Biological Forum – An International Journal **17(1): 14-26(2025)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Cardioprotective Potential of Lactic Acid Bacteria: An *in vitro* screening Study

Heena Parmar, Urmila Parmar, Sreeja V.*, Saroj Chaudhary and Kiran S.
Department of Dairy Microbiology,
SMC College of Dairy Science, Kamdhenu University, Anand (Gujarat), India.

(Corresponding author: Sreeja V.)*
(Received: 04 October 2024; Revised: 19 November 2024; Accepted: 05 December 2024; Published online: 04 January 2025)
(Published by Research Trend)

ABSTRACT: Hypertension, high cholesterol, abnormal lipid levels, obesity, and diabetes are modifiable risk factors for cardiovascular diseases (CVDs). Probiotics from lactic acid bacteria (LAB) may play a role in reducing these risks. Seven LAB strains, including *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* strains BM15 and BM24; *Lactiplantibacillus plantarum* strains F7 and F11, were screened for bile deconjugation, antioxidant activity, lipase inhibition, cholesterol assimilation, ACE inhibition, proteolytic activity, and antidiabetic potential. Strain V3 demonstrated superior bile deconjugation, while BM24 exhibited the highest antioxidant and lipase inhibition activities. F7 showed the strongest ACE inhibition, while I4 excelled in α-glucosidase inhibition. MD2 and BM24 had the highest α-amylase inhibition. Viability studies revealed strong survival under acidic (pH 2.0) and bile (0.5%) conditions, with log CFU/mL values of 4.11–5.41. Autoaggregation ranged from 31–66%, and coaggregation from 10–36% with various pathogens, highlighting strain-specific probiotic potential. Strains V3, MD2, I4, and BM24 were particularly effective across key parameters, including bile deconjugation (309–350 µg/mL), antioxidant activity (54–73%), and antidiabetic effects (α-amylase inhibition 80–94%, α-glucosidase inhibition 26–58%). Their antimicrobial effects further underscore their cardioprotective potential. This study emphasizes the strain-specific mechanisms of LAB in mitigating CVD risks, offering insights into developing multi-strain probiotics for cardioprotection and advancing probiotic research.

Keywords: Probiotics, Bile deconjugation, Antioxidant activity, ACE and Lipase inhibition, Cholesterol assimilation, α-amylase and α-glucosidase inhibition.

INTRODUCTION

Coronary heart diseases, are among the most prevalent cardiovascular diseases (CVDs), encompassing various pathological conditions that impact the heart and blood vessels. In 2023, the World Health Organization (WHO) reported that CVDs were responsible for around 17.9 million deaths globally, accounting for approximately 32% of all deaths worldwide (Tsao *et al.*, 2023). Modifiable risk factors for MI include hypercholesterolemia, hypertension, obesity, diabetes, smoking, environmental factors, and modern lifestyle changes. These factors are closely linked to alterations in gut microbiota and its metabolites (Cai & Hui 2022; Ghorbani *et al*., 2023). Previous research has shown a strong association between CVD pathogenesis and imbalances in intestinal microbiota and inflammatory responses. Probiotics, which positively influence the microbial and metabolic composition of gut microbiota, are considered a potential therapeutic strategy for CVD (Wu & Chiou 2021). The range of cardiovascular-related diseases that can be alleviated through probiotic supplementation has broadened to include conditions such as hypercholesterolemia, atherosclerosis, myocardial infarction, heart failure, type 2 diabetes mellitus, and obesity (Zhao *et al.*, 2021). Probiotics have been shown to protect against CVD by lowering cholesterol levels, reducing oxidative stress, balancing functional and structural changes in gut microbiota, and enhancing immune responses (Oniszczuk *et al*., 2021).

Oxidative stress plays a crucial role in the progression of CVDs. An increase in reactive oxygen species (ROS) production has been observed in various cardiac conditions (Satheesh Kumar *et al*., 2023; Dubois-Deruy *et al.*, 2020). Reducing oxidative stress is one of the protective effects attributed to probiotics. Probiotics exert antioxidant effects by scavenging ROS, enhancing superoxide dismutase activity, and reducing or preventing lipid peroxidation and ascorbic acid autooxidation (Amaretti *et al.*, 2013; Sadeghzadeh *et al.*, 2017). Specific strains of *Lactobacillus* and *Bifidobacterium* spp. exhibit antioxidant properties, helping to mitigate oxidative damage (Avila-Escalante *et al.*, 2020).

Bile salt deconjugation is a key factor in selecting probiotics due to its impact on cholesterol metabolism (Staley *et al.*, 2017; Foley *et al.*, 2019). Further, probiotics can reduce blood cholesterol by absorbing it, binding it to their cell membranes, or metabolizing it,

thereby reducing its absorption in the gut and potentially lowering coronary artery disease risk (Tomaro-Duchesneau *et al.,* 2014). The role of intestinal flora in atherosclerosis has gained attention as a potential target for preventing and treating CVDs. Pancreatic lipase, crucial for digesting 50 to 70% of dietary lipids, breaks down triglycerides for absorption. Finding effective pancreatic lipase inhibitors, particularly natural ones with fewer side effects, is increasingly important. Various lactic acid bacteria (LAB) strains, especially lactobacilli and bifidobacteria, have been shown to inhibit pancreatic lipase in several studies (Park *et al.*, 2014).

Hypertension is a major risk factor for cardiovascular events, driven by excess angiotensin II, a vasoconstrictor produced by angiotensin-converting enzyme (ACE) (Aluko, 2019). Inhibiting ACE is a strategy for preventing and treating conditions like diabetes, heart failure, and other CVDs. Probiotics have shown significant antihypertensive effects, reducing CVD risk by improving lipid levels, bile acid deconjugation, body mass index, nutrient absorption, and lowering plasma glucose levels, all contributing to better blood pressure regulation (Upadrasta & Sudha 2016). Many LAB strains have demonstrated potential hypoglycemic effects *in vitro*, including the inhibition of α-amylase, α-glucosidase (Wang & Li 2022).

This investigation explores the cardioprotective properties of LAB strains. Strains were screened based on bile deconjugation, antioxidant, lipase inhibition, cholesterol assimilation, ACE-inhibition, proteolytic activities, and antidiabetic potential to identify promising candidates for cardio protection. The strains with strong biofunctional activities were further evaluated for their probiotic potential.

MATERIAL AND METHODS

Lactic strains. The strains listed in Table 1 were obtained from the Culture Collection of Dairy Microbiology Department at SMC College of Dairy Science, Kamdhenu University, Anand, Gujarat, India. These strains were verified for purity and propagated in sterilized reconstituted skim milk (11% total solids) at 37ºC/24h before being stored at 7±1ºC. To maintain their activity throughout the study, the strains were regularly transferred to either sterile selective media or sterilized reconstituted skim milk on a weekly basis, depending on the study's requirements.

Table 1: Details of Lactic Acid Bacteria (LAB) strains used in this study.

Sr. No.	Strains with Lab code	Source of Isolation	NCBI accession
1.	*Lactobacillus helveticus* MTCC 5463 (V3)	Human vagina	GQ253960
2.	*Streptococcus thermophilus* MTCC 5460 (MD2)	Curd	GQ253961
3.	*Lacticaseibacillus rhamnosus* MTCC 5462 (I4)	Human feces	GQ253960
4.	*Limosilactobacillus fermentum* (BM15)	Breast milk	MW077436
5.	*Limosilactobacillus fermentum* (BM24)	Breast Milk	MW077438
6.	*Lactiplantibacillus plantarum* (F7)	Human feces	MG696187
7.	*Lactiplantibacillus plantarum* (F11)	Human feces	SAMN12060783

Assessment of Bile Deconjugation Ability. Bile deconjugation ability of the LAB strains was tested using the method of Ashar and Prajapati (1998). Selective broth media with 0.2% sodium thioglycolate and 0.3% conjugated bile salt were prepared for growing *Lactobacillus* and *Streptococcus* strains. The cell-free supernatant was adjusted to pH 1, diluted, and mixed with ethyl acetate (HiMedia, Mumbai, India) to extract bile salts. The ethyl acetate layer was evaporated, and the residue was dissolved in NaOH, treated with H_2SO_4 and furfuraldehyde, then heated. After cooling, glacial acetic acid was added to stop color development, and absorbance was measured at 660 nm using PC based double beam spectrophotometer, 2202 (Systronics, Ahmedabad, India). The free cholic acid content was determined using a standard curve, and results were expressed in µg/mL.

Assessment of Antioxidant Activity. Antioxidant activity was measured using the ABTS [(2, 2'-Azino-bis (3-ethylbenzothiazoline 6-Sulphonic acid (Sigma-Aldrich, Bangalore, India)] method of Re *et al.* (1999) with few modifications. The total radical scavenging capacity was assessed by the ability of a compound to scavenge the ABTS radical within 10 minutes. Selected cultures were inoculated in reconstituted skim milk at 2% and incubated at 37ºC for 24 hours. A 200 µL aliquot of culture supernatant, obtained after centrifuging at 14,000 rpm for 30 minutes, was mixed with 2300 µL ABTS and brought to a total volume of 2500 µL. The decrease in absorbance at 734 nm was recorded using PC based double beam spectrophotometer, 2206 (Systronics, Ahmedabad, India) over 10 minutes at 30-second intervals, and percent inhibition was calculated using the following formula.

$$\% \text{ Radical scavenging activity} = \frac{(\text{A control} - \text{A test}}{(\text{A control})} \times 100$$

Where, A control = Absorbance of phosphate buffer solution at 734 nm

A Test = Absorbance of bacterial suspension at 734 nm

Assessment of Lipase Inhibitory Activity. Lipase inhibition was evaluated using the method of Gil-Rodriguez and Beresford (2020). Strains were inoculated in reconstituted skim milk at 2% and incubated at 37ºC for 24 hours. A 500 µL fermented milk sample was mixed with 2 mL of Tris-HCl buffer (pH 8.25-8.75), followed by the addition of 50 µL 4-nitrophenyl octanoate and 50 µL lipase. The mixture was incubated at 37ºC for 30 minutes, then 1 mL of clarifying reagent (Sigma-Aldrich, Germany) was added and incubated for another 3 minutes. Absorbance

was measured at 412 nm against various controls using PC based double beam spectrophotometer 2206 (Systronics, Ahmedabad, India). Orlistat was used as a positive control, and samples were tested in triplicate. The lipase inhibitory activity was calculated as a percentage relative to the control.

$$\text{Lipase inhibition (\%)} = 100 - \frac{(\text{Absorbance of sample} - \text{Absorbance of blank})}{(\text{Absorbance of the 100 \% activity control} - \text{Absorbance of the blank of this control})} \times 100$$

Assessment of Cholesterol Assimilation. Cholesterol assimilation of LAB strains was assessed using a modified method from Ashar and Prajapati (1998). Strains were inoculated at 2% in a selective broth with bile salts (0.2% sodium taurocholate, 0.3% sodium thioglycolate) and 50 µg/mL cholesterol (Himedia Laboratories Pvt. Ltd, Mumbai, India). After anaerobic incubation at 37ºC for 24 hours, the tubes were centrifuged at 10,000 rpm for 10 minutes at 4ºC (Eppendorf centrifuge, US). 0.5 mL of the supernatant was mixed with 3 mL of 95% ethanol and 2 mL of 50% KOH, then heated at 60ºC for 10 minutes. After cooling, 5 mL of hexane(n-hexane 99% AR, LobaChemie Pvt. Ltd, Maharashtra, India) was added, mixed, and then 3 mL of distilled water was added. Phase separation was allowed at room temperature for 15 minutes, and 2.5 mL of the hexane layer was transferred to clean test tubes. The hexane was evaporated overnight at 60ºC. Four mL of O-phthalaldehydereagent (Himedia Laboratories Pvt. Ltd, Mumbai, India) was added to the dried extracts and allowed to stand for 10 minutes at room temperature. Then, 2 mL of concentrated sulfuric acid was added slowly, and the mixture was thoroughly mixed and left for another 10 minutes. Absorbance was measured at 550 nm using a PC-based double beam spectrophotometer (Systronics, Ahmedabad, India). A standard curve of absorbance versus cholesterol concentrations was generated. The percentage of cholesterol assimilation was calculated using the following formula.

Cholesterol assimilated (µg/mL) = [Cholesterol (µg/mL)]0 h – [Cholesterol(µg/mL)]24 h

$$\text{\% cholestrol assimilation} = \frac{\text{cholesterol assimilated (µg/mL)}}{\text{cholesterol (µg/mL)0 h}} \times 100$$

Assessment of Angiotensin-I-Converting Enzyme (ACE) Inhibitory Activity. ACE-inhibitory activity (%) was measured using the method of Hati *et al.* (2015). This involves hydrolyzing N-Hippuryl-His-Leu (HHL) into Hippuric Acid (HA) and His-Leu (HL) with ACE. The process includes mixing HHL solution (Sigma, USA) with deionized water and sample, adding ACE enzyme (Sigma, USA), and incubating at 37ºC. After the reaction, it's terminated with HCl, and HA is extracted using ethyl acetate(Himedia Laboratories Pvt. Ltd, Mumbai, India). The extract is evaporated, dissolved in water, filtered, and its absorbance at 228 nm is measured using spectrophotometer 2202 (Systronics, Ahmedabad, India). ACEi % is calculated by comparing HA produced with and without inhibitors under identical conditions.

$$\text{ACEi \%} = \frac{\text{Absorbance of HA control} - \text{Absorbance of HA sample}}{\text{Absorbance of HA control}} \times 100$$

Where,

HA control: The absorbance of concentration of hippuric acid produced by the ACE in buffer without lactic cultures

HA sample: The absorbance of the concentration of hippuric acid produced by the ACE in the presence of lactic cultures

Assessment of Proteolytic Activity. The degree of proteolysis during milk fermentation was assessed by measuring free NH_3 groups using the o-phthaldialdehyde (OPA) method (Hati *et al*., 2015). 3 mL sample of fermented milk was mixed with 5 mL of 0.75% trichloroacetic acid (TCA) (Himedia Laboratories Pvt. Ltd, Mumbai, India), vortexed (Thermo Fisher Scientific, India), and filtered through Whatman™ no. 42 paper (Sigma-Aldrich, supplied by Merck Life Science Private Limited, Mumbai, India). The filtrate (200 µL) was combined with 3 mL of OPA reagent, incubated at 20ºC for 2 min, and absorbance was measured at 340 nm using a Systronics spectrophotometer (Systronics, Ahmedabad, India). A standard curve was prepared with leucine.

Assessment of Antidiabetic Activity. Antidiabetic activity was assessed through the inhibition of α-amylase and α-glucosidase enzymes.

α-Amylase inhibition assay. α-Amylase inhibition was assessed using a spectrophotometric method with 3,5-dinitrosalicylic acid (Chaudhary & Mudgal 2020). In this assay, 300 µL of sample extract, 70 µL of 50% methanol, 50 µL of enzyme solution, and 1 mL of starch solution were mixed and incubated at 37°C for 5 minutes. After incubation, 2 mL of 3,5-dinitrosalicylic acid (Sigma-Aldrich, Mumbai, India) reagent was added, and the mixture was heated in a boiling water bath for 5 minutes, then cooled to room temperature. Absorbance was measured at 540 nm. Blank and control tubes were prepared by excluding the enzyme and sample, respectively

$$\text{\% inhibition of α-amylase} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

α-Glucosidase inhibition assay. The assay, based on Chaudhary and Mudgal (2020), involved mixing sample extracts (500 µg/mL) with 1000 µL of 0.1 M phosphate buffer (pH 6.9) containing α-glucosidase (1 U/mL) (Sigma-Aldrich, Mumbai, India) and incubating at 25°C for 10 minutes. After pre-incubation, 500 µL of 5 mM para-nitrophenyl-α-D-glucopyranoside (Sigma-Aldrich, Mumbai, India) in buffer was added, and the mixture was incubated at 25°C for 5 minutes. Absorbance at 405 nm was measured before and after incubation using a UV spectrophotometer and compared to a control with buffer instead of extract. α-glucosidase inhibitory activity (%) was calculated as follows.

$$\% \text{ inhibition of } \alpha\text{-glucosidase} = \frac{\text{O.D. Control} - \text{O.D. Sample}}{\text{O.D. Control}} \times 100$$

Assessment of the probiotic potential of selected strains. Probiotic potential of the strains *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), and *Limosilactobacillus fermentum* (BM24) was assessed according to ICMR-DBT guidelines (ICMR-DBT, 2011). Evaluation included resistance to gastric acidity and bile acid, antimicrobial activity against pathogenic bacteria, and pathogen adhesion reduction.

Tolerance of the strains to low pH was assessed using method from Kathiriya *et al.* (2015). Selective broths were adjusted to pH 1.0, 2.0, 3.0 using 1 N HCl, with pH 6.5 as the control. After mixing, the broth was distributed in 10 mL aliquots. Cultures were activated by inoculating in selective broth (2%) and incubated for 12 hours, then centrifuged at 10,000 rpm for 10 minutes at 4°C. The pellets were washed twice with phosphate buffer saline (PBS) and resuspended in PBS. These cultures (2%) were added to tubes with selective broth at pH 1.0, 2.0, 3.0, and 6.5. After incubation at 37°C, 1 mL samples were taken at 0, 1, 2 and 3 hours, diluted in PBS, and plated on selective agar. Plates were incubated at 37°C for 48-72 hours, and viable cell counts were recorded as log CFU/mL.

Bile tolerance of the strains was assessed using method from Kathiriya *et al.* (2015) Cultures were activated by inoculating in selective broth (2%) and incubated for 24 hours. After centrifugation at 10,000 rpm for 10 minutes at 4°C, the pellets were washed with PBS and resuspended in PBS. Suspended cultures (2%) were added to 10 mL selective broth containing 0.3%, 0.5%, and 1% bile salt, with a control containing no bile salt. Tubes were incubated at 37°C. Samples (1 mL) were taken at 0, 1, 2, and 4 hours, diluted in PBS, and plated on selective agar. Plates were incubated at 37°C for 48-72 hours, and viable cell counts were recorded as log CFU/mL.

In the methods for adhesion tests (Tuo *et al.,* 2013), each strain was centrifuged at 10,000 rpm for 10 minutes at 4°C, washed with PBS (pH 7.2), and resuspended in PBS buffer. The suspension was incubated at 37°C for 5 hours, and absorbance at 600 nm was measured for both the upper and total suspension. Auto-aggregation percentage was then calculated.

Auto aggregation %=[1 – A (Upper suspension)/A (Total suspension)]×100

Where,

$A_{\text{Upper suspension}}$=OD at 600nm of upper suspension after 5 hr

$A_{\text{Total suspension}}$=OD at 600 nm of Total bacteria suspension after 5 hr

To determine co-aggregation ability (Tuo *et al.,* 2013), 10 mL of culture was centrifuged at 5,000 rpm for 10 minutes. Equal volumes (2 mL) of each strain and pathogenic bacterial strains were mixed and incubated at 37°C for 5 hours. Optical density of the mixed cultures was measured at 600 nm. The co-aggregation percentage was calculated using the following formula

Co-aggregation %=[(A test bacteria + A strain) – 2 (A mixed strain)/(A test bacteria+ Astrain)] × 100

Where,

$A_{\text{test bacteria}}$ -OD_{600nm} Pathogenic Bacteria

A_{strain}-OD_{600nm} *strain*

$A_{\text{mixed strain}}$ -$OD_{600\text{ nm}}$ of *strain*+ Pathogenic Bacteria

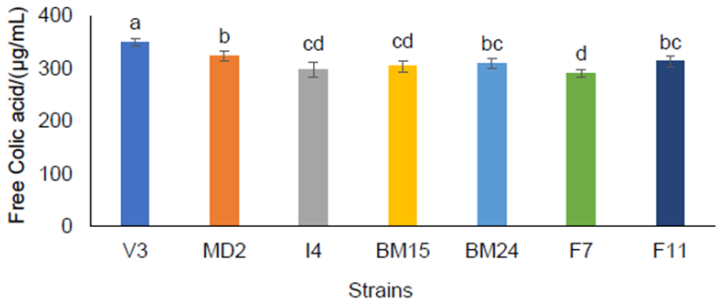
Antimicrobial activity of the strains was assessed using the agar well diffusion assay following Kathiriya *et al.* (2015). Cell-free supernatant (CFS) from each strain was tested. Indicator bacteria, grown in nutrient broth at 37°C for 12 hours, were mixed with 100 mL of 1% nutrient agar and poured into Petri dishes. Wells (6 mm diameter) were created in the solidified agar using a sterile borer. 100 µL of CFS was added to each well. Plates were incubated at 37°C for 24 hours and antimicrobial activity was measured by the diameter of the growth inhibition zone around the wells.

Statistical Analysis. Data obtained were analyzed by completely randomized design (CRD) as per the methods described by Steel and Torrie (1980). The significance of the influence of each parameter on the specific characteristic was tested at 5.0% level of significance.

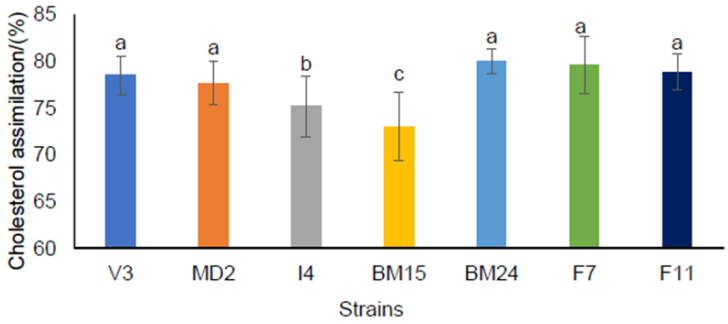
RESULTS AND DISCUSSION

CVDs are a leading cause of death and disability putting economic burden. While traditional risk factors like hypertension, diabetes, hyperlipidemia, obesity, smoking, and environmental influences are well-known, recent studies have highlighted the significant role of gut microbiota in the development and progression of CVDs. Research suggests that probiotics can mitigate health complications associated with or leading to CVDs. This study investigated the potential of LAB strains to manage CVD risk factors, including hypertension, hyperlipidemia, and oxidative stress. The probable probiotic mechanisms of action can be bile deconjugation, cholesterol assimilation, antioxidant capacity, ACE inhibition, antidiabetic potential and generation of bioactive metabolites through proteolytic activity in addition to being contributing to the gut microbiota health.

Bile deconjugation ability of LAB strains. The ability of the LAB strains to deconjugate bile was determined by measuring the production of free cholic acid from sodium taurocholate. The values were derived using a standard curve. The bile deconjugation ability of the lactic acid strains differed significantly ($p<0.05$). As shown in Fig. 1 (a), strain V3 exhibited the highest bile deconjugation ability (350.13 µg/mL), followed by MD2 (323.72 µg/mL), F11 (312.95 µg/mL), BM24 (309.62 µg/mL), BM15 (303.97 µg/mL), I4 (296.80 µg/mL), and F7 (291.15 µg/mL) after 24 hours at 37ºC. These results clearly indicate strain-specific variations in bile deconjugation ability.



(a)



(b)

Fig. 1(a) Bile deconjugation and (b) Cholesterol assimilation ability of LAB strains. Each observation is a mean±SD of (n=3). Different small case letters in the graph indicate statistically significant ($p<0.05$) differences. *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* (BM15), *Limosilactobacillus fermentum* (BM24), *Lactipantibacillus plantarum* (F7) and *Lactipantibacillus plantarum* (F11).

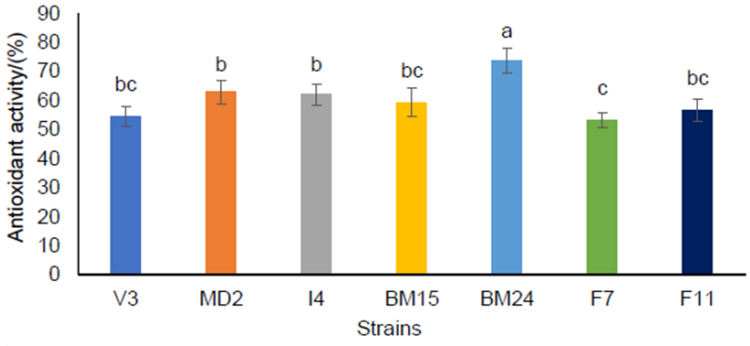
Cholesterol assimilation by LAB strains. Cholesterol assimilation by the strains, as detailed in Fig.1(b), revealed significant differences ($p<0.05$). Strains V3 (78.49%), MD2 (77.65%), BM24 (79.93%), F7 (79.56%), and F11 (78.84%) exhibited significantly better cholesterol assimilation, with their assimilation abilities being statistically comparable. The lowest cholesterol assimilation was observed in strain BM15 (73.01%), which was also statistically significant.

Bile salt hydrolysis is essential for regulating cholesterol and maintaining cholesterol homeostasis. Liong and Shah (2005) explain that bile salt hydrolase (BSH) enzymes catalyze the hydrolysis of conjugated bile salts into free bile acids and amino acids. At the intestinal lumen's physiological pH, some free bile salts precipitate, reducing serum cholesterol levels by increasing the need for new bile salts synthesized from cholesterol. This deconjugation process, facilitated by BSH enzymes from gut bacteria like *Bifidobacterium* and *Lactobacillus* species, reduces bile salt reabsorption in the terminal ileum and increases fecal excretion. BSH activity is noted in *Lactobacillus*, *Bifidobacterium*, and *Clostridium* species (11). Avci (2014) reported that *L. delbrueckii* subsp. *bulgaricus* (HL6) and *S. thermophilus* (HS12) deconjugated sodium taurocholate at 1.0-1.4 mg/ml and 0.9-1.3 mg/ml, respectively (Avci, 2014). Kathiriya *et al.* (2018) found that *L. rhamnosus* NS6 had the highest bile deconjugation ability, producing 364 µg/mL of cholic acid from sodium taurocholate, followed by *S. thermophilus* MD8 with 230 µg/mL. Additionally, Hernandez-Gomez *et al*. (2021) observed that *L. fermentum* K73, isolated from traditional fermented sour cream, showed deconjugation activity of 24%.

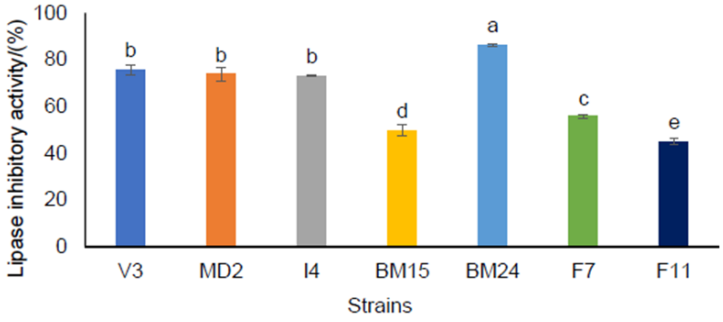
Kaplan *et al*. (1998) state that elevated blood cholesterol levels contribute to atherosclerosis, increasing the risk of MI and stroke. Tomaro-Duchesneau *et al*. (2014) suggest that probiotic bacteria in the gut can assimilate cholesterol, reducing its absorption by enterocytes and promoting its excretion, potentially lowering the risk of CVDs. The primary *in vitro* mechanisms proposed for how probiotics impact cholesterol levels include cholesterol adherence to cell surfaces and absorption into cellular membranes. Tomaro-Duchesneau *et al*. (2014) identified *L. reuteri* NCIMB 11951, *L. reuteri* NCIMB 701089, and *L. acidophilus* ATCC 314 as the top cholesterol-absorbing strains, with *L. acidophilus* ATCC 314 showing the highest assimilation at 41.20±1.92 µg/mL (Tomaro-Duchesneau *et al*., 2014). In an *in vitro* study by Ding *et al*. (2017) *L. plantarum* (Lp1) exhibited the highest cholesterol assimilation at 75.9%, while *Lactococcus lactis* subsp. *lactis* (L1) had the lowest at 61.2% (Ding *et al*., 2017). Bhat and Bajaj found that among the LAB, *L. casei* M6 showed the highest assimilation at 82.15%, followed by *L. casei* M5 at 76.51%, *L. paracasei* M3 at 67.4%, and *L. paracasei* M37 at 67.2% (Bhat & Bajaj 2020). Wang *et al*. (2021) reported significant variability among *L. plantarum* strains in their cholesterol-lowering abilities. In MRS broth, *L. plantarum* AR113 and AR171 showed the highest reductions at 27.89% and 19.90%, respectively, while strains AR501 and AR300 exhibited minimal reductions of 0.34% and 0.91%. All strains in our study demonstrated cholesterol reduction of more than 70%,

highlighting their potential as probiotics for CVD prevention.

Antioxidant potential of LAB strains. Antioxidant activity was assessed using the ABTS assay method, with results expressed as a percentage of free radical scavenging activity. The findings, presented in Fig. 2(a), indicate that strain BM24 exhibited significantly ($p<0.05$) higher antioxidant activity at 73.57% compared to all other strains. On the other hand, strain F7 showed the lowest antioxidant activity at 53.14%, which was statistically similar to that of BM15 at 59.24%. The antioxidant activities of strains V3 (54.48%), F11 (56.62%), BM15 (59.24%), I4 (62.05%), and MD2 (62.76%) were found to be comparable to each other. These results clearly demonstrate that antioxidant activity is highly strain dependent.



(a)



(b)

Fig. 2. (a) Antioxidant activity and (b) Lipase Inhibitory activity of LAB strains. Each observation is a mean±SD of (n=3). Different small case letters in the graph indicate statistically significant ($p<0.05$) differences. *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* (BM15), *Limosilactobacillus fermentum* (BM24), *Lactipantibacillus plantarum* (F7) and *Lactipantibacillus plantarum* (F11).

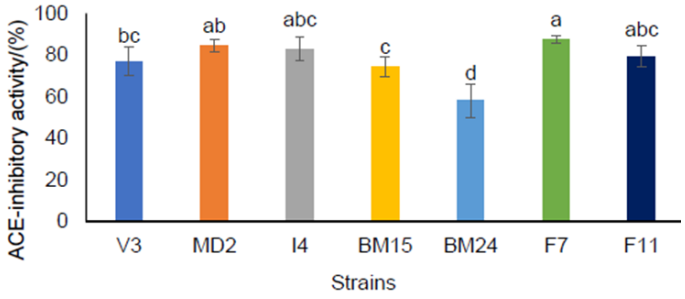
Oxidative stress, marked by elevated reactive oxygen species (ROS) and reactive nitrogen free radicals, occurs due to adverse conditions like ischemia and hypoxia, leading to apoptosis and tissue damage, which are risk factors for MI[35]. Normally, low ROS levels are balanced by detoxification processes that support cellular signaling and function. However, in pathological states such as atherosclerosis or hypertension, excessive ROS production overwhelms the body's antioxidant defenses, causing cell death. Numerous *in vivo* and *in vitro* studies have shown that *Lactobacilli* and *Bifidobacteria* possess strong antioxidant capacity, offering protection against oxidative stress (Rani *et al.*, 2016.) These bacteria are valuable natural antioxidants, producing enzymes and metabolites that combat free radicals. Amaretti *et al.* (2013) identified *Levilactobacillus brevis*, *L. acidophilus*, and *B. lactis* as strains with the highest antioxidant activity. Among the 9 LAB strains isolated from traditional Chinese dairy foods, *L. paracasei* had better scavenging activity of free radicals (Wang & Li 2022).

Lipase inhibitory activity of LAB strains. Significant differences ($p<0.05$) were observed in the lipase inhibitory activity of the LAB strains, as shown in Fig. 2(b). Among the strains, BM24 demonstrated the highest lipase inhibition at 86.21%, significantly higher than the other strains. This was followed by V3 (75.57%), MD2 (73.72%), I4 (73.03%), F7 (55.67%), BM15 (49.51%), and F11 (44.73%) after 24 hours. Additionally, lipase inhibition by strains V3, MD2, and I4 was statistically similar to each other.

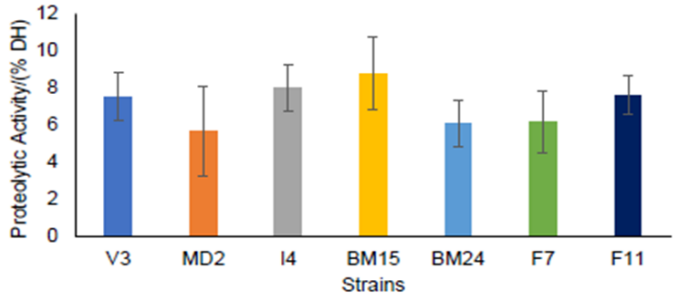
Lipase is essential for the digestion, transport, and processing of dietary lipids. In humans, pancreatic lipase is the key enzyme that breaks down dietary fats in the digestive system. Lipase inhibitors reduce fat absorption in the gastrointestinal tract, leading to fat excretion rather than absorption for energy, potentially resulting in weight loss. These inhibitors are often used to treat obesity (Maqsood *et al.*, 2017). This test was conducted because obese individuals are at a higher risk of developing diabetes and heart-related issues. Mudgil investigated the inhibitory effects of LAB strains from raw camel milk on pancreatic lipase, comparing 11 reference strains and 97 LAB isolates. Among 52 *Streptococcus* isolates, inhibition ranged from 3.0% to 99.0%, with 11 isolates showing strong effects comparable to or exceeding orlistat (83.0%). Of the 45

Lactobacillus isolates, 13 showed negative inhibition, while 32 exhibited inhibition between 4.0% and 81.0%. Reference cultures had inhibition levels from 3.0% to 37.0%, with *L. acidophilus* DSMZ 9126 showing the highest and *L. gasseri* 20243 the lowest (Mudgil *et al.*, 2016). Gil-Rodríguez and Beresford also found varying lipase inhibitory activities among lactic strains in fermented milk, with *L. plantarum* 70 (37.22%) showing the highest inhibition[21]. In our study, except BM 15 and F11, others exhibited over 50% lipase inhibition, indicating their potential against CVDs.

ACE-Inhibitory activity of LAB strains. ACE-inhibition ability of the strains is summarized in Fig. 3(a). After 24 hours of incubation, ACE inhibition by the strains ranged from 57.90% to 87.58%. Strain F7 demonstrated the highest ACE-inhibitory activity at 87.58%, followed by MD2 (84.19%), I4 (82.85%), F11 (79.27%), V3 (76.77%), BM15 (74.41%), and BM24 (57.90%). The strains F7, MD2, I4, and F11 showed similar levels of activity, while BM24 exhibited the lowest ACE inhibition, which was significantly different ($p<0.05$) from the other strains.



(a)



(b)

Fig. 3. (a) ACE-inhibitory and (b) Proteolytic activity of LAB strains. Each observation is a mean±SD of (n=3), Different small case letters in the graph indicate statistically significant ($p<0.05$) differences. DH=Degree of Hydrolysis. *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* (BM15), *Limosilactobacillus fermentum* (BM24), *Lactipantibacillus plantarum* (F7) and *Lactipantibacillus plantarum* (F11).

Hypertension contributes to cardiovascular disorders like arteriosclerosis, heart failure, coronary heart disease, MI, and stroke. The enzyme ACE produces angiotensin II, a vasoconstrictor that raises blood pressure. ACE inhibitors lower blood pressure by blocking the conversion of angiotensin I to angiotensin II and may also prevent the breakdown of bradykinin, which induces vasodilation. These inhibitors are used to manage and treat conditions such as diabetes, heart failure, MI, nephropathy, and other CVDs (Li *et al.*, 2017). Hati *et al*. (2015) reported the ACE-inhibitory activity of *Lactobacillus rhamnosus* (NS4) and *Lactobacillus bulgaricus* (009) strains to be 79.66% and 67.09%, respectively. According to Zhao *et al.* (2021) *Lactobacillus helveticus*, used in dairy fermentation, has long-term hypotensive effects in hypertensive patients. It produces two tripeptides, Val-Pro-Pro and Ile-Pro-Pro, that inhibit angiotensin-I converting enzyme and lower blood pressure without adverse effects. Furthermore, *L. helveticus* KII 13 isolated from fermented milk produced hypotensive peptides. Parmar *et al*. (2020) investigated five *Lactobacillus* strains *L. rhamnosus* (NK2) (KR080695), *L. casei* (NK9) (KR732325), *L. fermentum* (M5) (KU366365), *L. paracasei* (M16) (KU366368), and *L. fermentum* TDS030603 (MTCC 25067) (LF) from fermented goat milk for their ACE-inhibitory activities. After 24 hours, NK9 significantly outperformed the other cultures in terms of ACE-inhibitory activity (66.99%, $p<0.05$) (Parmar *et al.*, 2020).

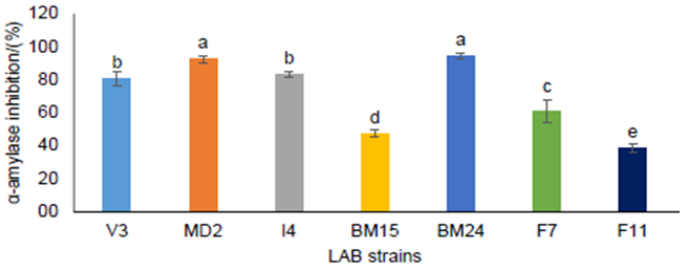
Proteolytic activity of LAB strains. Proteolytic activity of the strains ranged from 5.65% to 8.75% during 24 hours of incubation [Fig. 3(b)]. There was no significant difference observed in the proteolysis among the strains. BM15 demonstrated the highest proteolytic activity at 8.75%, followed by I4 (7.94%), F11 (7.59%), V3 (7.53%), F7 (6.14%), BM24 (6.06%), and MD2 (5.65%) after 24 hours at 37ºC.

LAB can degrade milk proteins through their proteolytic system, producing peptides with health benefits, including ACE-inhibitory bioactive peptides (Hati *et al.*, 2015). Ahmed and Bousmaha-Marroki (2014) reported the highest proteolytic activities in *Lactobacillus plantarum* strains LbMS16 (15.50%) and

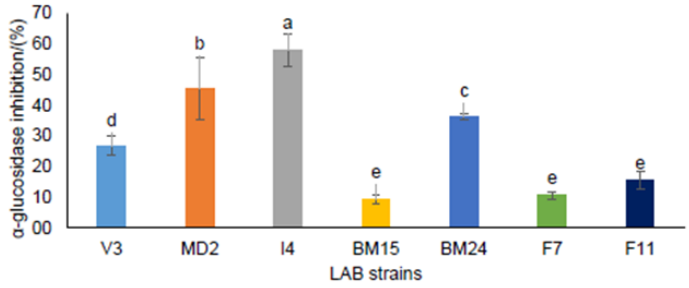
LbMS21 (19.00%), as well as in *Lactobacillus rhamnosus* strain LbMF25 (25.00%). Hati *et al*. (2015) found that the proteolytic activity of *Lactobacillus rhamnosus* (NS4 and NS6), *Lactobacillus helveticus* MTCC 5463 (V3), *Lactobacillus delbrueckii* (009), *Enterococcus faecalis* (ND3 and ND11), and *Lactobacillus rhamnosus* (SH8) increased in skim milk when cultured at a rate of 1% at 37ºC for 24 hours. Among these, NS4 released the highest number of amino acids after 24 hours of fermentation at 37ºC, with 009 and ND3 also showing efficient amino acid release. In contrast, V3, NS6, SH8, ND11, and I4 exhibited comparatively lower proteolytic activity. Their study concluded that NS4, 009, and ND3 had the most robust proteolytic systems, with the greatest capacity for producing proteolytic enzymes in skim milk.

Antidiabetic potential of LAB strains. Diabetes is recognized as a modifiable risk factor for CVDs. Inhibiting α-amylase activity can help mitigate postprandial blood glucose spikes. Additionally, α-glucosidase, an enzyme found in the intestine, hydrolyzes various sugars into glucose; thus, inhibiting α-glucosidase can help lower blood glucose levels (Wang & Li 2022).

α-amylase inhibition by LAB strains. α-amylase inhibitory activity of the LAB strains is shown in Fig. 4(a). Among the strains BM24 (94.28%) and MD2 (92.16%) showed significantly high inhibition followed by V3 (80.16%), I4 (83.05%), F7 (60.82%), BM15 (47.21%) and F11 (38.42%). Least inhibition was seen for strain F11. The α-amylase inhibitory activity of strains V3 and I4 were at par with each other.



(a)



(b)

Fig. 4. (a) Alpha amylase inhibition and (b) Alpha glucosidase inhibition by LAB strains. Each observation is a mean±SD of (n=3), Different small case letters in the graph indicate statistically significant ($p<0.05$) differences. *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* (BM15), *Limosilactobacillus fermentum* (BM24), *Lactipantibacillus plantarum* (F7) and *Lactipantibacillus plantarum* (F11).

α-glucosidase inhibition by LAB strains. α-glucosidase inhibition by the LAB strains is shown in Fig. 4(b). Among the strains I4 (57.73%) exhibited the highest ($p<0.05$) inhibition followed by MD2 (45.51%), BM24 (36.20%), V3 (26.75%) and others. Least inhibition was seen for strains BM15 (9.29%), F7 (10.56%) and F11 (15.60%) which were at par with each other.

Wang and Li (2022) reported that among all the LAB strains they studied, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus paracasei* and *Lactobacillus casei* exhibited significantly higher inhibition activity ($p<0.05$), with *Lactobacillus plantarum* showing the highest inhibition rate at 83.36% for α-amylase. Additionally, the CFS of *Lactobacillus plantarum* demonstrated the highest α-glucosidase inhibitory activity at 85.16%. Ayyash *et al*. (2018) reported that the probiotic cultures *L. reuteri* KX881777, *L. plantarum* KX881772, and *L. plantarum* KX881779 exhibited α-amylase inhibitions of over 34% in milk medium. Kwun *et al*. (2020) found that *L. sakei* MBEL1397, isolated from kimchi, exhibited an α-glucosidase inhibitory activity of 3.91±0.25%.

Probiotic potential of the selected strains. The strains V3, MD2, I4 and BM24 were capable of surviving at pH 2.0 after 3h of incubation and they had shown viability of 4.24, 4.11, 4.57 and 4.24 log CFU/mL respectively (Fig. 5). Similarly, the bile tolerance of the strains at 1% bile concentration after incubation period of 4h were 3.70, 3.78, 3.74 and 4.47 log CFU/mL respectively (Fig. 6).

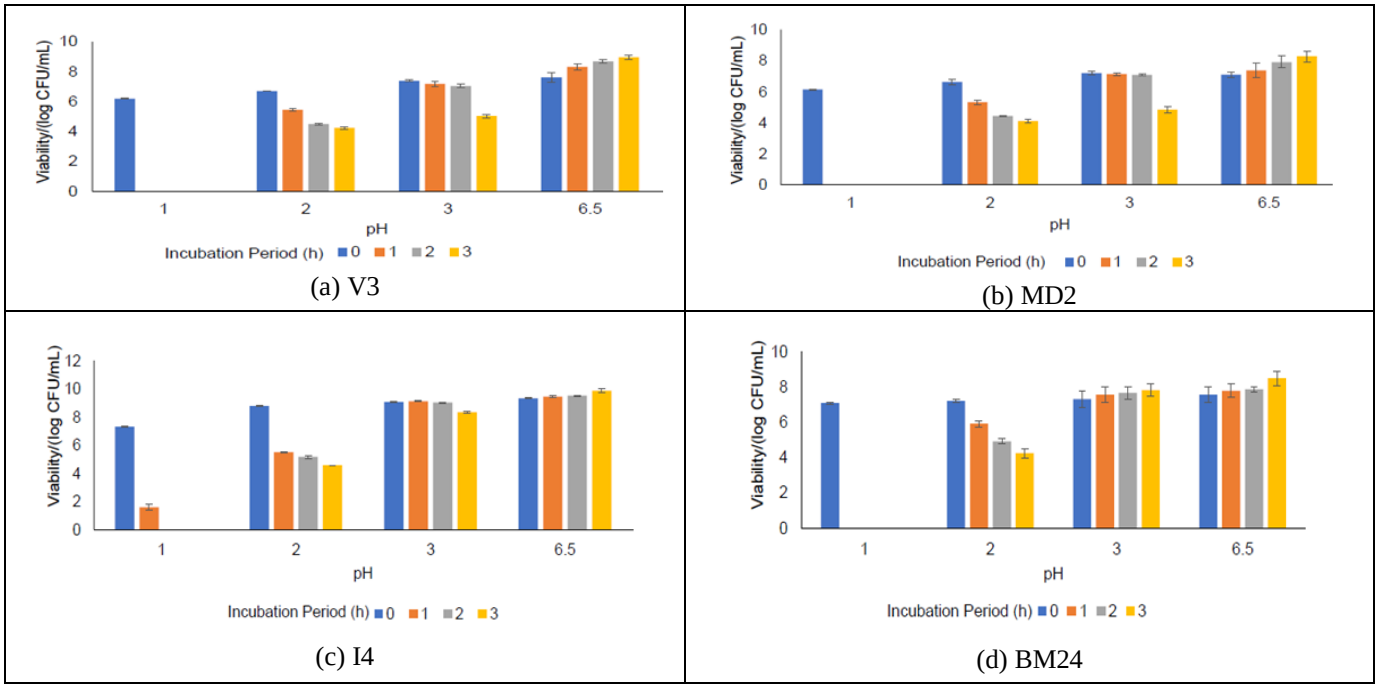


Fig. 5. pH tolerance of the strains. (a) *Lactobacillus helveticus* MTCC 5463 (V3), (b) *Streptococcus thermophilus* MTCC 5460 (MD2), (c) *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), (d) *Limosilactobacillus fermentum* (BM24). Each observation is a mean±SD of (n=4).

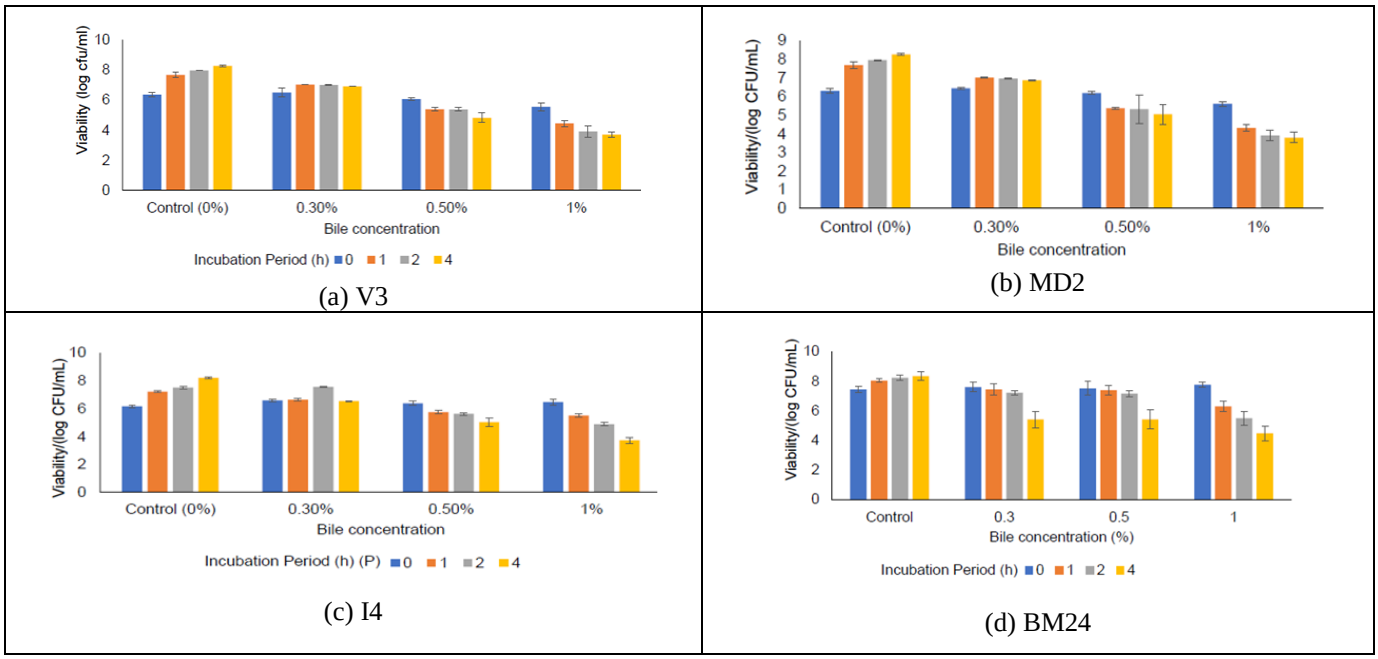


Fig. 6. Bile tolerance of the strains. (a) *Lactobacillus helveticus* MTCC 5463 (V3), (b) *Streptococcus thermophilus* MTCC 5460 (MD2), (c) *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), (d) *Limosilactobacillus fermentum* (BM24). Each observation is a mean±SD of (n=4).

Our results [Fig. 7(a)] revealed that the rate of auto-aggregation increases over time. After 2 hours, the auto-aggregation percentages for strains V3, MD2, I4, and BM24 were 56.57%, 14.71%, 34.19%, and 40.05%, respectively. These percentages significantly increased for all strains after 5 hours, reaching 66.42%, 31.97%, 44.77%, and 57.62%, respectively. As shown in Fig.7(b), the strain V3 exhibited the highest co-aggregation with all tested pathogens, with co-aggregation ability varying by strain. Co-aggregation of strains with the pathogens varied from 10-36%.

The results (Table 2) of the antimicrobial activity of the strains revealed that 3 strains had significant ($p<0.05$) antimicrobial activity against all 5 pathogens except for MD2, which has shown antimicrobial activity only against *S. enterica paratyphi* MTCC 735 and *B. cereus* MTCC 1272. V3 had shown highest inhibition against *Salmonella enterica ser. paratyphi* MTCC 735 (10.00±3.29), and *Bacillus cereus* MTCC 1272 (10.00±1.00). I4 showed highest inhibition against *Bacillus cereus* MTCC 1272 (10.33±1.52).

Table 2: Antimicrobial activity of LAB strains against pathogens (Inhibition zone in mm).

Strains	*S. aureus* MTCC 737	*E. coli* MTCC 1687	*S. paratyphi* MTCC 735	*B. cereus* MTCC 1272	*S. flexneri* MTCC 1457	Treatment Mean
V3	10.33 ± 0.57	11.00 ± 3.61	10.00 ± 3.29	10.00 ± 1.00	9.33 ± 0.57	10.13^{b}
MD2	0.00 ± 0.00	0.00 ± 0.00	9.33 ± 4.09	9.33 ± 0.57	0.00 ± 0.00	3.73^{d}
I4	9.00 ± 0.00	9.67 ± 3.31	9.33 ± 2.99	9.33 ± 0.57	10.33 ± 1.52	9.53^{c}
BM24	12.33 ± 0.57	13.00 ± 4.31	9.33 ± 3.89	9.33 ± 0.57	10.00 ± 1.00	10.80^{a}
Pathogens Mean	7.923	8.422	9.501	9.501	7.424	
CD (0.05) T=0.54, P=0.60, T×P=1.21; CV%=8.53						

Each observation is a mean±SD of (n=4). *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), *Limosilactobacillus fermentum* (BM24). *Staphylococcus aureus subsp. aureus* MTCC 737, *Escherichia coli* MTCC 1687, *Salmonella enterica ser. paratyphi* MTCC 735, *Bacillus cereus* MTCC 1272, *Shigella flexneri* MTCC 1457

In order to exert its beneficial effect on the host, a probiotic strain must be able to survive the gut passage of humans to reach to the action site in viable state and should be in sufficient population (ICMR-DBT, 2011). Acid tolerance of 255 isolates of LAB isolated from Greek traditional fermented products revealed that 133 isolates out of 255, exhibited final counts of $\geq 10^3$ CFU/mL at low pH for 3h. Probiotic potential of *Lactobacillus rhamnosus* NK2, *Lactobacillus casei* NK9, *Lactobacillus rhamnosus* NK10, *Lactobacillus pentosus* M20 and *Lactobacillus plantarum* M22 has been reported by Kathiriya *et al.* (2015). These isolates were able to tolerate upto pH 2.0 for 3h. Pino *et al.* (2019), in their investigation on detection of vaginal *lactobacilli* as probiotic candidates reported survival rates of ≥80% for *lactobacilli* isolate at both pH 3.0 and pH 2.0 conditions.

Bile salt tolerance is generally considered a prerequisite for the colonization and metabolic activity of bacteria in the host's intestine (CMR-DBT, 2011). The average concentration of bile salts in the small intestine ranges from 0.2% to 0.3%, but it can rise to as high as 2% (w/v) depending on the individual and the type and amount of food consumed (Menconi *et al*., 2014). Nagyzbekkyzy *et al*. (2016) examined the bile tolerance of *Lactobacillus* strains isolated from Kazakh dairy products after 24 hours of exposure to 1.0% bile acid and found that the cultures responded differently in the presence of bile acids. Several of the most tolerant LAB isolates showed growth percentages ranging from 40% to 71%, while the remaining isolates demonstrated intermediate tolerance, with cell growth ranging from 20% to 40% (Nagyzbekkyzy *et al.*, 2016). In a study by Pino *et al.* (2019), investigating vaginal lactobacilli as potential probiotics, it was found that a bile salt concentration of 0.5% (w/v) had no effect on most strains, except for *Lactobacillus crispatus* P10 and *Lactobacillus plantarum* C11 and V7 strains. At a bile salt concentration of 1.0% (w/v), 86% and 79% of the strains exhibited bile tolerance after 2 and 4 hours, respectively.

Bacterial aggregation, whether involving the same strain (auto-aggregation) or different species and strains (co-aggregation), plays a crucial role in the human gut, where probiotics are intended to function. A probiotic strain's ability to adhere to the oral cavity, gastrointestinal system, and urogenital tract is largely determined by its auto-aggregation capacity, while its co-aggregation ability helps form a barrier that prevents pathogen colonization (Divya *et al.*, 2012).

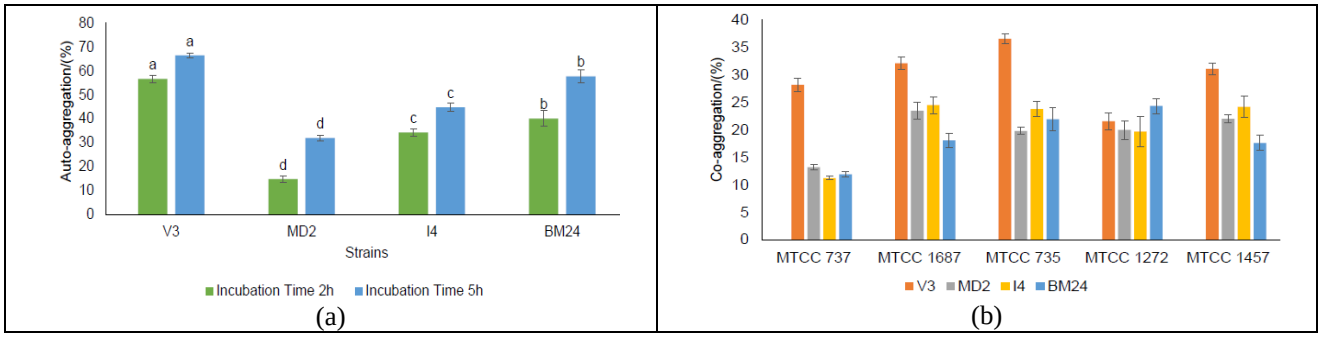


Fig. 7. (a) Autoaggregation and (b) Co-aggregation of the strains. Each observation is a mean±SD of (n=4). *Lactobacillus helveticus* MTCC 5463 (V3), (b) *Streptococcus thermophilus* MTCC 5460 (MD2), (c) *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), (d) *Limosilactobacillus fermentum* (BM24). *Staphylococcus aureus subsp. aureus* MTCC 737, *Escherichia coli* MTCC 1687, *Salmonella enterica ser. paratyphi* MTCC 735, *Bacillus cereus* MTCC 1272, *Shigella flexneri* MTCC 1457.

Li *et al*. (2015) studied the aggregation and adhesion abilities of 18 LAB strains isolated from traditional fermented foods and found significant ($p<0.05$) differences in co-aggregation among the strains. All tested LAB exhibited some level of co-aggregation with *Salmonella* sp., ranging from 5.15% to 29.54%, highlighting strain-specific characteristics. *Lactobacillus fermentum* 9 showed the highest co-aggregation ability (29.54%) with *Salmonella* sp., followed by *Lactobacillus fermentum* AB4 (19.45%). *Enterococcus faecalis* 5 displayed the lowest co-aggregation ability. Among the 18 strains, 66.67% showed co-aggregation above 15%, while only 11.11% had co-aggregation below 10%.

LAB are well known for producing a variety of antimicrobial compounds that have significant antagonistic effects against various microorganisms, including pathogenic and spoilage organisms. These

antimicrobial compounds include organic acids such as lactic acid and acetic acid, along with substances like hydrogen peroxide, acetoin, diacetyl, reuterin, helveticin, carbon dioxide, and bacteriocins. Menconi *et al*. (2014) identified and characterized LAB in a commercial probiotic culture, discovering that the strains LAB 18 and LAB 48 demonstrated *in vitro* antibacterial activity against *Salmonella enterica* serovar Enteritidis, *Escherichia coli* O157, and *Campylobacter jejuni* (Divya *et al*., 2012). Shokryazdan *et al*. (2014) explored the probiotic potential of *Lactobacillus* strains with antimicrobial activity against several human pathogens, finding that nine *Lactobacillus* strains could be considered potential probiotics. Many of these strains exhibited stronger antagonistic effects against the test pathogens compared to the reference strain *Lactobacillus casei* Shirota. Notably, *L. casei* BF1, *L. casei* BF2, and *L. casei* BF3 showed significantly higher inhibitory effects on *Helicobacter pylori* and *Staphylococcus aureus* than *L. casei* Shirota. Karimi *et al*. (2017) evaluated the antimicrobial effects of probiotic bacterial strains isolated from various natural sources against two pathotypes of pathogenic *E. coli*. They observed that *Lactobacillus plantarum*, *Lactobacillus gasseri*, *Enterococcus faecium*, *Bacillus subtilis*, and *Weissella paramesenteroides* strains exhibited considerable antimicrobial effects against the *Escherichia coli*O157strain but had no inhibitory effect against Enterohemorrhagic *Escherichia coli*.

CONCLUSIONS

Among the seven LAB strains evaluated for cardioprotective potential, *Lactobacillus helveticus* MTCC 5463 (V3), *Streptococcus thermophilus* MTCC 5460 (MD2), *Lacticaseibacillus rhamnosus* MTCC 5462 (I4), and *Limosilactobacillus fermentum* (BM24) stood out for their superior performance across a range of bio-functional properties. These strains showed the most effective bile deconjugation, potent antioxidant and lipase inhibition, significant cholesterol assimilation, strong ACE inhibition, notable proteolytic activity, and antidiabetic potential. As a result, they have been identified as promising candidates for use as a multi-strain probiotic. The observed variation in biological activities among LAB strains highlights the importance of selecting specific strains based on their unique properties to address targeted health issues. Leveraging the unique capabilities of individual LAB strains will be key to optimizing therapeutic efficacy and expanding their use in health-promoting and disease-preventing products.

FUTURE SCOPE

Further *in vivo* studies using animal models, along with human clinical trials, are essential to confirm the cardioprotective effects of these strains. Research should prioritize understanding their mechanisms of action, optimizing effective dosages, and evaluating long-term safety to ensure regulatory compliance and build consumer confidence.

REFERENCES

Ahmed, M. & Bousmaha-Marroki, L. (2014). Lactobacilli isolated from Algerian goat's milk as adjunct culture in dairy products. *Brazilian Archives of Biology and Technology, 57*(3), 1678-4324.

Aluko, R. E. (2019). Food protein-derived renin-inhibitory peptides: In vitro and in vivo properties. *Journal of Food Biochemistry, 43*, 12648.

Amaretti, A., di Nunzio, M., Pompei, A., Raimondi, S., Rossi, M. & Bordoni, A. (2013). Antioxidant properties of potentially probiotic bacteria: In vitro and in vivo activities. *Applied Microbiology and Biotechnology, 97*(2), 809-817.

Ashar, M. N. & Prajapati, J. B. (1998). Bile tolerance, bile deconjugation, and cholesterol reducing properties of dietary lactobacilli. *Indian Journal of Microbiology, 38*(3), 145-148.

Avci, G. A. (2014). Bile salts deconjugation using microencapsulated lactic acid bacteria isolated from handmade yogurt. *Journal of Food and Nutrition Research, 2*(7), 340-343.

Avila-Escalante, M. L., Coop-Gamas, F., Cervantes-Rodríguez, M., Méndez-Iturbide, A. & Aranda-González, I. I. (2020). The effect of diet on oxidative stress and metabolic diseases—clinically controlled trials. *Journal of Food Biochemistry, 44*, 13191.

Ayyash, M., Al-Nuaimi, A. K., Al-Mahadin, S. & Liu, S. Q. (2018). In vitro investigation of anticancer and ACE-inhibiting activity, α-amylase and α-glucosidase inhibition, and antioxidant activity of camel milk fermented with camel milk probiotic: A comparative study with fermented bovine milk. *Food Chemistry, 239*, 588-597.

Bhat, B., & Bajaj, B. K. (2020). Multifarious cholesterol lowering potential of lactic acid bacteria equipped with desired probiotic functional attributes. *Biotechnology, 10*(5), 1-16.

Cai, J. J. & Hui, J. (2022). Application potential of probiotics in acute myocardial infarction. *Cardiovascular Innovation and Applications, 7*(1).

Chaudhary, J. K. & Mudgal, S. (2020). Effect of incorporation of finger millet (*Eleusine coracana*) on the antimicrobial, ACE inhibitory, antioxidant, and antidiabetic potential of a milk millet composite probiotic fermented product. *Indian Journal of Dairy Science, 73*(3), 222-230.

Ding, W., Shi, C., Chen, M., Zhou, J., Long, R. & Guo, X. (2017). Screening for lactic acid bacteria in traditional fermented Tibetan yak milk and evaluating their probiotic and cholesterol-lowering potentials in rats fed a high-cholesterol diet. *Journal of Functional Foods, 32*, 324-332.

Divya, J. B., Varsha, K. K. & Nampoothiri, K. M. (2012). Newly isolated lactic acid bacteria with probiotic features for potential application in food industry. *Applied Biochemistry and Biotechnology, 167*(5), 1314-1324.

Dubois-Deruy, E., Peugnet, V., Turkieh, A. & Pinet, F. (2020). Oxidative stress in cardiovascular diseases. *Antioxidants, 9*(9), 864.

Foley, M. H., O'Flaherty, S., Barrangou, R. & Theriot, C. M. (2019). Bile salt hydrolases: Gatekeepers of bile acid metabolism and host-microbiome crosstalk in the gastrointestinal tract. *PLoS Pathogens, 15*(3), 1007581.

Ghorbani, Z., Kazemi, A., Bartolomaeus, U. P., Martami, F., Noormohammadi, M., Salari, A., Lober, U., Balou, H. A., Forslund, S. K. & Mahdavi-Roshan, M. (2023). The effect of probiotic and synbiotic supplementation

on lipid parameters among patients with cardiometabolic risk factors: A systematic review and meta-analysis of clinical trials. *Cardiovascular Research, 119*, 933-956.

Gil-Rodriguez, A. M. & Beresford, T. P. (2020). Lipase inhibitory activity assay for fermented milk. *Methods, 7*, 100999.

Hati, S., Sreeja, V., Solanki, J. & Prajapati, J. B. (2015). Significance of proteolytic microorganisms on ACE-inhibitory activity and release of bioactive peptides during fermentation of milk. *Indian Journal of Dairy Science, 68*(6), 584-591.

Hernandez-Gomez, J. G., Lopez-Bonilla, A., Trejo-Tapia, G., Avila-Reyes, S. V., Jimenez-Aparicio, A. R. & Hernandez-Sanchez, H. (2021). In vitro bile salt hydrolase (BSH) activity screening of different probiotic microorganisms. *Foods, 10*(3), 674.

Indian Council of Medical Research, & Department of Biotechnology. (2011). *Guidelines for evaluation of probiotics in food*. Indian Council of Medical Research, New Delhi.

Kaplan, M. L., Goldberg, B. A., Wingard, D. L. & Ellison, R. C. (1998). Cholesterol lowering by oat bran with varying fiber content in hypercholesterolemic men. *American Journal of Clinical Nutrition, 67*(4), 577-583.

Karimi, G., Jamaluddin, R., Mohtarrudin, N., Ahmad, Z., Khazaai, H. & Parvaneh, M. (2017). Single-species versus dual-species probiotic supplementation as an emerging therapeutic strategy for obesity. *Nutrition, Metabolism, and Cardiovascular Diseases, 27*(10), 910-918.

Kathiriya, M. R., Hati, S., Prajapati, J. B. &Vekariya, Y. V. (2018). Assessment of in vitro probiotic potential of lactic acid bacteria. *Journal of Dairy Science and Technology, 5*(1), 17-30.

Kathiriya, M., Sreeja, V., Hati, S. & Prajapati, J. B. (2015). Evaluation of probiotic potential of lactic acid bacteria isolated from traditional fermented milks by in vitro study. *International Journal of Fermented Foods, 4*(1-2), 61-72.

Kwun, S. Y., Bae, Y. W., Yoon, J. A., Park, E. H. & Kim, M. D. (2020). Isolation of acid-tolerant lactic acid bacteria and evaluation of α-glucosidase inhibitory activity. *Food Science and Biotechnology, 29*(8), 1125-1130.

Li, C., Kwok, L. Y., Mi, Z., Bala, J., Xue, J., Yang, J., Ma, Y., Zhang, H. & Chen, Y. (2017). Characterization of the angiotensin-converting enzyme inhibitory activity of fermented milks produced with *Lactobacillus casei*. *Journal of Dairy Science, 100*(12), 9495-9507.

Li, Q., Liu, X., Dong, M., Zhou, J. & Wang, Y. (2015). Aggregation and adhesion abilities of 18 lactic acid bacteria strains isolated from traditional fermented food. *International Journal of Agricultural Policy and Research, 3*(2), 84-92.

Liong, M. T. & Shah, N. P. (2005). Acid and bile tolerance and cholesterol removal ability of lactobacilli strains. *Journal of Dairy Science, 88*(1), 55-66.

Maqsood, M., Ahmed, D., Atique, I. & Malik, W. (2017). Lipase inhibitory activity of *Lagenaria siceraria* fruit as a strategy to treat obesity. *Asian Pacific Journal of Tropical Medicine, 10*(3), 305-310.

Menconi, A., Kallapura, G., Latorre, J. D., Morgan, M. J., Pumford, N. R., Hargis, B. M. & Tellez, G. (2014). Identification and characterization of lactic acid bacteria in a commercial probiotic culture. *Bioscience, Biotechnology, and Biochemistry, 33*(1), 25-30.

Mudgil, P., Mustafa, M. K. Y. N., Kaabi, A. M., Ketbi, A. M. & Abubaid, A. (2016). Pancreatic lipase inhibitory activities of lactic acid bacteria isolated from raw camel milk. *Conference Proceedings of IPC2016*. Paper presented at the International Scientific Conference on Probiotics and Prebiotics, Budapest. (49)

Nagyzbekkyzy, E., Abitayeva, G., Anuarbekova, S., Shaikhina, D., Li, K., Shaikhin, S. & Marotta, F. (2016). Investigation of acid and bile tolerance, antimicrobial activity, and antibiotic resistance of Lactobacillus strains isolated from Kazakh dairy foods. *Asian Journal of Applied Sciences, 9*(4), 143-158.

Oniszczuk, A., Oniszczuk, T., Gancarz, M. & Szymańska, J. (2021). Role of gut microbiota, probiotics, and prebiotics in cardiovascular diseases. *Molecules, 26*(4), 1172.

Park, S. Y., Cho, S. A., Kim, S. H. & Lim, S. D. (2014). Physiological characteristics and anti-obesity effect of Lactobacillus plantarum Q180 isolated from feces. *Korean Journal of Food Science and Animal Resources, 34*(5), 647-652.

Parmar, H., Hati, S., Panchal, G. & Sakure, A. A. (2020). Purification and production of novel Angiotensin I-Converting Enzyme (ACE) inhibitory bioactive peptides derived from fermented goat milk. *International Journal of Peptide Research and Therapy, 26*(2), 997-1011.

Pino, A., Bartolo, E., Caggia, C., Cianci, A. & Randazzo, C. L. (2019). Detection of vaginal lactobacilli as probiotic candidates. *Scientific Reports, 9*(1), 3355-3358.

Rani, V., Deep, G., Singh, R. K., Palle, K. & Yadav, U. C. (2016). Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sciences, 148*, 183-193.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9-10), 1231-1237.

Sadeghzadeh, J., Vakili, A., Sameni, H. R., Shadnoush, M., Bandegi, A. R. & Khorasani, M. Z. (2017). The effect of oral consumption of probiotics in prevention of heart injury in a rat myocardial infarction model: A histopathological, hemodynamic, and biochemical evaluation. *Iranian Biomedical Journal, 21*(3), 174-181.

Satheesh Kumar, A., Manikgantan E. & Ravichandran, M. (2023). *In Vitro* Evaluation of anti cancer and antioxidant activities of siddha polyherbal formulation – Maramanjal Chooranam. *Biological Forum – An International Journal, 15*(3a), 34-39.

Shokryazdan, P., Sieo, C. C., Kalavathy, R., Liang, J. B., Alitheen, N. B., Jahromi, F. M. & Ho, Y. W. (2014). Probiotic potential of *Lactobacillus* strains with antimicrobial activity against some human pathogenic strains. *Biomed Research International*.

Staley, C., Weingarden, A. R., Khoruts, A. & Sadowsky, M. J. (2017). Interaction of gut microbiota with bile acid metabolism and its influence on disease states. *Applied Microbiology and Biotechnology, 101*(1), 47-64.

Steel, R. G. D., & Torrie, J. H. (1980). *Principles and procedures of statistics: A biometrical approach* (2nd ed.). McGraw-Hill Kogakusha Ltd.

Tomaro-Duchesneau, C., Jones, M. L., Shah, D., Jain, P., Saha, S. & Prakash, S. (2014). Cholesterol assimilation by Lactobacillus probiotic bacteria: An in vitro investigation. *Biomedical Research International, 2014*, 380316.

Tsao, C. W., Aday, A. W., Almarzooq, Z. I., Anderson, C. A. M., Arora, P., Avery, C. L., Baker-Smith, C. M., Beaton, A. Z., Boehme, A. K., Buxton, A. E., Commodore-Mensah, Y., Elkind, M. S. V., Evenson, K. R., Eze-Nliam, C., Fugar, S., Generoso, G., Heard, D. G., Hiremath, S. & Ho, J. E. (2023). Heart disease and stroke statistics-2023 update: A report from the American Heart Association. *Circulation, 147*(8), 93-621.

Tuo, Y., Yu, H., Ai, L., Wu, Z., Guo, B. & Chen, W. (2013). Aggregation and adhesion properties of 22 Lactobacillus strains. *Journal of Dairy Science*, *96*(7), 4252-4257.

Upadrasta, A. & Sudha, R. (2016). Probiotics and blood pressure: Current insights. *Integrative Blood Pressure Control, 9*, 33-42.

Wang, H. & Li, L. (2022). Comprehensive evaluation of probiotic property, hypoglycemic ability, and antioxidant activity of lactic acid bacteria. *Foods*, *11*, 1363.

Wang, Y., He, L., Liu, Y. & Wang, F. (2021). Effects of *Lactobacillus plantarum* B1449 on cholesterol concentration in hypercholesterolemic rats. *Frontiers in Microbiology, 12*, 704603.

Wu, H. & Chiou, J. (2021). Potential benefits of probiotics and prebiotics for coronary heart disease and stroke. *Nutrients*, *13*(8), 2878.

Zhao, X., Zhong, X., Liu, X., Wang, X. & Gao, X. (2021). Therapeutic and improving function of lactobacilli in the prevention and treatment of cardiovascular-related diseases: A novel perspective from gut microbiota. *Frontiers in Nutrition, 8*, 693412.

How to cite this article: Heena Parmar, Urmila Parmar, Sreeja V., Saroj Chaudhary and Kiran S. (2025). Cardioprotective Potential of Lactic Acid Bacteria: An *in vitro* screening Study. *Biological Forum – An International Journal, 17*(1): 14-26.