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Research Article

PROBIOTIC PROPERTY OF *Lactobacillus* spp ISOLATED FROM DIFFERENT FOOD SAMPLES

Swesha Adhikari[1], Sushma Adhikari[1], Chandra Bohara[1], Sushan Adhikari[1], Surendra Neupane[1], Om Prakash Pant[2], Sarita Manandhar[1,*]

[1]Tri-Chandra Multiple Campus, Institute of Science and Technology, Tribhuvan University, Kathmandu, Nepal
[2]Central Campus of Technology, Institute of Science and Technology, Tribhuvan University, Dharan, Nepal
**Correspondence: sarita.manandhar@trc.tu.edu.np*

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ABSTRACT

Lactic acid bacteria (LAB) are Gram-positive, non-sporing cocci or rod-shaped bacteria that produce lactic acid as a primary metabolic product and are recognized for their probiotic potential. This study aimed to isolate, screen, and identify LAB from 20 indigenous fermented food samples, including fermented leafy vegetable (*Gundruk*), yogurt (*Dahi*), bamboo shoot (*Tama*), pickle (*Achar*), and fresh cabbage. A total of 15 LAB isolates were identified based on Gram staining and biochemical tests. Their probiotic properties were evaluated through resistance to gastric pH 3.5 (9 isolates survived for 90 min), bile salt tolerance at 0.3% concentration (4 isolates exhibited strong growth), and NaCl tolerance at 2% and 4% concentrations (all isolates survived, but none at 6.5%). Antibiotic susceptibility testing revealed that all isolates were sensitive to ampicillin, gentamicin, erythromycin, ciprofloxacin, and oxacillin. Antibacterial activity assays demonstrated that LAB strains inhibited pathogenic bacteria, with inhibition zones ranging from 10 to 20 mm. Notably, two LAB isolates exhibited the strongest probiotic properties based on acid, bile, and NaCl tolerance, as well as antibacterial activity. These findings highlight the potential of indigenous fermented foods as a natural source of probiotic LAB strains.

Key words: Fermented foods, lactic acid bacteria, pathogenic bacteria, probiotic

INTRODUCTION

Lactic acid bacteria (LAB) are a heterogenous group of bacteria which plays a significant role in a variety of fermentation processes. Lactobacilli is the largest genus within the group of lactic acid bacteria that are Gram-positive, facultative anaerobic or microaerophilic, catalase-negative, oxidase-negative, non-spore-forming, rod shaped and are natural inhabitants of the human gastrointestinal tract. They have an important role in maintaining the microbial ecosystem of the colon (Kirtzalidou *et al.*, 2011). They are assumed to be extremely advantageous non-pathogenic species for the human population, currently being assessed as possible probiotic microorganisms (Fijan, 2014). In the food industry, LAB is widely used as starters to achieve favorable changes in texture, aroma, flavor and acidity (Leroy & De Vuyst, 2004).

Probiotics are defined as "live microorganism which when administered in adequate amount confers a health benefit to the host" (Reuben *et al.*, 2019). Probiotics are selected viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human through their effects in the intestinal tract (Grajek *et al.*, 2005). They are non-pathogenic organisms (yeast or bacteria, especially lactic acid bacteria) in food that can exert a list of positive influences on the health of the host (Brown & Valiere, 2004). Those positive influences on the host can be described based on their health benefits, antimicrobial activity, improvement in lactose metabolism, reduction in serum cholesterol, immune system stimulation, anti-mutagenic properties, anti-carcinogenic properties, anti-diarrheal properties, improvement in inflammatory bowel disease and suppression of *Helicobacter pylori* infection by the addition of selected strains to food product (Fijan, 2014). The most important properties of strains to be considered as probiotic include non-pathogenic and non-toxic to host, resistance to gastric acidity, bile acid resistance, adherence to mucus or epithelial cell and cell lines. They should also offer certain health benefits like antimicrobial activities against potentially pathogenic microorganisms, toxin reducing effects and boosting immune response (Somashekaraiah *et al.*, 2019). The most used organism in probiotic products is *Lactobacillus* spp., *Bifidobacterium* spp., *Streptococcus* spp., *Enterococcus* spp., *Lactococcus* spp., *Leuconostoc* spp. etc. Besides these other genera like *Bacillus* spp., *Escherichia* spp. and yeast like *Saccharomyces* spp. have been reported to have probiotic potential (Fuller, 2012; Medina *et al.*, 2007).

LAB is the most commonly used probiotics, with an important role in the protection of the host against harmful microorganisms and also strengthens the immune system in human and animal. These can be recovered naturally from the fermented foods and beverages, vegetables, milk and milk products. They have ability of the bio preservation of the food and can be used as starter culture in the fermentation process under controlled conditions. Therefore, the isolation and characterization of LAB from different traditional fermented foods and products have gained research

interest in recent years (Alonso García *et al*., 2021). LAB are the most important bacteria in desirable food fermentation, being responsible for the fermentation of fermented food, vegetables, milk and beverages. The LAB strains can be used in the formulation of fermented foods with functional characteristics to manage growth of adverse pathogenic microbes, which would help in the prevention and treatment of diseases in consumers (Batista *et al*., 2017).

Nepalese fermented foods, such as *Gundruk*, *Kinema*, *Dahi*, and *Tama*, are rich sources of lactic acid bacteria (LAB), particularly *Lactobacillus* spp., which exhibit probiotic potential. These foods undergo natural fermentation, promoting the growth of beneficial microbes that enhance gut health, improve digestion, and inhibit pathogens. Compared to fermented foods from other countries, such as *Kimchi* (Korea), *Sauerkraut* (Germany), and *Natto* (Japan), Nepalese fermented foods share similarities in microbial composition, primarily dominated by LAB and *Bacillus* spp. However, differences in raw ingredients, fermentation conditions, and traditional processing methods contribute to variations in microbial diversity and nutritional profiles. While studies on the probiotic properties of Nepalese fermented foods are limited, their unique microbial communities suggest significant potential for health benefits, comparable to well-studied probiotic-rich foods worldwide (Bhattarai *et al*., 2016). The purpose of this study is to isolate and evaluate the probiotic potential of *Lactobacillus* spp. from 20 different fermented food samples in Nepal, aiming to identify strains with beneficial properties for human health. Nepalese fermented foods are naturally rich in lactic acid bacteria (LAB), yet their probiotic potential remains largely unexplored. By focusing on *Lactobacillus* spp., which are widely recognized for their probiotic benefits, this study seeks to assess their acid and bile tolerance, antimicrobial activity, and antibiotic susceptibility. Further research is needed to fully characterize their probiotic strains and functional properties.

MATERIALS AND METHODS

Isolation and Identification of LAB

A total of 20 different food samples [four samples each of fermented leafy vegetables (*Gundruk*), yoghurt (*Dahi*), bamboo shoot (*Tama*), pickle (*Achar*) and fresh cabbage] were collected from the local market of Kathmandu for isolation of lactic acid bacteria. The selected food samples: *Gundruk*, *Dahi*, *Tama*, *Achar*, and fresh cabbage, were chosen due to their traditional fermentation methods, cultural significance, and widespread consumption in Nepal. These foods naturally undergo lactic acid fermentation, fostering the growth of *Lactobacillus* spp., which are key probiotics. Their deep-rooted presence in Nepalese diets and their role in nutrition and food preservation make them ideal for studying indigenous probiotic strains with potential health benefits.

Pour plate method with 10-fold dilutions in de Man, Rogosa and Sharpe (MRS) broth (Hi-Media, Mumbai, India) was used to isolate the lactic acid bacteria (LAB). The morphologically discrete colonies (white/creamy, oval/round shaped and having soft smooth consistency forming clear zone) were further sub-cultured onto MRS agar incorporated with 1% $CaCO_3$ plates.

Fifteen individual colonies showing clear zone in MRS agar media were preliminarily screened as LAB. The pure isolates were identified as LAB based on their phenotypic and biochemical characteristics that included Gram-staining, catalase test, oxidase test, indole test, nitrate test, TSIA test, MR-VP test and sugar fermentation ability (Boone *et al*., 2001).

Antagonistic Activity of LAB

The antibacterial activity of lactobacilli isolates was determined by agar overlay and agar well diffusion method. The test organisms included common human pathogens isolated from different clinical specimens; *Salmonella enterica* serovar Typhi isolated from blood, *Escherichia coli* from urine, *Klebsiella pneumoniae* from pus and *Staphylococcus aureus* from pus.

Agar Overlay Method

The fresh culture of lactobacilli was prepared by inoculating pure culture to MRS broth and incubated at 37°C for 48 h. A loop-full (≈10^5 CFU/spot) of culture were spot inoculated onto the MRS agar plates and incubated at 37^{0}C for 48 h. The spots were then overlaid with a soft Mueller Hinton Agar (MHA with 0.8% agar) seeded with 10^8 cfu/ml test organism and after solidification incubated at 37 ^{0}C for 24 h.

The zone of inhibition (ZOI) >20 mm, 10–20 mm and <10 mm was considered as strong, intermediate and weak inhibitions, respectively (Ait Chait *et al*., 2021; Shokryazdan *et al*., 2014). All tests were performed in triplicate.

Agar Well Diffusion Method

Fresh culture of test organism was prepared on nutrient broth (0.5 McFarland standard) and carpet cultured on surface of MHA media and wells (6 mm diameter) were prepared using sterile cork borer. The wells were filled with culture filtrates (75 µL/well) of the isolated lactobacilli. Following 24 h incubation at 37◦C, ZOI values nearest the well were recorded, and interpreted as less active, moderately active and highly active with ZOIs ≤10 mm, 11–14 mm, and ≥15 mm, respectively (Shokryazdan *et al*., 2014; Ait Chait *et al*., 2021). The tests were performed in triplicate.

Screening for Probiotic Property

Acid tolerance test

The acid tolerance test was assayed as described earlier with slight modification (Ramos *et al*., 2013). Each test isolate was grown at 37°C for 24 h in GYP (Glucose yeast extract peptone) broth and cells were harvested by centrifugation at 5800 rpm for 10 minutes. Centrifuged cells were washed once with sterile saline solution. Harvested cells were resuspended in acidified GYP

broth, one pH 3.5 and other pH 7.4 respectively and incubated at 37°C. After time interval of 0 and 90 minutes, samples were taken and serially diluted in phosphate buffered saline (PBS). Viable counts were made at 0 and 90 minutes from both sets of tubes by pour plating technique into GYP agar and incubated at 37°C for up to 48 hours. Cell viability was assessed by the plate count method and the results were expressed as log cfu/ml. The assay was performed in triplicate for each isolate.

Bile tolerance test

The bile tolerance test of the isolate was assayed as described by Ramos *et al.* (2013) with slight modification. Overnight culture of each LAB strain, adjusted to a final concentration of 7 to 8 log CFU/mL, was inoculated (1%, v/v) into 10 mL of fresh GYP broth containing 0.3%, 0.5% and 0.15% of bile salts or without (control) 0.3% (w/v) and incubated at 37°C for 24 h with shaking (100 rpm). Bile tolerance was estimated by comparing growth in GYP broth with and without bile (oxgall).

Sodium chloride tolerance test

The sodium chloride tolerance test was performed using the protocol of Chowdhury *et al.* (2012), with slight modifications. Briefly, the isolated lactobacilli were grown (for 24 h at 37°C) in MRS broth with sodium chloride supplementation of 2%, 4% and 6.5%, and thereafter the growth of lactobacilli, following subculture of the MRS broth cultures, on MRS agar (for 24 h at 37°C), indicated their tolerance to sodium chloride.

Safety Profiling

Antibiotic Susceptibility Test

The antibiotic susceptibility of the LAB isolates was assessed on MHA agar plates by disc diffusion method using various antibiotics as per CLSI (Clinical and Laboratory Standards Institute) guidelines (CLSI, 2016). The antibiotics used were ampicillin (10µg), gentamicin (30µg), erythromycin (15µg), ciprofloxacin (5µg) and oxacillin (1µg). Freshly prepared Lactobacilli culture (0.5 McFarland standards) was spread on MHA plates, selected antibiotic discs were placed and incubated at 37°C for 48 hours. The results were expressed as susceptible or resistant by comparing the diameter of zone of inhibition produced by lactobacilli around antibiotic disc (CLSI, 2016).

Hemolytic Activity of LAB

The hemolytic activity of the LAB isolates was determined by streaking the overnight grown MRS broth culture of the lactobacilli strains on blood agar plate (Hi-Media, India). The plates were observed for the presence of hemolysis around the lactobacilli colonies after incubation at 37^{0}C for 72 h (Yadav *et al.*, 2016).

RESULTS

A total of 30 bacterial colonies that showed white, round, edge, centrally slightly raised, with thick and opaque, smooth surface in the MRS culture medium were subjected for various physiological and biochemical tests for identification of LAB. Out of this, 15 isolates that were Gram-positive with long rod arranged in clusters or chains, non-spore forming, non-motile, non-hemolytic and negative test result to catalase test and oxidase test were preliminarily identified as LAB isolates.

The isolates were subjected to a series of physiological and biochemical tests to confirm *Lactobacillus plantarum* and *Lactobacillus* spp. All LAB isolates were able to ferment glucose without CO_2 hence considered as homolactic and identified as homo-fermentative (Table 1).

Table 1. Sugar fermentation pattern of Lactic acid bacteria

LAB isolates	Gl	Ga	La	Ra	Xy	Fu	Su	Ml	Ar	Remark
G_1	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_2	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_3	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_4	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_5	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_7	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_8	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_9	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_{10}	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_{11}	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_{12}	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_{13}	+	+	+	+	+	+	+	+	+	*L. plantarum*
G_{14}	+	+	+	+	+	+	+	+	+	*L. plantarum*
AC	+	+	+	+	+	+	-	-	-	*Lactobacillus* spp.
AA	-	-	+	+	-	+	-	-	-	*Lactobacillus* spp.

Gl=Glucose, Ga=Galactose, La=Lactose, R=Raffinose, Xy=Xylose, Fu=Fructose, Su=Sucrose, Ar=Arabinose, Ml=Maltose, G_1-G_{14}= isolates from sample *Gundruk,* AC= isolates from sample Cabbage, AA= isolates from sample *Achar*

All LAB isolated from *Gundruk, Achar* and fresh cabbage were sensitive to all five antibiotics tested (Table 2). Some LAB isolated from *Gundruk* showed moderate sensitivity towards ciprofloxacin and ampicillin.

Table 2. Antibiotic susceptibility test of bacteria

Isolates	Antibiotics				
	CIP	**E**	**GEN**	**OX**	**AMP**
G_1	MS	S	S	S	S
G_2	MS	S	S	S	S
G_3	MS	S	S	S	MS
G_4	S	S	S	S	S
G_5	S	S	S	S	S
G_7	S	S	S	S	S
G_8	S	S	S	S	S
G_9	S	S	S	S	S
G_{10}	MS	S	S	S	S
G_{11}	MS	S	S	S	S
G_{12}	S	S	S	S	S
G_{13}	S	S	S	S	S
G_{14}	S	S	S	S	S
AC	S	S	S	S	S
AA	S	S	S	S	S

MS- moderately sensitive, S- Sensitive, G_1-G_{14}= isolates from sample *Gundruk*, AC- isolates from sample Cabbage, AA- isolates from sample *Achar*

Table 3. Tolerance of Lactobacillus to pH 3.5 and pH 7

Sample Code	Dilution	Growth at pH 3.5		Growth at pH 7.4	
		T0 (min)	T90 (min)	T0 (min)	T90 (min)
G_1	10^6	+	+	+	w
G_2	10^6	+	+	+	+
G_3	10^6	+	-	+	w
G_4	10^6	+	-	+	w
G_5	10^6	+	-	+	+
G_7	10^6	+	+	+	+
G_8	10^6	+	+	+	+
G_9	10^6	+	+	+	+
G_{10}	10^6	+	+	+	+
G_{11}	10^6	+	+	+	+
G_{12}	10^6	+	+	+	+
G_{13}	10^6	+	+	+	+
G_{14}	10^6	+	-	+	w
AC	10^6	+	-	+	w
AA	10^6	+	-	+	w

+ = resistant/tolerant, - = sensitive/non tolerant, w= weakly tolerant, AA- isolates from sample *Achar*, AC- isolates from sample Cabbage, G- isolates from sample *Gundruk*

Table 4. Tolerance of Lactic Acid Bacteria to different concentration of bile salt

Sample Code	0.15%	0.3%	0.5%
G_1	-	-	-
G_2	+	w	-
G_3	-	-	-
G_4	-	-	-
G_5	-	-	-
G_7	-	+	+
G_8	+	w	-
G_9	+	+	+
G_{10}	-	-	-
G_{11}	+	+	+
G_{12}	+	+	+
G_{13}	+	w	-
G_{14}	-	-	-
AC	-	-	-
AA	-	-	-

+ = resistant/tolerant, - = sensitive/non tolerant, w= weakly tolerant, AA- isolates from sample *Achar*, AC- isolates from sample Cabbage, G- isolates from sample *Gundruk*

To better evaluate the probiotic potential of the LAB isolates, several characteristics were tested. Among 15 isolates, nine LAB isolated from different samples were able to survive at pH 3.5 for 90 minutes to be identified as "acid tolerant" (Table 3). These isolates were further tested for bile salt tolerance with bile concentration of

0.3%, 0.5% and 0.15%. In the present study, four lactic acid bacteria were considered as "bile tolerant" being able to grow in bile salt concentration 0.3% while three showed moderate tolerance (Table 4).

The tolerance of LAB isolates to NaCl was determined by using MRS broth containing 2%, 4% and 6% of NaCl concentration. All isolates of *Gundruk* showed growth at 2% and 4% of NaCl concentration. None of the isolates showed growth at 6% NaCl concentration (Table 5).

Table 5. Tolerance of Lactic Acid Bacteria to different concentration of NaCl

Sample code	NaCl concentration		
	2%	4%	6.5%
G_1	+	+	-
G_2	+	+	-
G_3	+	+	-
G_4	+	w	-
G_5	+	+	-
G_7	+	+	-
G_8	+	+	-
G_9	+	+	-
G_{10}	+	+	-
G_{11}	+	+	-
G_{12}	+	w	-
G_{13}	+	w	-
G_{14}	+	+	-
AC	+	-	-
AA	+	-	-

+ = resistant/tolerant, - = sensitive/non tolerant, w= weakly tolerant, AA- isolates from sample *Achar*, AC- isolates from sample Cabbage, G- isolates from sample *Gundruk*

In this study, antimicrobial activity of the isolates was analyzed against four indicator human bacterial pathogens (*Escherichia coli, Salmonella* Typhi*, Klebsiella pneumoniae, Staphylococcus aureus*) using agar overlay and agar well diffusion methods. The antagonist activity of the LAB isolates against selected human pathogens was demonstrated by the zone of inhibition produced. Results showed that all LAB isolated from samples had inhibitory effect against selected pathogens, but inhibitory effect of G_5, G_9, G_{13} to *Escherichia coli*, G_2, G_7 to *Klebsiella pneumoniae*, G_7, G_{11}, G_{12} to *Staphylococcus aureus* and G_3, G_{11} to *Salmonella* Typhi were more considerable than others (Table 6).

Table 6. Antagonistic test against selected pathogenic organisms

Sample code	Test organism			
	Escherichia coli	***Klebsiella pneumoniae***	***Staphylococcus aureus***	***Salmonella* Typhi**
G_1	+	++	+	+
G_2	++	+++	+	+
G_3	+	+	++	+++
G_4	+	+	++	+
G_5	+++	++	+	++
G_7	++	+++	+++	++
G_8	+	+	+	+
G_9	+++	+	++	++
G_{10}	++	+	++	+
G_{11}	++	++	+++	+++
G_{12}	+	++	+++	++
G_{13}	+++	+	+	++
G_{14}	++	+	+++	+
AC	+	++	+	+
AA	+	+	+	+

+ = weak inhibition (≤10 mm), ++ = strong inhibition with detectable clear zone (11mm -14mm), +++ = very strong inhibition with large, clear zone (≥15mm)

DISCUSSION

Lactic acid bacteria (LAB) are heterogenous groups of bacteria playing a significant role in a variety of fermentation processes with a well-known probiotic property. They create acidic condition by converting carbohydrates to organic acids producing preservative effects. Thus, most of the fermented foods are rich sources of LAB. Lactic acid bacteria are known by their ability to ferment carbohydrate. Isolates from *gundruk* fermented all nine different types of carbohydrates tested and were identified as homo fermentative *Lactobacillus plantarum*. These findings align with previous research on probiotic *Lactobacillus* strains isolated from different fermented foods across the world.

The study identified *Lactobacillus plantarum* as the dominant isolate, which is consistent with previous reports on fermented vegetables and dairy products. For instance, *L. plantarum* has been identified as a major LAB species in Nepalese *Gundruk* (Ghimire *et al*., 2020; Tamang *et al*., 2005) and Chinese pickles (Zeng *et al*., 2020). Similar findings were reported in other fermented vegetable products, including *kimchi* from Korea and *sauerkraut* from Germany (Reuben *et al*., 2019). The ability of these isolates to ferment various carbohydrates supports their classification as homofermentative LAB, a trait commonly associated with *Lactobacillus* strains isolated from different fermented foods worldwide.

All LAB isolates were found to be sensitive to the tested antibiotics. Antibiotic resistance profile of a proposed probiotic should be evaluated to prevent the spread of antibiotic resistant genes via food chain. Several studies reported the sensitivity of natural isolates of lactobacilli (*L. acidophilus, L. brevis, L. fermentum and L. plantarum*) to ampicillin, gentamicin, erythromycin, tetracycline and intrinsic resistance to vancomycin (Fijan, 2014; Halder *et al*., 2017; Georgieva *et al*., 2015; Yadav *et al*., 2016). The antibiotic sensitivity property of lactobacilli helps formulate safe probiotic products for human consumption.

Acid and bile tolerance are crucial attributes of probiotic bacteria, as they must survive harsh gastrointestinal conditions (Hoque *et al*., 2010). In many studies, pH 3 has been considered as a standard pH for investigation of acid tolerance of probiotic strains (Georgieva *et al*., 2015; Halder *et al*., 2017; Hoque *et al*., 2010). Our study found that several *Lactobacillus* isolates exhibited strong resistance to pH 3.5 and bile salt concentration of 0.3%, which aligns with previous studies on LAB from fermented dairy and vegetable products. Similar results have been reported by Shokryazdan *et al.* (2014) and Halder *et al.* (2017), where *Lactobacillus* strains isolated from dairy products and fermented foods survived acidic conditions and bile salt concentrations up to 0.5%.

The ability to tolerate bile salt at a concentration of 0.3% has a physiological significance because it is a level normally encountered in human intestine (Halder *et al*., 2017). In our study, four lactic acid bacteria showed bile tolerance while three showed moderate tolerance. Many studies also demonstrate the ability of Lactobacilli to grow at 0.3% bile salt (Halder *et al*., 2017; Hoque *et al*., 2010; Sahadeva *et al*., 2011). Rahman (2015) reported the growth and survivability of *L. fermentum* and *L. acidophilus* isolates from buffalo milk at the bile salt concentrations of 0.3–0.5%. This study shows growth of lactobacilli in 0.3% bile salt supplement but poor tolerance at 0.2% bile salt concentration which is in agreement with other studies (Hoque *et al*., 2010; Jose *et al*., 2015). These findings reinforce the potential of Nepalese fermented foods as sources of probiotic strains with resilience to gastrointestinal stress.

All isolates of *Gundruk* showed growth at 2% and 4% of NaCl concentration while growth was not observed at 6% NaCl concentration. Forhad *et al.* (2015) also agreed that *Lactobacillus* spp. is able to grow in high concentration of NaCl. The *Lactobacillus* strains isolated from river buffalo milk cheese showed viability in presence of NaCl (1–7%), indicating their high sodium chloride tolerance (Rahman, 2015). The salt tolerance gives lactobacilli an advantage of initiating lactic acid fermentation for the production of acid as salt inhibits the growth of non-desirable organisms.

Antimicrobial activity against pathogens is another important attribute to be considered in the selection of potential probiotic strains for maintaining a healthy microbial balance in the gut. All LAB isolated from samples showed inhibitory effect against selected pathogens. Manzoor *et al.* (2016) reported broad antibacterial spectrum by *Lactobacillus* isolated from fermented fruits and vegetables against food-borne bacterial pathogens. The probiotic *Lactobacillus* strains: *L. fermentum, L. casei* and *L. acidophilus*, isolated from buffalo milk showed growth inhibitory activity against *Vibrio cholerae, S*. Typhi, *E. coli* and *Shigella* species (Rahman, 2015). But the study of Jose *et al.* (2015) showed that lactobacilli strains, including *L. rhamnosus* and *L. plantarum*, procured from dairy food products (commercially available yoghurt and cheese) and rumen contents of cow did not show growth inhibitory activity against *E. coli*, while *Salmonella menston* was found sensitive to all the test lactobacilli. Thus, the antagonism of pathogenic bacteria with lactobacilli is strain/isolate as well as pathogen (target bacteria) specific.

Antimicrobial activity is another key characteristic of probiotic LAB, as they can inhibit the growth of pathogenic bacteria. The isolates in this study demonstrated antagonistic activity against *E. coli, Salmonella Typhi, Klebsiella pneumoniae,* and *Staphylococcus aureus*, similar to previous findings. Manzoor *et al.* (2016) reported broad-spectrum antibacterial effects of *Lactobacillus* isolates from fermented fruits and vegetables against foodborne pathogens. Likewise, Rahman (2015) observed strong inhibitory effects of *L. fermentum, L. casei,* and *L. acidophilus* against *Vibrio cholerae*, *S. Typhi*, *E. coli*, and *Shigella* spp. The production of organic acids, hydrogen peroxide, and bacteriocins likely contributed to the observed antimicrobial effects, as noted in prior studies (Adikari *et al*., 2021; Shokryazdan *et al*., 2014).

The antagonistic effects exerted by LAB are mainly due to the production of organic acid such as lactic acid, acetic acid and other compounds such as ethanol, formic acid, hydrogen peroxide, diacetyl, reuterin, reutericyclin and bacteriocin or related compounds. These compounds together with the mechanism of competition, in which probiotic strains compete with pathogens for nutrients and attachment sites, would prevent colonization of the intestine by pathogens (Shokryazdan *et al*., 2014; Adikari *et al*., 2021).

Additionally, none of the LAB isolates in this study exhibited hemolytic activity, reinforcing their safety as potential probiotics. This aligns with findings by

Kaktcham *et al.* (2012), who reported the absence of hemolysis among LAB isolates from dairy products. The isolates also displayed sensitivity to commonly used antibiotics such as ampicillin, erythromycin, and gentamicin, reducing concerns about antibiotic resistance transmission through the food chain, which has been emphasized in previous research (Halder *et al.*, 2017; Georgieva *et al.*, 2015).

Some limitation of the study was that the probiotic characterization did not include adherence assays to intestinal epithelial cells, which is crucial for colonization. Identification of LAB was based on biochemical and physiological tests, lacking molecular confirmation through 16S rRNA sequencing. Additionally, the sample size was relatively small, which may not fully capture the diversity of LAB in indigenous fermented foods.

CONCLUSIONS

The increasing demand for probiotic-rich foods highlights the significance of lactic acid bacteria (LAB) as natural probiotics with potential health benefits. This study isolated and identified LAB from indigenous fermented food products, demonstrating their probiotic properties through acid and bile tolerance, antibiotic susceptibility, and antagonistic activity against pathogenic bacteria. The findings confirm that certain LAB strains, particularly *Lactobacillus plantarum*, exhibit strong probiotic potential, reinforcing their suitability for use in functional food applications. Nevertheless, further exploration may be performed to confirm their potential health benefits and applications.

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AUTHOR CONTRIBUTIONS

Sarita Manandhar and Om Prakash Pant conceived the study. Swesha Adhikari, Sushma Adhikari; Formal analysis and investigation: Swesha Adhikari, Chandra Bohora, Susan Bhandari, Surendra Neupane and Chandra Bohora carried out experiments and analyzed data. Sarita Manandhar, Om Prakash Pant and Swesha Adhikari drafted the manuscript, and all authors contributed to preparing the final version of the manuscript. All authors read and approved of the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interests.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author, upon reasonable request.

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