			CL of LAB2		
	MIC (µg/mL)	MBC (µg/mL)	Ratio MIC/MBC	MIC (µg/mL)	MBC (µg/mL)	Ratio MIC/MBC
S. aureus	50	50	1	50	50	1
S. hominis	25	50	2	25	50	2
P. aeruginosa	25	50	2	25	50	2
E. coli	50	50	1	100	100	1
S. typhi	12.5	50	4	50	50	1

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Bacterial growth reduction

Table 4 shows the growth reduction of pathogenic bacteria exposed to LAB cell lysate after 12 and 24 h at an extract concentration of MIC. *S. aureus* and *S. hominis* cells were reduced to 86.86% and 83.56%, respectively, after 12 h exposure to LAB1 cell lysate (LAB1-CL). The viable cell counts were significantly reduced to 99.44% and 98.76%, respectively, after the media were incorporated with LAB1-CL for 24 h. For Gram-negative bacteria, the viable cell counts declined between 78.95% and 81.78% after 12 h of exposure to LAB1-CL. However, the bacterial cells of *P. aeruginosa* and *E. coli* were dramatically reduced to 91.05% and 98.98%, respectively, when treated with LAB1-CL for 24 h. On the other hand, the viable cell counts of Gram-positive bacteria (*S. aureus* and *S. hominis*) were reduced to 87.28 and 86.05% after being exposed to LAB2 cell lysate (LAB2-CL) for 12 h. However, when the incubation time was extended to 24 h, the bacterial cells were sharply reduced to 99.12% and 99.11%. For *P. aeruginosa* and *E. coli*, the viable cells were reduced to 76.59 % and 82.91%, respectively, after 12 h exposure to the LAB2-CL. The reduction percentage of bacterial cells increased to 90.34 (*P. aeruginosa*) and 98.44% (*E. coli*) after 24 h exposure to the cell lysate.

Table 5 exhibits the reduction of viable cell counts after 24 h exposure to LAB cell lysate. At MIC concentration, the viable cell counts of *S. aureus* and *S. hominis* were reduced to 99.44% and 98.76% after being treated with LAB1-CL. However, the viable cell counts of *P. aeruginosa* and *E. coli* decreased to 91.05% and 98.98%, respectively, after 24 h of exposure to LAB1-CL. For LAB2-CL, the reduction percentage of bacterial cells (*S. aureus* and *S. hominis*) treated with cell lysate at the MIC value was 99.12 and 99.11%, respectively. After being treated with cell lysate, the viable cell counts for *P. aeruginosa* and *E. coli* were reduced to 90.34% and 98.44%, respectively.

At higher extract concentration (2 × MIC), the bacterial cells of *S. aureus* and *S. hominis* exposed to LAB1-CL were reduced to 99.44% and 98.76%, respectively, whereas for Gram-negative bacteria, *P. aeruginosa* and *E. coli,* the viable cell count were reduced to 91.05% and 99.49%, respectively. On the other hand, the bacterial growth of *S. aureus* and *S. hominis* was reduced to 99.12% and 99.11%, respectively, after being exposed to LAB2-CL at 2 × MIC value. Meanwhile, for *P. aeruginosa* and *E. coli*, their cell growth was eliminated to approximately 90.34% and 98.44%, respectively.

Table 4 Growth reduction of pathogenic bacteria treated with LAB extracts after 12 and 24 h of exposure.

Test bacteria	Incubation period (h)					
	12h			24h		
	Control	With extract	Bacterial reduction (R%±SD)	Control	With extract	Bacterial reduction (R%±SD)
LAB1						
Gram-positive bacteria						
S. aureus	2.11×10^8	2.81×10^7	86.86	2.11×10^8	1.18×10^6	99.44
S. hominis	2.02×10^8	3.30×10^7	83.56	2.02×10^8	2.51×10^6	98.76
Gram-negative bacteria						
P. aeruginosa	1.81×10^8	3.80×10^7	78.95	1.81×10^8	1.62×10^7	91.05
E. coli	1.97×10^8	3.60×10^7	81.78	1.97×10^8	2.01×10^6	98.98
LAB2						
Gram-positive bacteria						
S. aureus	2.35×10^8	2.99×10^7	87.28	2.35×10^8	2.06×10^6	99.12
S. hominis	2.23×10^8	3.10×10^7	86.05	2.23×10^8	1.99×10^6	99.11
Gram-negative bacteria						
P. aeruginosa	2.05×10^8	4.80×10^7	76.59	2.05×10^8	1.98×10^7	90.34
E. coli	1.99×10^8	3.40×10^7	82.91	1.99×10^8	3.10×10^6	98.44

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Table 5 Growth reduction of pathogenic bacteria treated with LAB extracts after 24 h of exposure at different extract concentrations.

Test bacteria	Extract concentrations					
	MIC			2 × MIC		
	Control	With extract	Bacterial reduction (R%±SD)	Control	With extract	Bacterial reduction (R%±SD)
LAB1						
Gram-positive bacteria						
S. aureus	2.11×10^8	1.18×10^6	99.44	2.11×10^8	1.18×10^6	99.44
S. hominis	2.02×10^8	2.51×10^6	98.76	2.02×10^8	2.51×10^6	98.76
Gram-negative bacteria						
P. aeruginosa	1.81×10^8	1.62×10^7	91.05	1.81×10^8	1.62×10^7	91.05
E. coli	1.97×10^8	2.01×10^6	98.98	1.97×10^8	1.01×10^6	99.49
LAB2						
Gram-positive bacteria						
S. aureus	2.35×10^8	2.06×10^6	99.12	2.35×10^8	1.56×10^5	99.93
S. hominis	2.23×10^8	1.99×10^6	99.11	2.23×10^8	1.33×10^5	99.94
Gram-negative bacteria						
P. aeruginosa	2.05×10^8	1.98×10^7	90.34	2.05×10^8	1.28×10^6	99.38
E. coli	1.99×10^8	3.10×10^6	98.44	1.99×10^8	1.08×10^6	99.46

CONCLUSION

The present study has successfully demonstrated isolation and characterization, as well as determining the probiotic properties of two LAB isolated, which are *L. rhamnosus* and *L. acidophilus* from the commercialized yoghurt. The antibacterial activity of cell-free supernatant (CFS) and cell lysate (CL) against five skin and food-borne bacteria was observed via disc diffusion assay. The inhibition zone sizes range from 8.5 ± 0.15 mm to 12.0 ± 0.82 mm in diameter. A few recommendations that may be useful in this study are the Time Kill Analysis, which can be carried out to evaluate an antimicrobial test material and assess the reduction of a microbial population of test organisms after exposure to MIC and MBC. The test sample or its dilution is brought into contact with a known population of microorganisms for a specified period at a specified temperature, and the surviving organisms are enumerated. We can also study the mode of action of the antibacterial compounds, which have six major modes of action, including interference with cell wall and nucleic acid synthesis, inhibition of protein synthesis, metabolic pathway, membrane function, and ATP Synthase.

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AUTHOR'S CONTRIBUTION

Nur Husna Auni Mohamad Zawawi conducted the research and wrote and revised the article. Mohd Taufiq Mat Jalil conceptualized the central research idea and provided the theoretical framework. Mohd Taufiq Mat Jalil and Nurul Aili Zakaria designed the research and supervised its progress. Syarifah Ab Rashid, Mohd Fakharul Zaman Raja Yahya, and Mohd Taufiq Mat Jalil anchored the review and revisions and approved the article submission.

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CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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