Nepal J Biotechnol. 2024 Jul; 12 (1):58-66 Research article DOI: https://doi.org/10.54796/njb.v12i1.321

Nepal Journal of Biotechnology
Publisher: Biotechnology Society of Nepal ISSN (Online): 2467-9313
Journal Homepage: https://nepjb.com/index.php/NJB ISSN (Print): 2091-1130

Functional Attributes of Gut Guardians: Probiotic Potential of *Lactobacillus* strains isolated from Traditional Nepalese Fermented Foods

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Received: 19 May 2024; Revised: 11 Jul 2024; Accepted: 22 Jul 2024; Published online: 31 Jul 2024

Abstract
The interplay between the gut microbiome and fermented foods has significant implications for human health and nutrition. Fermented foods, rich in beneficial microorganisms such as Lactic Acid Bacteria (LAB) including *Lactobacillus* spp., have attracted considerable research interest due to their potential probiotic effects. The six Nepalese fermented foods selected for this research—homemade yogurt, commercial yogurt, *gundruk*, *tama*, *chhurpi*, and *achaar*—are recognized for their diverse microbial profiles and purported health benefits, commonly consumed across various regions of Nepal. This study aims to elucidate their probiotic potential through tests such as acid and salt tolerance and their antimicrobial effects against five pathogens (*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella* spp.). A total of 120 samples of these six different fermented foods were collected from households and local markets in Chitwan District. Among 120 fermented food samples, 103 samples (85.83%) were found to be LAB positive. Out of the total sample, 64 samples (53.30%) were found to be *Lactobacillus* spp. positive. These positive isolates were further tested for their pH tolerance, NaCl tolerance, and antibacterial potential. Upon research, 18 isolates were found to be tolerant at pH 2, and 21 isolates were found to be tolerant at 8% NaCl concentration. The highest antibacterial effect of *Lactobacillus* spp. was found against *Staphylococcus aureus* with ZOI 18 mm and lowest against *Escherichia coli*. The study here contributes to the importance of utilizing the microbial diversity present in fermented foods, along with providing valuable insights for developing novel probiotic interventions for enhancing gut and overall health. Moreover, the research highlights the potential of traditional fermented foods as a valuable source of probiotic strains, paving the way for innovative approaches to improve health through diet.

Keywords: Probiotic, Lactic Acid Bacteria, *Lactobacillus*, Gut Health, Fermented Food

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Introduction
Lactic Acid Bacteria (LAB) refer to a diverse group of bacteria, including both cocci and rods, distinguished by their ability to ferment carbohydrates, resulting in the production of lactic acid. They have been considered of great importance because of their role in food fermentations and are considered safe and have been used in making fermented foods for a long time [1]. LAB are crucial for their ability to enhance the shelf life, safety, and sensory qualities of fermented products. These bacteria are also known to produce vitamins and other bioactive compounds that can enhance the nutritional profile of fermented foods. Today, LAB are known for their probiotic benefits and are added to many foods [2]. Furthermore, LAB have been integral to traditional food processing and preservation methods, contributing significantly to the culinary heritage of various cultures around the world.

The term "probiotic" comes from the Latin word "pro" and the Greek word "bios," meaning "for life" [3]. The idea of probiotics started in the early 20th century from a theory by Nobel Prize-winning Russian scientist Elie Metchnikoff. He suggested that the long and healthy lives of Bulgarian peasants were due to their consumption of fermented milk products [4]. Metchnikoff believed that the fermenting bacteria, when consumed, positively affected the gut's microflora, reducing harmful microbial activities. This early hypothesis paved the way for modern probiotic research, emphasizing the importance of beneficial bacteria in maintaining gut health. Today, probiotics are recognized not only for their gastrointestinal benefits but also for their potential to boost the immune system and reduce the risk of certain infections. Lately, probiotics have been defined more precisely as single or mixed cultures of live microorganisms that benefit the host by improving the natural microflora. While one can find LAB naturally in foods like yogurt and cheese, selecting and testing different LAB strains is important for creating good cultures for research and commercial use. In fact, choosing the right LAB is crucial for making any fermented products with the right texture and flavor [5]. However, people consume probiotic products to get more than just good flavor; they are equally sought after for their health benefits. According to the United Nations Food and Agriculture Organization and the World Health Organization, these live microorganisms, primarily bacteria, when consumed, positively influence the host's health or physiology. LAB produce a substance called exopolysaccharide (EPS), which is important because it adds useful properties to foods and has benefits for people from a health perspective [6, 7]. EPS can improve the texture of food products and also has prebiotic effects, promoting the growth of beneficial gut bacteria. LAB also produce bacteriocins, which can kill harmful bacteria. Bacteriocins produced by LAB exert broad spectra of inhibitory activity among bacteria, and their proteinaceous nature implies their degradation in the gastrointestinal tracts of humans and animals without harming the gut as antibiotics do [8].

Coming to *Lactobacillus*, these bacterial species belong to the Lactobacillaceae family. *Lactobacilli* are Gram-positive rods or coccobacilli and can sometimes form short chains [9]. They naturally inhabit the human mouth, gastrointestinal tract, and female genital tract as components of the normal flora [10]. These fermentative organisms are chemo-organotrophic, microaerophilic, non-spore-forming, and non-motile [11]. *Lactobacilli* play a crucial role in maintaining the balance of the gut microbiota, which is essential for overall health. They are also involved in the production of various vitamins, including B-group vitamins and vitamin K, contributing to the nutritional value of the diet. For *Lactobacillus* strains to provide the anticipated probiotic benefits, they must meet specific criteria. They need to survive in the gastrointestinal tract and tolerate low pH levels and bile acids like glycocholic or taurocholic acid and sodium deoxycholate [12]. They also need to adhere to the intestinal mucus and epithelial cells. This is important because *Lactobacillus* bacteria can produce antimicrobial substances like lactic acid and bacteriocins and adhere to mucus to form a barrier that blocks harmful pathogens. Recent studies have also suggested that *Lactobacillus* strains can play a role in preventing and managing conditions such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), and even some allergic reactions [13].

It is evident that *Lactobacillus* and other LAB are well known for their use in dairy products as well as in the fermentation of sauerkraut, pickles, sausage, and breads, and also, in the malolactic fermentation of wine [14]. Fermentation can be defined as anaerobic biochemical reactions in which an enzyme produced by microorganisms catalyzes the conversion of one substance into another, especially the conversion of sugars to any form of acid, such as lactic acid. The food produced by this process is called fermented foods [15]. The fermentation process was likely developed to preserve fruits and vegetables during times of shortage by using organic acids and alcohols to extend shelf life, enhance flavor and texture, reduce toxicity, and shorten cooking times. Today, it has become a crucial dietary part of the global population [16]. The health benefits of fermented foods, including improved digestion, enhanced nutrient absorption, and the presence of beneficial bioactive compounds, have contributed to their popularity worldwide. Fruits and vegetables are widely used for fermentation by lactic acid bacteria as they provide a suitable environment due to their nutrients like vitamins and minerals, and their acidic nature. The main lactic acid bacteria (LAB) involved in these ethnic fermented processes include *Lactobacillus brevis*, *L. plantarum*, *Pediococcus pentosaceus*, *P. acidilactici*, and *Leuconostoc fallax* [17]. Some of these LAB strains have protective and functional properties, making them suitable candidates to use as starter cultures for the controlled and optimized production of fermented vegetable products. The variety of LAB used in different fermentation processes highlights the adaptability and versatility of these bacteria, ensuring the production of diverse and flavorful fermented products [18].

Fermentation offers many benefits, including food security, improved nutrition, and enhanced social well-being for people in marginalized and vulnerable communities [19]. Nepal's topographical and ecological niche variations welcome several ethnic communities, each with its own diverse culture, resulting in various types of fermented foods. Such fermented vegetables are not only consumed as traditional products in Nepal but are also popular in Sikkim and Bhutan. Several traditional Nepalese fermented foods, including *sinki*, *gundruk*, *mesu*, *khalpi*, and pickled tama, are widely celebrated and have been consumed for centuries. In the eastern Himalayan regions of Nepal, different types of fermented vegetables are made to preserve perishable produce for storage and later use [20]. The selected food samples for this research include homemade yogurt, commercial yogurt, *gundruk*, *tama*, *chhurpi*, and *achaar*, each representing a distinct aspect of Nepalese culinary heritage. Homemade and commercial yogurts are renowned for their live cultures, primarily consisting of *Lactobacillus* and *Bifidobacterium* species, which confer various digestive health benefits [21]. *Gundruk*, a fermented leafy green vegetable, and Tama, fermented bamboo shoots, staple foods in Nepal, are rich in diverse microbial populations that contribute to gut health by enhancing digestion and nutrient absorption [22]. Chhurpi, a traditional cheese, and achaar, a type of pickle, both undergo lactic acid fermentation, introducing beneficial probiotics that can outcompete pathogenic bacteria and support the immune system [23]. The rationale behind this study was to find practical screening techniques to identify food sources with probiotic microbes. This approach ultimately aimed to uncover Nepalese traditional fermented foods as potential sources of beneficial bacteria, particularly *Lactobacillus* spp. Understanding the unique probiotic attributes of these foods can shed light on their potential health benefits through the consumption of traditional fermented foods.

Materials and Methods

Study site and Study duration

This study was conducted at microbiology lab of Balkumari College in the period of four months.

Sample Collection and Processing

Dahi (homemade), *Dahi* (commercial yogurt), Tama, *Gundruk*, and *Achaar* were aseptically collected in alcohol-disinfected zip-seal plastic bags. *Chhurpi* was collected as available in commercial plastic package. Samples if not immediately processed were stored at 4°C and processed next day after bringing to room temperature. The respectively samples were processed as mentioned here:

***Dahi* (Commercial and Homemade)**

Each sample of *dahi* weighing twenty-five grams was transferred into a conical flask filled with peptone water.

Gundruk

Gundruk weighing twenty five grams was grinded in alcohol-disinfected mortar and pestle using 5 mL sterile autoclaved water and was then processed for the isolation of *Lactobacillus* spp.

Achaar

Achaar weighing twenty five grams was grinded in alcohol-disinfected mortar and pestle using 5 mL sterile autoclaved water and was then processed for the isolation of *Lactobacillus* spp.

Chhurpi

Chhurpi pieces were soaked in sterile water overnight and cut into smaller pieces (nearly 0.5 cm) by alcohol-disinfected knife. Twenty five grams were processed for isolation of *Lactobacillus* spp.

Tama

Tama weighing twenty five grams was grinded in alcohol-disinfected mortar and pestle using 5 mL sterile autoclaved water and was then processed for the isolation of *Lactobacillus* spp.

Isolation and identification of *Lactobacillus* spp. from fermented food samples

Each sample, weighing twenty-five grams after the respective processing steps mentioned above, was homogenized in 225 mL of peptone water for approximately 20 minutes using a magnetic stirrer. One milliliter of the homogenate was inoculated into MRS (de Man–Rogosa–Sharpe) broth and incubated anaerobically for 24 hours at 37 °C. Loopful samples from the MRS broth were streaked onto plates of MRS agar using the streaking method. The plates were then incubated anaerobically for 48 hours at 37 °C. After incubation, colonies with typical characteristics were randomly selected from the plates and tested for Gram staining, oxidase test, motility test, and catalase test [24].

pH tolerance test of isolated *Lactobacillus* spp.

The gram-positive, acid-producing rods (identified by a clear zone around the bacterial colony) were the test isolates for the pH tolerance test. Strains were grown in MRS broth at 37 °C overnight, and 0.1 mL aliquots of each active culture were adjusted to pH 2, 3, 4, and 5 with 5N HCl and incubated anaerobically at 37 °C for 48 hours. A loopful suspension from MRS broth was inoculated onto MRS agar and incubated anaerobically at 37 °C for 24 hours. The grown colonies on the MRS agar exhibited acid-tolerant characteristics [25].

NaCl tolerance test of isolated *Lactobacillus* spp.

Isolated strains of *Lactobacillus* spp. were grown anaerobically in MRS broth at 37 °C for 24 hours; saturated NaCl solution was prepared separately by dissolving NaCl powder. The concentration of NaCl was adjusted to 2%, 4%, 6%, and 8%, which were then sterilized. A loopful sample from MRS broth was inoculated into the adjusted concentration of NaCl and incubated anaerobically at 37 °C for 48 hours. Then, a loopful sample from each concentration was inoculated onto MRS agar plates and incubated anaerobically at 37 °C for 24 hours. The grown colonies on the MRS agar plates exhibited NaCl tolerance [25].

Antibacterial effect of isolated *Lactobacillus* spp. on different pathogens

The inhibitory effect of *Lactobacillus* strains on selected clinical reference strains (*Salmonella* spp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Escherichia coli*) was determined by the well-diffusion method. For the agar well diffusion assay, an overnight culture of the indicator strains was used to inoculate Muller Hinton Agar (MHA) growth media at 37 °C. The turbidity of the indicator strains was compared with 0.5 McFarland solution before being inoculated onto MHA growth media. Wells of 5 mm diameter were cut into agar plates with a cork borer, and 50 µL of *Lactobacillus* culture supernatant, which likely contains antibacterial activity, was added to each well. The plates were then incubated at 37 °C overnight, and the inhibitory zone produced by *Lactobacillus* was measured [25].

Purity Plate

The purity plate was used to ensure that the inoculum used for the biochemical tests was a pure culture and also to verify whether the biochemical tests were performed under aseptic conditions or not. Thus, while performing biochemical tests, the same inoculum was sub-cultured onto the respective medium and incubated. The media were then checked for the appearance of pure growth of organisms.

Statistical Analysis

The experiment was carried out in triplicate, and the data were analyzed using SPSS version 20.0. The Chi-square test was used to determine the significant association of dependent variables. A P-value < 0.05 was considered significant for the analyses. Graphical and tabular representations were also created for descriptive analysis.

Table 1. Frequency of lactic acid bacteria isolated from fermented foods

Fermented Food	Frequency of LAB isolated		P-value
	Number	Percentage (%)	
Commercial *Yogurt*	18	17.5	0.297
Achaar	20	19.5	
Homemade *Yogurt*	17	16.5	
Tama	16	15.5	
Chhurpi	15	14.5	
Gundruk	17	16.5	
Total	103	100	

Results

Isolation and Distribution of Lactic Acid Bacteria from fermented food samples

Among 120 samples, Lactic Acid Bacteria (LAB) were isolated from 103 (85.83%) samples. Among those 103 LAB isolates, 18 were obtained from commercial yogurt, followed by 20 isolates from *Achaar*, 17 from Homemade *Yogurt*, 16 from *Tama*, 15 from *Chhurpi*, and 17 from *Gundruk* (**Table 1**).

Isolation of *Lactobacillus* spp. from fermented food samples

Among 103 LAB isolated, 64 (53.3%) *Lactobacillus* spp. were isolated, confirmed with the morphological tests. While viewed under the microscope, all isolates were observed to be Gram positive and appeared rod-shaped (**Figure 1** and **Figure 2**).

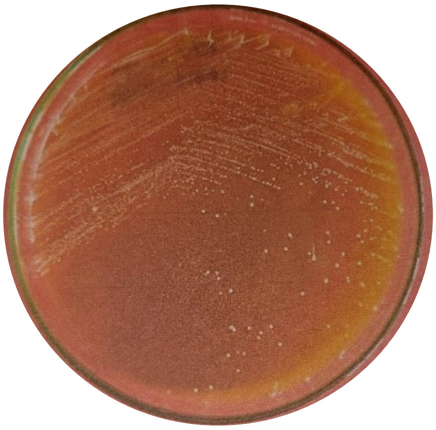

Figure 1: Isolation of *Lactobacillus* spp. on MRS Agar

Figure 2: Gram staining of *Lactobacillus* spp. showing slender rod morphology

Distribution of *Lactobacillus* spp. in different fermented foods

Among the 64 positive *Lactobacillus* spp. samples, 16 were isolated from commercial *yogurt*, followed by 15 samples from *Achaar*, 12 from Homemade *Yogurt*, 10 from *Tama*, 6 from *Chhurpi*, and 5 from *Gundruk*. The types of fermented foods were found to have a statistically significant impact on the growth of *Lactobacillus* spp. ($P \leq 0.05$)(**Table no 2**).

Table 2: Frequency of *Lactobacillus* spp isolated from fermented foods

Fermented Food	Frequency of *Lactobacillus* spp. isolated		P Value
	Number	Percentage (%)	
Commercial *Yogurt*	16	25	0.001
Achaar	15	23.4	
Homemade *Yogurt*	12	18.8	
Tama	10	15.6	
Chhurpi	6	9.4	
Gundruk	5	7.8	
Total	64	100	

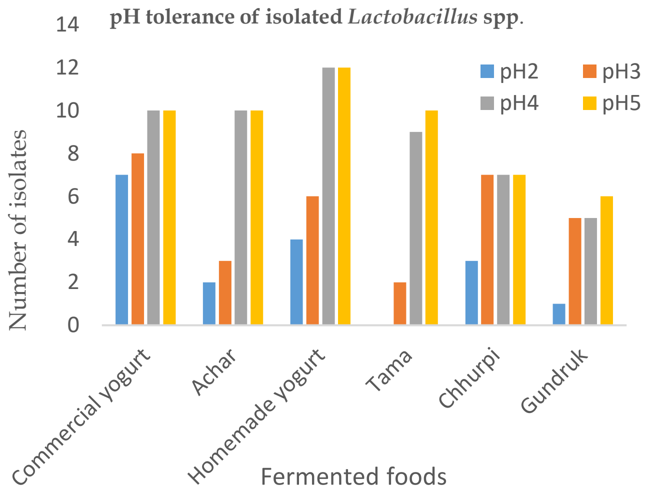

Figure 3. pH tolerance of isolated *Lactobacillus* spp

Figure 4. pH tolerance shown by a *Gundruk* Sample (G19)

pH tolerance of isolated *Lactobacillus* spp.

For the pH tolerance test, a total of 64 *Lactobacillus* isolates were examined. In commercial yogurt, seven isolates were found to be tolerant at pH 2. Similarly, only one isolate from *Gundruk* was found to be tolerant at pH 2. The maximum number of tolerant isolates was found in homemade yogurt at pH 4 and 5. No tolerant isolates were found in the *tama* sample at pH 2. This demonstrates that homemade yogurt contains a greater number of *Lactobacillus* spp. capable of thriving in various pH levels, especially compared to other fermented foods like tama. (**Figure 3** and **Figure 4)**

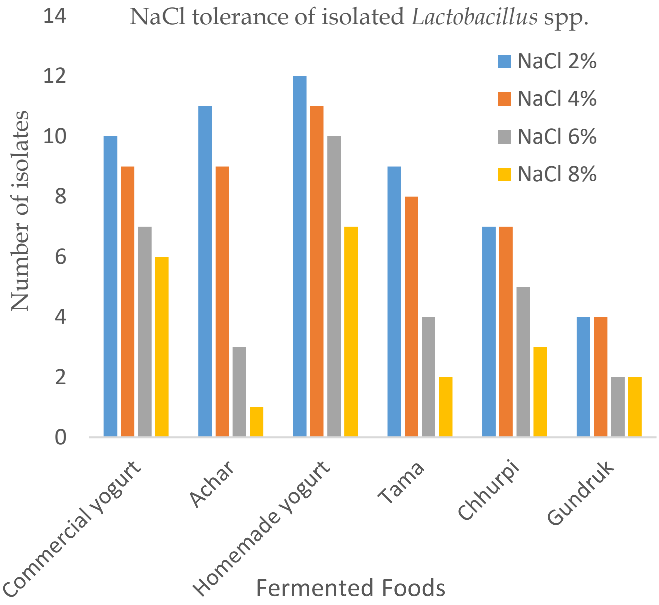

Figure 5. NaCl tolerance of isolated *Lactobacillus* spp.

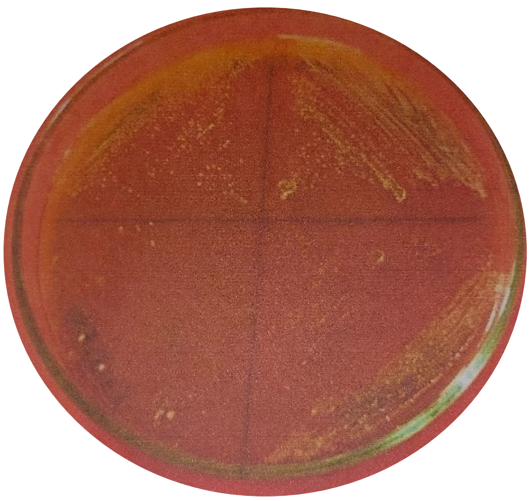

Figure 6. Nacl tolerance shown by a *Gundruk* Sample (G19)

NaCl tolerance of isolated *Lactobacillus* spp.

For the next test, the NaCl tolerance of 64 isolates was tested. In homemade yogurt, seven isolates were found to be tolerant at 8% NaCl. Similarly, only 1 isolate of *Achaar* were found to be tolerant at 8% NaCl. The maximum number of tolerant isolates was found in homemade yogurt at 2% NaCl. This shows that homemade yogurt has a higher number of salt-tolerant *Lactobacillus* spp. compared to other fermented foods, like *achaar*, which had only one isolate able to tolerate 8% NaCl (**Figure 5** and **Figure 6**).

Inhibitory potential of the isolated *Lactobacillus* spp. against Pathogenic Bacteria

Total *Lactobacillus* spp. isolates from different fermented foods were furtherproceeded to analyze their probiotic potential against clinically isolated five pathogenic bacteria (*Staphylococcus aureus, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Salmonella* spp.) Among the 64 *Lactobacillus* spp. isolates, those from homemade yogurt demonstrated notable inhibitory effects, with zones of inhibition ranging from 5-20 mm, including a significant effect against *S. aureus* and *P. aeruginosa* (**Table 3**).

Discussion

According to Fuller (1989), probiotics are live microbial supplements that improve the host animal's intestinal microbial balance. Later, the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) defined probiotics as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host." Historically, probiotics have been utilized for the treatment of various mucosal infections, such as those in the gut and vagina, due to their ability to colonize the gastrointestinal tract and provide long-term benefits without ongoing medical treatment. While their use declined with the advent of antibiotics, fermented and fortified probiotic foods are increasingly popular in today's market [26]. With the rise of antibiotic resistance and the necessity to reduce treatment costs, probiotics are now considered a viable alternative to antibiotics [27]. This shift is driven by the growing recognition of the negative impacts of antibiotics on the microbiome, which probiotics can help restore and maintain.

The findings of this research suggest that certain *Lactobacillus* isolates exhibit strong resilience in harsh conditions and possess significant antibacterial properties, which could be harnessed to formulate effective probiotic products or we can simply include these probiotic foods in our daily diet to reap its benefit [28]. These beneficial bacteria can produce various compounds that inhibit the growth of pathogens, including organic acids (such as lactic and acetic acids), bacteriocins, and reuterin [29]. This multifaceted inhibitory action highlights the potential of *Lactobacillus* strains to serve as natural preservatives and health-promoting agents. As established earlier, *Lactobacillus* strains to be effective as probiotics, they need to survive the high acidity of the gastrointestinal tract, tolerate bile salts, adhere to and persist in the gut, and resist pathogens through the production of antimicrobial substances, among other mechanisms [30].

The study aimed to evaluate the acid and salt tolerance of these probiotic bacteria and determine their inhibitory capacity against certain clinically significant pathogens.

Table 3: Inhibitory Potential of *Lactobacillus* spp. isolated from fermented foods against pathogens

Samples	Zone of Inhibition (mm)	*S aureus*	*K. pneumoniae*	*E. coli*	*P. aeruginosa*	*Salmonella* spp.
Commercial Yogurt	1-5	-	-	-	-	-
	5-10	2	1	2	-	-
	10-15	2	-	-	-	2
	15-20	1	-	-	1	1
Homemade Yogurt	1-5	-	-	-	-	-
	5-10	3	2	-	3	1
	10-15	-	-	-	1	1
	15-20	2	-	1	1	-
Achaar	1-5	1	1	-	-	-
	5-10	2	1	-	1	-
	10-15	1	1	1	3	-
	15-20	1	-	-	-	-
Tama	1-5	-	-	-	-	-
	5-10	-	-	-	1	3
	10-15	2	-	-	1	-
	15-20	-	-	-	-	-
Chhurpi	1-5	-	1	-	-	-
	5-10	2	-	1	1	1
	10-15	-	-	-	-	-
	15-20	1	-	-	-	-
Gundruk	1-5	-	-	-	-	-
	5-10	2	2	-	1	-
	10-15	-	-	-	-	-
	15-20	-	-	-	-	-

Out of the 120 processed samples, 103 tested positive for lactic acid bacteria, with 64 of them testing positive for *Lactobacillus* spp. This prevalence of *Lactobacillus* in the analyzed fermented foods suggests the potential of traditional fermented foods as a rich source of probiotic strains for enhancing human health. Moreover, as two different types of yogurts were included in the study, it demonstrated the nearly equal benefits of homemade yogurt compared to commercial yogurt. As a matter of fact, based on the results, homemade yogurt was found to be the most potent source of probiotics. Among the 64 *Lactobacillus* spp. isolates identified, 12 were from homemade yogurt. This food also exhibited the highest pH tolerance, with the maximum number of tolerant isolates at pH levels 4 and 5, and it had the most isolates (7) that tolerated 8% NaCl. This highlights the superior quality of homemade yogurt in providing resilient probiotic strains. Additionally, the *Lactobacillus* spp. isolated from homemade yogurt demonstrated significant inhibitory effects against pathogenic bacteria, showing substantial zones of inhibition in the 10-15 mm and 15-20 mm ranges against several pathogens. Given the traditional value of homemade yogurt in Nepalese culture, this finding highlights the potential health benefits associated with this age-old practice. Similarly, the other food samples included in the study, such as *gundruk, achaar, and tama*, represent other preserved delicacies of Nepalese gastronomy. This emphasizes how probiotics have been incorporated into our diets and promotes the consumption of these traditional foods for their potential.

NaCl acts as an inhibitory substance that can impede the growth of certain types of bacteria. Probiotic organisms must be able to withstand high salt concentrations in the human gut. In this study, *Lactobacillus* spp. isolated from various fermented foods demonstrated tolerance to NaCl concentrations ranging from 1% to 8%, with optimal growth observed at 2% NaCl. This suggests their robust nature and adaptability to various environmental stresses. Conversely, another study by Chowdhury in 2012 found that *Lactobacillus* spp. isolated from yogurts could tolerate NaCl concentrations of 1% to 9%, with optimal growth occurring at 1% to 5% NaCl [31]. Similarly, another study [32] showed that lactic acid bacteria from fermented foods exhibited optimal growth in NaCl concentrations ranging from 2% to 6.5%. These findings underline the importance of salt tolerance for probiotics to survive and function effectively in the human digestive system.

pH is another critical factor that significantly influences bacterial growth. For organisms to serve as probiotics, they must withstand the low pH of the human gut. The isolated *Lactobacillus* strains demonstrated tolerance to a wide range of pH (1-9), with optimal growth observed at pH 4 and pH 5. This suggests their ability to thrive in acidic environments characteristic of the human gut. These findings are consistent with previous studies, highlighting the sturdy nature of *Lactobacillus* strains in withstanding harsh conditions. For instance, Hawaz et al. (2014) found that the lactic acid bacteria isolated from curd were able to survive at pH 3 during three hours of incubation, with *L. casei* and *L. delbrueckii* showing greater resistance to low pH than other strains [33]. Similarly, in the research done by Argyri and the team in 2013 showed nine strains of *Lactobacillus* with very high resistance to low pH [34]. Also, Bassyouni and other researchers in 2012 revealed that eight out of eleven tested *Lactobacillus* isolates were resistant to pH 3 during a three-hour incubation period [35]. These findings collectively highlight the strong acid tolerance of *Lactobacillus* strains, reinforcing their potential as effective probiotics in the human gut environment. The consistent ability of these strains to endure acidic conditions positions them as strong candidates for probiotic applications.

The next crucial selection criterion for probiotics is showcasing antimicrobial activity, and this study revealed promising results in this aspect as well. All *Lactobacillus* isolates exhibited the ability to inhibit the tested pathogens, albeit with variations in the diameter of the inhibition zones. The highest antibacterial effect was observed against *Staphylococcus aureus*, with a zone of inhibition (ZOI) measuring 18 mm, while the lowest was against *Klebsiella*, with a ZOI of 4 mm. This variation could be attributed to the different mechanisms of action and production levels of antimicrobial substances. In comparison, Gharaei-Fathabad et al. (2011) found that *Lactobacillus paraplantarum* isolated from tea leaves showed strong inhibitory activity against *Salmonella typhi* (65 mm), E. coli (30 mm), *Staphylococcus aureus* (56 mm), *Enterococcus faecalis* (55 mm), and *Citrobacter* spp. (60 mm) [1]. Osuntoki et al. (2008) also reported that *Lactobacillus* spp. isolated from fermented dairy products exhibited antimicrobial activity against Enterotoxigenic *E. coli* (4.2 mm), *Salmonella typhimurium* (4.3 mm), and *Listeria monocytogenes* (5 mm) [36]. Similarly, in the research conducted by Chowdhury et al. in 2012, he took eight buffalo yogurt samples among which four isolates (Y1, Y2, Y3, and Y4) were identified to be *Lactobacillus plantarum* based on their growth and biochemical characteristics [37]. Among these four identified isolates, the highest inhibitory effect of isolate Y1 was observed against *Bacillus cereus* (52 mm), with the lowest zone of inhibition being 24.05 mm against *Staphylococcus aureus* after 72 hours of incubation. For isolate Y2, the largest inhibition zone was against *E. coli* ATCC 8739 (42.35 mm), while the smallest zone was 25.81 mm against *Staphylococcus aureus*. In the similar manner, the highest inhibition zone for isolate Y3 was against *Salmonella paratyphi* (44 mm), with the smallest zone being 25.81 mm against *Staphylococcus aureus*. In the case of isolate Y4, the largest inhibition zone was found against *Salmonella paratyphi* (48.26 mm), and the smallest zone was 27.73 mm against *Staphylococcus aureus* after 72 hours of incubation. The consistent inhibitory effects against a range of pathogens underline the potential of these *Lactobacillus* strains as effective biocontrol agents.

With the results of this research and other published literature in similar study, we can confirm the probiotic potential of *Lactobacillus* strains and their ability to inhibit various pathogenic bacteria. While some pathogenic strains, such as *E. coli*, *Klebsiella pneumoniae*, and *Salmonella*, showed less inhibition, likely due to their drug resistance, strains like *Staphylococcus aureus* and *Pseudomonas* spp. were significantly inhibited. This variability in inhibition effectiveness could guide targeted probiotic applications. The overall findings suggest the significant probiotic ability of Lactobacilli, attributed to their inhibitory action through the production of lactic acid, bacteriocins, H2O2, and other antimicrobial compounds [38, 39]. Future studies could explore the molecular mechanisms underlying these antibacterial effects to assess the probiotic properties of these strains.

Conclusion

This study underscores the probiotic potential of *Lactobacillus* strains isolated from traditional Nepalese fermented foods. The findings reveal that these strains exhibit significant acid and salt tolerance, thriving in conditions similar to the human gut environment. They further demonstrate antimicrobial activity against different Gram positive and Gram negative bacteria, highlighting their potential for enhancing gut health and combating pathogens. Additionally, the result has suggested us that homemade yogurt is particularly rich in *Lactobacillus* spp. strains, showing its strong probiotic potential. Commercial yogurt also showed considerable inhibitory activity against *S. aureus* and *Salmonella* spp. While *chhurpi* and *gundruk* displayed the least inhibitory capacity, *achaar* and *tama* exhibited moderate inhibition, particularly against *E. coli* and *Salmonella* spp. The inhibitory action of *Lactobacillus* spp. is primarily due to the production of lactic acid, bacteriocins, and other antimicrobial substances. The reason for *Escherichia coli* being the least inhibited pathogenic strain could be its inherent resistance to these chemicals. As drug resistance is increasingly prevalent, both pharmaceutical treatments and natural microbiota often fail to inhibit many pathogenic strains. Nevertheless, *Lactobacillus* spp. can be effective in reducing the risks of diseases caused by pathogenic strains such as *Staphylococcus aureus* and *Pseudomonas spp*. In overall, the resilient nature and antimicrobial properties of these strains support the inclusion of traditional fermented foods in the diet as a source of beneficial probiotics. Further research is required to comprehensively evaluate the probiotic properties of these strains, providing insights into utilizing their beneficial characteristics for practical applications.

Author's Contribution

AP is the principal investigator; she drafted and edited the manuscript. SS, SB, AS, and AP helped in laboratory. PS conceptualized the research and guided during the lab work.

Competing Interest

This study does not involve any competing interests.

Funding

There is no funding provided by any internal or external sources.

Ethical Approval and Consent

Ethical approval is not applicable.

Acknowledgement

The supervisor Ms. Pabitra Shrestha and department head, Mr. Anup Muni Bajr*achaar*ya, along with all of the faculty members at Balkumari College is acknowledged by the authors for their technical and academic support.

References

1. Gharaei Fathabad E and Eslamifar M (2011). Isolation and Applications of one strain of Lactobacillus paraplantarum from tea leaves (Camellia sinensis). American Journal of Food Technology. 6:429-434.
2. Birollo GA, Reinheimer JA and Vinderola CG (2000). Viability of lactic acid microflora in different types of yoghurt. Food Research International. 33(9):799–805.
3. Hamilton-Miller JM, Gibson GR and Bruck W (2003). Some insights into the derivation and early uses of the word 'probiotic'. British Journal of Nutrition. 90:845.
4. Metchnikoff E (1908). The prolongation of life. Optimistic studies. New York: Putman's Sons. 9(39):161-83.
5. Sanders ME (2000). Considerations for use of probiotic bacteria to modulate human health. Journal of Nutrition. 130:384-390.

6. Tallon R, Bressollier P and Urdaci MC (2003). Isolation and characterization of two exopolysaccharides produced by Lactobacillus plantarum EP56. Research Microbiology. 154(10):705-712.
7. Welman AD and Maddox IS (2003). Exopolysaccharides from lactic acid bacteria: perspectives and challenges. Trends in Biotechnology 2(6):269-73.
8. Bromberg, Renata & Moreno, I. & Zaganini, Cíntia & Delboni, Roberta & Oliveira, Josiane. (2004). Isolation of bacteriocin-producing lactic acid bacteria from meat and meat products and its spectrum of inhibitory activity. Brazilian Journal of Microbiology. 35. 137-144.
9. Felis GE, and Dellaglio F (2007). Taxonomy of Lactobacilli and Bifidobacteria. Current Issues in Intestinal Microbiology. 8(2):44-61.
10. Axelsson, L. 2004. Lactic acid bacteria: classification and physiology. In Advance in Lactic Acid Bacteria: Microbiological and Functional Aspects, S. Salminen, A. Von Wright and A. Ouweland, editors. Marcel Dekker, New York, U.S.A. ppa. 1-66.
11. Cannon JP, Lee TA, Bolanos JT and Danziger LH (2005). Pathogenic relevance of Lactobacillus: a retrospective review of over 200 cases. European Journal of Clinical Microbiology & Infectious. Official Publication of the European Society of Clinical Microbiology. 24(1):31-40.
12. Madigan MT, Martinko JM and Parker J (2003). Brock Biology of Microorganisms. Upper Saddle River, New Jersey: Pearson Education. 10.1- 34.
13. Ehrmann, M.A., Kurzak, P., Bauer, J., & Vogel, R.F. (2002). Characterization of lactobacilli towards their use as probiotic adjuncts in poultry. Journal of Applied Microbiology, 92, 966-975.
14. Parajuli IP (2011). Antimicrobial Activity of Bacteriocin of Lactic Acid Bacteria Isolated from the Fermented Food. 1-64.
15. Maria LM, Dustin H, Sylvie B, Christopher JC, Paul DC, Benoit F, Michael G, Remco K, Gonca P, Anne P, Eddy J and Robert Hutkins (2017). Health benefits of fermented foods: microbiota and beyond. Institute of Agriculture and Natural Resources. 78:1496–1505.
16. Rolle R and Satin M (2002). Basic requirements for the transfer of fermentation technologies to developing countries. International Journal of Food Microbiology. 75(3):181–187.
17. Tamang JP, Tamang B, Schillinger U, Franz CM, Gores M and Holzapfel WH (2005). Identification of predominant lactic acid bacteria isolated from traditionally fermented vegetable products of the Eastern Himalayas. International Journal of Food Microbiology. 105(3):347–356.
18. Tamang JP, Tamang B, Schillinger U, Franz CM, Gores M and Holzapfel (2009). Functional properties of lactic acid bacteria isolated from ethnic fermented vegetables of the Himalayas. International Journal of Food Microbiology. 135(1):28–33.
19. Tamang, J. P., Watanabe, K., & Holzapfel, W. H. (2016). Diversity of Microorganisms in Global Fermented Foods and Beverages. Frontiers in Microbiology, 7, 377.
20. Tamang, J. P. (2010). Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values. CRC Press.
21. Dubey, R. C., & Maheshwari, D. K. (2014). Practical Microbiology. S. Chand Publishing.
22. Tamang, J. P. (2010). Himalayan Fermented Foods: Microbiology, Nutrition, and Ethnic Values. CRC Press.
23. Holzapfel, W. H., & Schillinger, U. (2002). Introduction to pre- and probiotics. Food Research International, 35(2-3), 109-116.
24. Montet D, Loiseau G and Zakhia-Rozis N (2006). Microbial technology of fermented vegetables. Microbial Biotechnology in Horticulture. 1:309-343.
25. Tika K, Saeed A and Ojha KS (2016). Ethnic Fermented Foods of Nepal. Ethnic Fermented Foods and Alcoholic Beverages of Asia. Journal of Ethnic Foods. Springer India. 91-117.
26. Orlem Erdogrul and Feryal Erbilir (2006). Isolation and Characterization of Lactobacillus bulgaricus and Lactobacillus casei from Various Foods. Turk J Biol. 30:39-44.
27. Hassan H, Ali E, Karim M and Javad H (2012). Investigation of antibacterial, acid and bile tolerance properties of lactobacilli isolated from Koozeh cheese. Veterinary Research Forum. 3(3):181–185.
28. O'May GA and Macfarlane GT (2005). Health Claims Associated With Probiotics. Probiotic Dairy Products. Blackwell Publishing, Oxford, UK. Wiley Online Library. 1-15.
29. Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJ, Panagou EZ and Tassou CC (2013). Selection of potential probiotic Lactic Acid Bacteria from fermented olives by in vitro tests. Food Microbiology. 33:282-291.
30. Pundir RK, Rana S, Kashyap N and Kaur A (2013). Probiotic Potential of Lactic acid Bacteria Isolated from food Samples – an in Vitro study. Journal of Applied Pharmaceutical Science. 3(03):085-093.
31. Dhanasekaran D, Subhasish Saha, Thajuddin N and Rajalaksmi M (2009). Probiotic Effect of Lactobacillus isolates against Bacterial Pathogens in Fresh Water Fish. Journal of Coastal Development. 13(2):103-112.
32. Demir N, Bahceci KS and Acar J (2006). The effects of different initial Lactobacillus plantarum concentrations on some properties of fermented carrot juice. Journal of Food Processing and Preservation. 30(3):352–363.
33. Chowdhury A, Hossain Md.N, Mostazir NJ, Fakruddin Md and Billah M (2012). Screening of Lactobacillus spp. from Buffalo Yoghurt for Probiotic and Antibacterial Activity. Journal of Bacteriology and Parasitology. 3:156.
34. Ishola RO and Adebayo-Tayo BC (2012). Screening of Lactic Acid Bacteria Isolated from Fermented Food for Bio-molecules Production. Thai Science. 1-13.
35. Hawaz E (2014). Isolation and identification of probiotic lactic acid bacteria from curd and in vitro evaluation of its growth inhibition activities against pathogenic bacteria. African Journal of Microbiology Research 8(13):1419-1425.
36. Argyri AA, Zoumpopoulou G, Karatzas KA, Tsakalidou E, Nychas GJ, Panagou EZ and Tassou CC (2013). Selection of potential probiotic Lactic Acid Bacteria from fermented olives by in vitro tests. Food Microbiology. 33:282-291.
37. Bassyouni RH, Abdel-all WS, Fadl MG, Abdel-all S and Kamel Z (2012). Characterization of Lactic Acid Bacteria Isolated from Dairy Products in Egypt as a Probiotic. Life Science Journal. 9:2924-2930.
38. Osuntoki AA, Ejide OR and Omonigbehin EA (2008). Antagonistic effects on Enteropathogenic and plasmid analysis of Lactobacilli isolated from fermented Dairy products. Biotechnology. 7:311-316.
39. Chen CC, Lai CC, Huang HL, Huang WY, Toh HS, Weng TC, Chuang YC, Lu YC, Tang HJ (2019). Antimicrobial Activity of Lactobacillus Species Against Carbapenem-Resistant Enterobacteriaceae. Frontiers in Microbiology. 10:789. doi: 10.3389/fmicb.2019.00789.