ISSN 2989-0705 (Online)|Vol 3|Issue 2|July 2025

Encapsulation of *Lactobacillus* sp. isolated from yoghurt and evaluation of their antibacterial activity

Zainab Fathima Abdul Cader[1], Punsisi Weerasooriya[1*] and Daniel George[1]

[1]Faculty of Life and Medical Sciences, Business Management School (BMS), Sri Lanka

[*]punsisi@bms.ac.lk

Abstract

Probiotics are living microorganisms that when ingested in the suitable quantities, provide numerous health benefits to the host organism. This study focused on *Lactobacillus* strains isolated from five different brands of yoghurt found in local markets and the effect of encapsulation on its antibacterial activity was observed. The isolated strains were labelled A to E and gram staining, catalase test, PCR (polymerase chain reaction), agarose gel electrophoresis and sequencing were done to identify *Lactobacillus* species. The bacteria were observed to be rod-shaped, gram positive and catalase negative and upon species specific PCR and sequencing, samples A and B were identified to be *Lactobacillus delbrueckii* subsp. *jakobsenii* and Sample E was *Lactobacillus rhamnosus* but samples C and D were identified to be *Streptococcus thermophilus* and *Streptococcus macedonicus* respectively. The identified *Lactobacillus* were then encapsulated in sodium alginate beads coated with chitosan. The CFS (cell-free supernatant) of encapsulated as well as non-encapsulated bacteria were subjected to a well-diffusion assay to assess the antibacterial activity of *Lactobacillus* against pathogenic *Escherichia coli* and *Staphylococcus aureus*. The results showed no antibacterial activity by both encapsulated and non-encapsulated bacteria against *Escherichia coli and Staphylococcus aureus*. This study helps understand that all *Lactobacillus* spp. may not have antibacterial activity. Therefore, finding mechanisms to improve the antibacterial activity of *Lactobacillus* and the use of those improved methods in dairy products would be more beneficial to human health.

Keywords: Antibacterial activity, Encapsulation, *Lactobacillus* spp., Probiotics, Yoghurt

1. Introduction

Probiotics are living microorganisms that when ingested in the suitable quantities, provide numerous health benefits to the host organism.[1] They help maintain the gut microflora. Bacteria that are commonly used as probiotics belong to the genera *Bifidobacterium, Lactobacillus, Saccharomyces, Enterococcus, Streptococcus and Lactococcus*.[1] Most commonly used probiotics in the food industry are lactic acid bacteria (LAB) which include the genera *Bifidobacterium* and *Lactobacillus*.[2] The type of probiotic bacteria that was focused on this study was *Lactobacillus*. This genus consists of various rod-shaped, gram-positive, catalase negative and non-spore forming bacteria. *Lactobacillus* spp. are classified as generally regarded as safe (GRAS) and are considered safe for human consumption. These bacteria are also naturally found in the GI (gastrointestinal) tract as part of the human microbiota. *Lactobacillus* are commonly found in dairy products and also added as starter cultures in the production of certain food products to improve the flavour and to impart health benefits to the consumer. Probiotic genus, *Lactobacillus* is widespread for its numerous medical functions which include the decrease of enteric infections, cholesterol infections, intestinal tumours, lactose intolerance and to boost immunity.[3,4] *Lactobacillus* has many probiotic properties which include acid tolerance, bile tolerance, antibacterial activity, antibiotic susceptibility, hydrophobicity, co-aggregation and auto-aggregation.[2,5] This study focus antibacterial activity as the main probiotic characteristic.

Yoghurt is one of the main sources of probiotics. Many studies have observed

Lactobacillus spp. in yoghurt. A study observed *Lactobacillus* strains in commercially available yoghurt samples which exhibited probiotic properties.[6] Another study also observed and isolated bacteriocin producing *Lactobacillus* spp. which exhibited antibacterial activity.[7] Since yoghurt is considered healthy for consumption and due to the presence of *Lactobacillus* spp. in it, yoghurt was chosen as the sample in this study.

Lactobacillus species play a major role in maintaining a healthy gut microflora by inhibiting the growth and activity of pathogenic bacteria. This is achieved by various mechanisms such as production of antibacterial compounds like bacteriocins, organic acids and hydrogen peroxide, competition for nutrients, competition for colonizing sites, competitive exclusion and modulation of host immune system.[8,9] Among the various mechanisms bacteriocin and organic acid production is considered the most common mode of maintaining a healthy gut microflora.[8,10]

Bacteriocins are low molecular weight peptides or proteins that inhibit the growth of pathogenic bacteria, particularly those associated with GI infections. They can function as a colonizing peptide, killing peptide or signalling peptide. For example, Plantaricin A, a bacteriocin secreted by *Lactobacillus plantarum* can function as both a killing peptide as well as a signalling peptide.[10, 11] A study observed Lactobacillus *plantarum* was able to produce bacteriocins which inhibited common pathogenic bacteria.[12] Moreover, another study observed that the bacteriocin produced by *Lactobacillus acidophilus* had inhibitory activity against some pathogens.[13]

Organic acids are end products of the fermentative metabolism of *Lactobacillus*. They have an antagonistic effect on pathogenic bacteria by causing intracellular acidification of cells and permeabilizing the membrane by disrupting the lipopolysaccharide layer of the outer membrane.[14] The main metabolic product released by *Lactobacillus* strains as observed by Serrano-Nino *et al.,*[15] was lactic acid which causes the decrease in pH in the environment creating unfavourable conditions for the growth of pathogenic bacteria. As lactic acid has the potential to permeabilize the membrane, it could lead to the entry of hydrogen peroxide into the bacterial cells which produces reactive and cytotoxic oxygen species which can damage the nucleic acids, proteins and lipids.[16,17]

Encapsulation is a technique to immobilize the bacterial cells within a protective matrix. It increases cell density, improve resistance to contamination, protect the probiotics from external disturbances during storage and processing, enhance the production and secretion of secondary metabolites and to protect the chemical and physical stability of the cell. Encapsulation is an efficient method to improve the viability of probiotics in food and GI tract. The most common material used for microencapsulation is alginate.[18, 19] Alginate is considered as a GRAS material that can be safely incorporated into food. Microencapsulation with alginate alone has low effectiveness as alginate has low stability in acidic conditions and in the presence of chelating agents.[20] Therefore, coating alginate microbeads with a polycation such as chitosan can decrease the porosity of the bead and enhance its stability thereby increasing the viability of the microencapsulated bacterial cells.[20] Moreover, through encapsulation probiotics can be released in a controlled manner at the required target site. A study observed an increased viability in microencapsulated *Lactobacillus rhamnosus* cells compared to free cells. They also showed greater tolerance to bile salts and acid, higher survival rates in stimulated gastric juice conditions and higher inhibition zones were observed in the antibacterial assay by the microencapsulated cells.[21] Another study also observed microencapsulated *Lactobacillus casei* cells to show an increased resistance to GI conditions. The significance of this study is to investigate the effect of encapsulation in the

antibacterial activity of *Lactobacillus* spp. The findings will be useful for manufacturers as they can aid in enhancing the quality of probiotic products. This study can help manufacturers to understand how encapsulation improves the viability and enhances the antibacterial activity of probiotic bacteria.

2. Methodology

2.1 Culturing of samples. The samples collected were cultured on De Man, Rogosa and Sharpe (MRS) agar under aseptic conditions and were subjected to gram staining and catalase tests.[6]

2.2. Biochemical test and staining. Lactobacillus species were identified by observing the colony morphology (size, shape and elevation) and through tests such as gram staining and catalase tests.

2.2.1 Gram staining. A bacterial smear was prepared and was stained with Gram-staining. The bacteria were then observed under the microscope at 100x magnification.[6]

2.2.2 Catalase test. A bacterial smear was prepared and a few drops of 3% H_2O_2 were added to the smears. The smears were observed for the bubble formation to confirm the presence of catalase enzymes.[6]

2.3 DNA extraction (QIAGEN DNeasy Kit method). The DNA was extracted by following the manufacturer's instructions provided. The extracted DNA was then stored in -20^{o}C.

2.4 Identification of Lactobacillus by PCR. The extracted DNA was amplified using universal primers 27F and 1492R (IDT) (Table 1). Master mix was prepared for five samples, positive control (known *Lactobacillus* DNA), negative control (autoclaved distilled water) and an extra reaction and 12.25µL of it was aliquoted into each PCR tube. Autoclaved distilled water (11.75µL) and DNA were then added into the PCR tubes. The final volume of PCR mix was 25µL. The PCR thermal cyclic conditions followed are mentioned in Table 2.

2.5 Visualization of PCR products. In 2% agarose gel, 6µL of the PCR products, positive and negative control and 2µL of 100bp DNA ladder was loaded into the wells. The gel was first run for 20 mins at a voltage of 45V and then run for 30 mins at 50V. The DNA bands were then visualized under UV.

2.6 Sequencing. Samples confirmed from PCR were sent to Macrogen, Korea to be sequenced (16SrRNA sequencing) to identify the bacterial species. The results were interpreted using BioEdit and NCBI BLAST. The bacteria identified as *Lactobacillus* were carried forward to the assay.

2.7 Encapsulation

2.7.1 Sodium alginate and chitosan preparation. Sodium alginate with a concentration of 3% (w/v) was prepared and sterilized. Upon cooling, 5 mL of *Lactobacillus* bacteria suspension was added into it slowly and mixed well. Chitosan with a concentration of 0.2% (w/v) was also prepared and sterilized.

*2.7.2 Gel beads formation***.** The prepared 3% sodium alginate was transferred to a 50mL syringe. The sodium alginate solution was released from the syringe drop-wise slowly into 100mL of $CaCl_2$ solution (32g/L) while gently swirling the beaker. The beads formed were filtered, washed with distilled water and immersed in the 0.2% chitosan solution for 15 mins. The beads were filtered again, washed with distilled water and was stored in MRS broth in the refrigerator This method was modified from Djaenudin *et al.,* 2020.[19]

2.8 Antibacterial assay. To compare the antibacterial activity of encapsulated and non-encapsulated bacteria, cell-free supernatants (CFS) of both bacteria were initially prepared.

Table 1. Universal primers used for DNA amplification.

Primer	Sequence (5' to 3')	Expected PCR product	References
Forward primer (27F)	AGAGTTTGATCMTGGCTCAG	1500bp	Dharmasiri *et al.*[29]
Reverse primer (1492R)	GGTTACCTTGTTACGACTT	1500bp	Dharmasiri *et al.*[29]

Table 2. PCR thermal cycling conditions

Steps	Temperature (°C)	Duration (minutes)	Number of cycles
Initial denaturation	94	5	
Denaturation	94	1	35
Annealing	60	1	
Extension	72	2	
Final extension	72	12	
Final hold	4	∞	

2.8.1 Encapsulated bacteria CFS preparation. First, 2.00g of beads were transferred to a 50 mL falcon tube and were dissolved in 15mL of phosphate buffer saline (PBS) buffer by mixing by vortex gently. The solution was centrifuged at 4000 rpm for 5 mins and the supernatant was discarded. Then, 5mL of MRS broth was added to the pellet and the tubes were left to incubate at 37°C for 20 hours. The mixture was then heated at 100°C for 20 mins and immediately frozen at -20°C for 20 mins. Finally, it was centrifuged at 4000 rpm for 5 mins to get the CFS.[18]

2.8.2 Non-encapsulated bacteria CFS preparation. Initially, 5mL of subculture was transferred into 50mL falcon tube, centrifuged at 4000 rpm for 5 mins and the supernatant was discarded. Then, 5mL of MRS broth was added to the pellet and incubated at 37°C for 20 hours. The mixture was then heated at 100°C for 20 mins and immediately frozen at -20°C for 20 mins. Finally, it was centrifuged at 4000 rpm for 5 mins to get the CFS.[18]

2.8.3. Pathogenic bacterial suspension preparation. *Escherichia coli* and *Staphylococcus aureus* were inoculated in nutrient broth and left to incubate at 37°C for 20 hours. The broth cultures were then centrifuged at 4000 rpm for 5 mins to obtain the pellet. PBS buffer was added to the pellet and the absorbance of the suspension was adjusted to 0.2 at a wavelength of 600nm.[23]

2.8.4 Antibacterial Assay. Antibacterial activity was assessed through well-diffusion method.[24] The CFS of the samples were heated at 100°C for 20 mins. The Mueller-Hinton agar was prepared, poured into the petri plates and was left to solidify under UV for 15 mins. Pathogenic bacterial suspensions (100µL) were spread onto the plate and the wells were prepared. The heat-treated CFS (100µL) were added into the appropriate wells along with a negative (autoclaved distilled water) and positive (gentamycin) control. The plates were incubated at 37°C for 48 hours (modified from Trabelsi *et al.*[24], 2014). The diameter of the inhibition zones formed were measured.

3. Results

3.1 Culturing of samples

Yoghurt samples were cultured on MRS agar exhibited milky white colonies with circular form, entire margin and convex elevation (Figure 1).

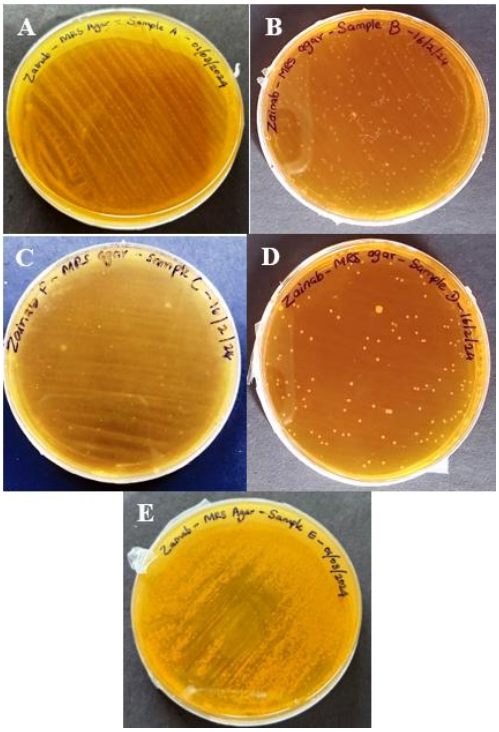

Figure 1. Yoghurt samples cultured on MRS agar (A to E – bacterial colonies from the yoghurt samples)

3.2 Gram staining

Microscopic observation of Gram-stained samples (Figure 2) revealed purple colour, rod-shaped bacteria in all the samples.

Table 3. Gram stain results

Bacterial isolates	Gram stain	Shape
A	+	rod-shaped
B	+	rod-shaped
C	+	rod-shaped
D	+	rod-shaped
E	+	rod-shaped

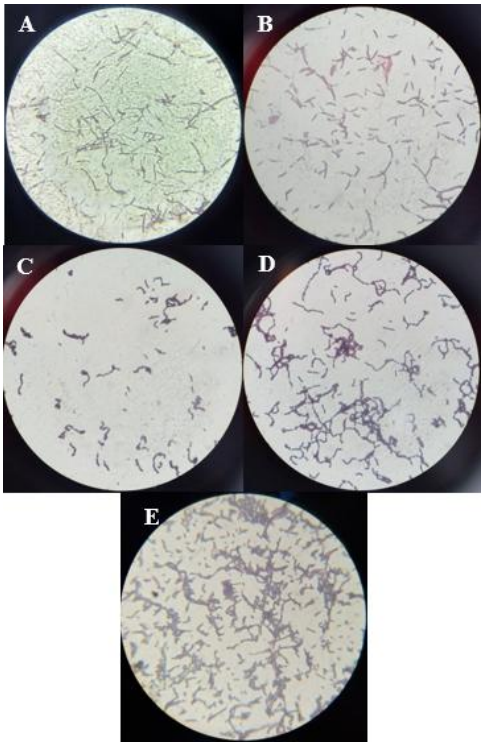

Figure 2. Microscopic images of bacterial isolates after gram staining (100x). (A to E – bacteria cultured on MRS agar)

3.3 Catalase test

Catalase test results revealed no bubble formation in any of the samples even under microscope.

3.4 Visualization of PCR products

PCR products visualized by agarose gel electrophoresis (Figure 3) showed bands of approximately 1500 bp in all samples and the positive control, but no band was observed in the negative control.

Figure 3. PCR products visualized on gel image. Lane 1: 100bp DNA ladder, Lanes 2-6: Samples A to E respectively, Lane 7: positive control, Lane 8: negative control.

3.5 Sequencing

Results of bacterial species identification through sequencing (Macrogen, Korea) is mentioned in Table 4. Sample A and B were identified to be *Lactobacillus delbrueckii* subsp. *jakobsenii* and Sample E was *Lactobacillus rhamnosus* but samples C and D were identified to be *Streptococcus thermophilus* and *Streptococcus macedonicus* respectively. Samples C and D were not carried forward to the assay.

Table 4. Sequencing results

Sample	Bacterial species	Accession number
A	*Lactobacillus delbrueckii* subsp. *jakobsenii* ZN7a-9 = DSM 26046	NZ_CP018218.1
B	*Lactobacillus delbrueckii* subsp. j*akobseni*i ZN7a-9	CP018218.1
C	*Streptococcus thermophilus* isolate STH_CIRM_65	NZ_LR822015.1
D	*Streptococcus macedonicus* strain NWAFU7001	MG550989.1
E	*Lactobacillus rhamnosus* strain 330	KJ939337.1

3.6 Encapsulation

Lactobacillus isolates encapsulated in sodium alginate coated with chitosan is shown below. Encapsulated beads of the isolates were observed to be small and spherical in shape (Figure 4).

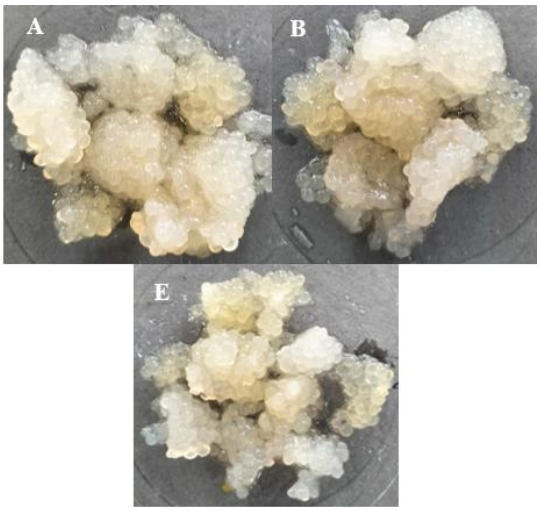

Figure 4. Bacterial isolates encapsulated in sodium alginate coated with chitosan.

3.7 Antibacterial Assay

Antibacterial activity of CFS of bacterial sample against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* in well-diffusion assay after incubation for 48 hours is depicted in Table 5. Encapsulated and non-encapsulated bacterial samples showed no antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Only the positive control showed a ZOI.

4. Discussion

The bacteria *Lactobacillus* has various probiotic properties and play a major role in maintaining the gut microflora. Therefore, *Lactobacillus* is used in food industry to improve human health. This study isolated *Lactobacillus* from yoghurt and the antibacterial activity of encapsulated and non-encapsulated bacteria were compared.

The yoghurt samples were cultured on MRS agar as they are selective for *Lactobacillus*. MRS agar contains sodium acetate and ammonium citrate that prevent the growth of other microorganisms like *Escherichia coli* but allow the growth of *Lactobacillus*.[25] Amphotericin B was also added to MRS agar during MRS agar preparation. This prevents the growth of fungi and yeast thereby preventing contamination.[26]

Table 5. Inhibition zone sizes against *Escherichia coli* and *Staphylococcus aureus*.

Pathogenic Bacteria	Sample	Encapsulated bacteria	Non-encapsulated bacteria	Positive control (mm)	Negative control
Escherichia coli	A	-	-	20.00	-
	B	-	-	20.00	-
	E	-	-	20.00	-
Staphylococcus aureus	A	-	-	18.00	-
	B	-	-	18.00	-
	E	-	-	18.00	-

Bacteria were initially identified by observing colony morphology. Similar colony morphology was observed by Taye *et al*. [27] and Kaboosi [28] for *Lactobacillus* spp. Gram staining results as seen in figure 2 observed the bacteria to be rod-shaped gram-positive bacteria. The following results is supported by Prabhurajeshwar and Chandrakanth [7] who observed *Lactobacillus* as gram-positive rod-shaped bacteria. Catalase test results showed no bubbles formation even when observed under a microscope. This indicates that all the samples are catalase negative. Hoque *et al*. [29] and Abid *et al*., [30] also observed similar results for *Lactobacillus* spp. These results strongly correlated to the results observed in this study thereby suggesting the bacteria isolated could be a *Lactobacillus* sp.

Upon species specific PCR and sequencing, samples A and B were identified to be *Lactobacillus delbrueckii* subsp. *jakobsenii* and sample E was identified as *Lactobacillus rhamnosus*. The remaining samples C and D were identified to be *Streptococcus thermophilus* and *Streptococcus macedonicus* respectively which are also types of bacteria found in probiotic food (table 4). Since this study is focused on *Lactobacillus*, samples C and D were not carried forward to the assay.

Lactobacillus are widely studied and observed to exhibit many probiotic properties and this study focused on their antibacterial activity. Release of antibacterial compounds such as organic acids, bacteriocins and hydrogen peroxide is an important mechanism as mentioned previously.[8, 9] Some organic acids produced by *Lactobacillus* spp. are lactic acid, acetic acid, citric acid and butyric acid.[33] The most common organic acid involved is lactic acid, which is a main metabolic compound released by *Lactobacillus*.[34] The isolated *Lactobacillus* bacterial samples were encapsulated to compare the effect of encapsulation on the antibacterial effect of *Lactobacillus* with non-encapsulated bacteria. Encapsulation of bacteria has been observed to show improved viability and activity compared to non-encapsulated bacteria in various studies. Oberoi *et al.* [21] and Iznaga *et al*.,[22] observed better activity in microencapsulated cells compared to free cells. Larger zones of inhibition (ZOI) of encapsulated cells against pathogenic bacteria were observed by Oberoi *et al*. [21] and Phoem *et al*.[35] compared to free non-encapsulated cells.

In this study, none of the isolates *Lactobacillus delbrueckii* subsp. *jakobsenii* and *Lactobacillus rhamnosus* showed antibacterial activity. Both encapsulated and non-encapsulated bacteria were observed to show no ZOI against *Escherichia coli* and *Staphylococcus aureus*. These results are supported by Sharma *et al*.,[36] in which the CFS of *Lactobacillus* strains isolated from curd and breast milk showed no antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. The *Lactobacillus* strains isolated

included *Lactobacillus delbrueckii* which is the same species isolated in this study as well. Sharma *et al*.,[36] also tested the antibacterial activity of the CFS of *Lactobacillus rhamnosus* as a reference strain which also showed no ZOI against *Escherichia coli* and *Staphylococcus aureus*. A study conducted by Hawas[32] observed that the CFS of some *Lactobacillus* species isolated from curd showed no ZOI. *Lactobacillus delbrueckii* at a low concentration showed no ZOI. CFS of *Lactobacillus rhamnosus* were also observed to show no ZOI against *Escherichia coli* and *Staphylococcus aureus* even when the CFS concentrations were increased. Another study by Jose, Bunt and Hussain[37], observed no inhibition of *Escherichia coli* by all *Lactobacillus* isolates which included *Lactobacillus rhamnosus*. They also observed that the *Lactobacillus* isolated from rumen of cows had better growth inhibition of pathogenic bacteria compared to the *Lactobacillus* isolated from dairy products. They thought it was probably because the *Lactobacillus* found in the gut may have to actively fight against these pathogens when encountered. Serrano-Niño *et al*.,[15] observed *Lactobacillus delbrueckii* to have antibacterial activity against *Escherichia coli* but not *Staphylococcus aureus* but Lopes *et al*.,[38] reported that *Lactobacillus delbrueckii* showed no ZOI against *Escherichia coli*.

In this study, the CFS was heat treated at 100ºC to check the antibacterial activity of antibacterial compounds on treating it with heat. The results showed no ZOI as mentioned before but, various studies have observed antibacterial compounds in CFS to be heat insensitive. Pompilio *et al*.,[39] heated the CFS at 70ºC and 100ºC to check if heat affects it and it was observed that there was no significant difference in the antibacterial potency between the heated and unheated CFS samples. Trabelsi *et al.,*[24] also heated the CFS of the samples up to 121ºC but observed no change on the antibacterial effect and clear ZOI were observed. Therefore, the reason for not observing an antibacterial activity in this study may not be due to the heat treatment of CFS of the samples.

One of the reasons for not observing ZOI could be due to the resistance of *Escherichia coli* and *Staphylococcus aureus* against the antibacterial compounds. Yang *et al*.[40], observed tolerance mechanisms of evolved acid-tolerant *Escherichia coli* against organic acids. The evolved strains of *Escherichia coli* showed an ability to maintain the intracellular ATP at high concentrations in increased acidic conditions, showed better cell membrane integrity compared to parental strains and showed increased accumulation of peptidoglycan in their cell wall by upregulating the respective genes resulting in a denser peptidoglycan layer.[40] This could block the entry of acid into the cells thus maintaining a better intracellular pH (pHi). *Escherichia coli* also can use acid resistance mechanisms that utilize protons and reduce proton influx by membrane modifications. Similar mechanisms are also present in *Staphylococcus aureus.*[41] In anaerobic conditions, protons in cytoplasm are converted to hydrogen gas by formate hydrogen lyase complex thereby preventing intracellular acidification.[42]

In *Staphylococcus aureus*, acetic acid dissociates in cytosol and the acetate ions interact with D-alanyl-D-alanine ligase (Ddl) and inhibit their activity. Panda *et al*.[43] observed that *Staphylococcus aureus* could resist acetate toxicity by alanine racemase which can increase the D-Ala-D-Ala pools. Some other studies have identified changes in gene expression in *Staphylococcus aureus* on exposure to acidic stress which help alleviate the intracellular acidification thereby ensuring survival.[44,45] A common response of *Staphylococcus aureus* to acid stress is increased urease activity, NADH mediated proton excretion and macromolecule repair mechanisms.[46]

Another reason could be because the potency of antibacterial activity is strain dependent. Qian *et al.,*[47] used *Lactobacillus*

delbrueckii as a reference strain and observed it to have a weaker activity compared to the other *Lactobacillus* spp. isolated from yoghurt. Jose, Bunt and Hussain[37] also observed some *Lactobacillus* spp. having antibacterial activity while some did not show any inhibition. Same was observed by Hawas[32] in which some *Lactobacillus* species exhibited ZOI against pathogenic bacteria while others did not. This could be due to the differences in gene expression. Some *Lactobacillus* spp. may contain a gene which has better antibacterial activity compared to others. Qian *et al.,*[47] also identified certain genes responsible for bacteriocin production. The *Lactobacillus* spp. that showed inhibitory activity had expressed these genes. Maybe the *Lactobacillus* isolated in this study lacks the genes involved in bacteriocin production and may also have reduced gene expression due to a mutation leading to poor antibacterial activity. An interesting observation reported by Osset *et al*.,[48] was that the inhibitory activity of the *Lactobacillus* spp. in solid and liquid medium were greatly different. *Lactobacillus* showed much better inhibition in liquid medium compared to solid medium. The species which showed good inhibitory activity in liquid medium failed to inhibit pathogen growth in solid medium. A similar observation was observed by Jamalifar *et al.,*[49]. The reason for this was thought to be due to better diffusion of antibacterial compounds in liquid medium. In this study, the antibacterial activity was assessed in solid medium thereby poor diffusion of antibacterial compounds in agar could be a potential reason for not observing any ZOI.

Above research evidences provide the possible reasons for no antibacterial activity. Identifying *Lactobacillus* species with good antibacterial activity and incorporating them in food products would be much more beneficial. Since antibacterial activity is strain dependent, use of different species together would give out a better result. The antibacterial activity of *Lactobacillus* could also be enhanced by incorporating genes responsible for bacteriocin and organic acid production. Ma *et al*.,[50] observed enhanced antibacterial activity of *Lactobacillus reuteri* after genetically modifying it through random mutagenesis. Bartkiene *et al*.,[51] utilized the byproducts of berries and fruits to improve the antimicrobial activity of some lactic acid bacteria. Similar mechanisms can be followed to enhance the antibacterial activity of *Lactobacillus* and incorporate them in the manufacture of probiotic food products thereby providing better health benefits to the consumer.

5. Conclusion

The *Lactobacillus* spp isolated from yoghurt were identified to be *Lactobacillus delbreuckii* and *Lactobacillus rhamnosus*. Both encapsulated and non-encapsulated *Lactobacillus delbreuckii* and *Lactobacillus rhamnosus* showed no antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*. Further research with a larger sample size is required to confirm the results. The antibacterial activity of *Lactobacillus* can be further improved by genetic modification and combining different strains together to confer better probiotic properties to humans.

Acknowledgement

I would like to extend my gratitude to BMS for providing the opportunity to conduct this research project.

References

1 X. Zhang, G. A. Esmail, A. F. Alzeer, M. V. Arasu, P. Vijayaraghavan, K. C. Choi and N. A. Al-Dhabi. *Saudi Journal of Biological Sciences*, 2020;**27**;35.5-3513.

2 Y. Bao, Y. Zhang, Y. Zhang, Y. Liu, S. Wang, X. Dong, Y. Wang, and H. Zhang. *Food Control,* 2010;**21**;6695-6701.

3 E. Motevaseli, A. Dianatapour, and S. G. Fard. *Int J Mol Cell Med.,* 2017;**6**(2);66-76.

4 S. Delgado, B. Sánchez, A. Margolles, P. R.Madiedo and L. Ruiz. *Nutrients*, 2020;**12**;391.

5 M. H. Hassan, H. Nayab, F. Shafique, M. P. Williamson, T. S. Almansouri, N. Asim, N. Shafi, S.

Attacha, M. Khalid, N. Ali and N. Akbar. *BioMed Research International*, 2020;**2020**;8889198;1-17.

6 C. Prabhurajeshwar, and K. Chandrakanth. *Clinical Nutrition Experimental*, 2019;**23**;97-115.

7 A. Chowdhury, R. Malaker, N. Hossain, Fakruddin, R. Noor, and M. M. Ahmed. *International Journal of Pharmaceutical Chemistry*, 2013;**3**;50-56.

8 W. F. Zyl, S. M. Deane and L. M. T. Dicks. *Gut Microbes*, 2020;**12**(1);e1831339.

9 X. Yang, Z. Peng, M. He, Z. Li, G. Fu, S. Li, and J. Zhang. *Science of the Total Environment*, 2024;**906**;167587;1-12.

10 A. Dobson, P. D. Cotter, R. P. Ross and C. Hill. *Applied and Environmental Microbiology*, 2024;**78**(1);1-6.

11 S. S. M. Al-Rawi, O. D. Salahdin, F. T. Al-Alaq, L. Abdulazeem, M. H. Kzar, E. S. Khattab and A. S. Naje. *Journal of Medicinal and Chemical Sciences*, 2023;**6**;702-709.

12 Y. Bu, Y. Liu, S. Wang, Q. Liu, H. Hao and H. Yi. *Foods*, 2022;**11**(11);1575.

13 C. Gaspar, G. G. Donders, R. Palmeira-de-Oliveira, J. A. Queiroz, C. Tomaz, J. Martinez-de-Oliveira and A. Palmeira-de-Oliveira. *AMB Express*, 2018;**8**;153.

14 H. L. Alakomi, E. Skytta, T. Mattila-Sandholm, K. Latva-Kala and I. M. Helander. *Applied Environmental Microbiology,* 2000; **66**(5);2001-2005.

15 J. C. Serrano-Niño, J. R. Solís-Pacheco, J. A. Gutierrez-Padilla, A. Cobián-García, A. Cavazos-Garduño, O. González-Reynoso and B. R. Aguilar-Uscanga. *Journal of Food and Nutrition Research*, 2016;**4**(3);170-177.

16 R. Georgieva, L. Yochevab, L. Tserovskab, G. Zhelezovab, N. Stefanova, A. Atanasova, A. Danguleva, G. Ivanova, N. Karapetkova, N. Rumyana and E. Karaivanova. *Biotechnology & Biotechnological Equipment*, 2015;**29**(1);84-91.

17 V. L. L. Moal, and A. L. Servin. *Clinical Microbiology Reviews*, 2024;**27**(2);167-199.

18 I. Trabelsi, D. Ayadi, W. Bejar, S. Bejar, H. Chouayekh and R. B. Salah. *International Journal of Biological Macromolecules*, 2014;**64**;84-89.

19 Z. Zhang, J. Liu, M. Li, B. Yang, W. Liu, Z. Chu, B. Cui and X. Chen. *Front. Nutr.*, 2022;**9**;872808.

20 Djaenudin, E. Budianto, E. Saepudin and M. Nasir. *Earth and Environmental Science*, 2020;**483**;012043.

21 K. Oberoi, A. Tolun, Z. Altintas and S. Sharma. *Foods*, 2021;**10**;1999.

22 T. B. Iznaga, R. V. Carvajal, E. S. Armesto and J. L. Vega. *Food Research International*, 2021;**141**;110136.

23 J. A. Myers, B. S. Curtis and W. R. Curtis. *BMC Biophys*, 2013;**6**(4)

24 I. Trabelsi, D. Ayadi, W. Bejar, S. Bejar, H. Chouayekh and R. B. Salah. *International Journal of Biological Macromolecules*, 2014;**64**;84-89.

25 K. Mizuno, M. Mizuno, M. Yamauchi, A. J. Takemura, V. M. Romero and K. Morikawa. *Scientific Reports*, 2017;**7**;12880;1-9.

26 F. B. Cavassin, J. L. Bau´-Carneiro, R. R. Vilas-Boas and F. Queiroz-Telles. *Infectious Diseases and Therapy*, 2021;**10**;115-147.

27 Y. Taye, T. Degu, H. Fesseha and M. Mathewos. *The Scientific World Journal,* 2021;4697445;1-6.

28 H. Kaboosi. *African Journal of Microbiology Research*, 2011;**5**(25);4363-4367.

29 M. Z. Hoque, F. Akter, K. M. Hossain, M. S. M. Rahman, M. M. Billah and K. M. D. Islam. *World Journal of Dairy & Food Sciences*,2010;**5**(1);39-46.

30 S. Abid, A. Farid, R. Abid, M. U. Rehman, W. F. Alsanie, M. Alhomrani, A.S. Alamri, S. M. B. Asdaq, D. I. Hefft, S. Saqib, M. Muzammal, S. A. Morshedy, M. W. Alruways and S. Ghazanfar. *Microorganisms*, 2022;**10**(5);954.

31 R. B. N. Dharmasiri, L. J. S. Undugoda, A. H. L. Nilmini, N. N. R. N. Nugara, P. M. Manage and D. Udayanga. *International Journal of Environmental Science and Technology*, 2023;**20**;13359–13372.

32 E. Hawas. *African Journal of Microbiology Research*, 2014;**8**(13);1419-1425.

33 D. Szczerbiec, J. Piechocka, R. Głowacki and A. Torzewska. *Molecules*, 2022; **27**(17);5557.

34 Y. Zhang, L. Zhang, M. Dua, H. Yi, C. Guo, Y. Tuo, X. Han, J. Li, L. Zhang and L. Yang. *Microbiological Research*, 2011;**167**;27-31.

35 A. N. Phoem, A. Mayiding, F. Saedeh and P. Permpoonpattana. *Brazilian Journal of Microbiology*, 2019;**50**(1);237-246.

36 C. Sharma, B. P. Singh, N. Thakur, S. Gulati, S. Gupta, S. K. Mishra, and H. Panwar. *3 Biotech*, 2017;**7**(1);31.

37 N. M. Jose, C. R. Bunt and M. A. Hussain. *Microorganisms*, 2015; **3**(2);198-212.

38 E. G. Lopes, D. A. Moreira, P. Gullon, B. Gullon, A. Cardelle-Cobas and F. K. Tavaria. *Journal of Applied Microbiology*, 2017;**122**(2);450-461.

39 A. Pompilio, E. Kaya, V. Lupetti, E. Catelli, M. Bianchi, G. Maisetta, S. Esin, G. D. Bonaventura and G. Batoni. *Microbes and Infection*, 2024;**26**(4);105301.

40 J. Yang, J. Zhang, Z. Zhu, X. Jiang, T. Zheng and G. Du. *Applied Microbiology and Biotechnology*, 2022;**106**;7577-7594.

41 P. D. Cotter, and C. Hill. *Microbiology and Molecular Biology Reviews*, 2003;**67**(3);429-453.

42 U. Kanjee and W. A. Houry. *Annual Review of Microbiology,* 2013;**67**;65-81.

43 S. Panda, Y. P. Jayasinghe, D. D. Shinde, E. Bueno, A. Stastny, B. P. Bertrand, S. S. Chaudhari, T. Kielian, F. Cava, D. R. Ronning and V. C. Thomas. *bioRxiv*, 2024.

44 B. Weinrick, P. M. Dunman, F. McAleese, E. Murphy, S. J. Projan, Y. Fang and R. P. Novick. *Journal of Bacteriology*, 2004; **186**(24);8407-8423.

45 C. Zhou and P. D. Fey. *Current Opinion in Microbiology*, 2020; **55**;67-73.

46 E. Bore, S. Langsrud, O. Langsrud, T. M. Rode and A. Holck. *Micrology*, 2007; **153**(7);2289-2303.

47 Z. Qian, D. Zhao, Y. Yin, H. Zhu and D. Chen. *BioMed Research International*, 2020; **2020**(1);3548618.

48 J. Osset, R. M. Bartolome, E. Garcia and A. Andreu. *The Journal of Infectious Diseases*, 2001;**183**(3);485-491.

49 H. Jamalifer, H. R. Rahimi, N. Samadi, A. R. Shahverdi, Z. Sharifian, F. Hosseini, H. Eslahi and M. R. Fazeli. *Iranian Journal of Microbiology*, 2011;**3**(1);21-25.

50 E. Ma, Y. An, G. Zhang, M. Zhao, M. W. Iqbal, H. M. Zabed and X. Qi. *Food Bioscience*, 2023;**51**;102209.

51 E. Bartkiene, V. Lele, V. Sakiene, P. Zavistanaviciute, M. Ruzauskas, J. Bernatoniene, V. Jakstas, P. Viskelis, D. Zadeike and G. Juodeikiene. *J Sci Food Agric*, 2019;**99**;3992-4002.