



Bioprospecting of Lactic Acid Bacteria from Fermented Foods of Pakyong, Sikkim: Documentation and Functional Assessment

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Abstract

Background: Fermentation is an ancient process of food processing that promotes the preservation of food, its taste, and nutrients. Some traditional Himalayan communities, such as those in the Pakyong district of Sikkim, are culturally dependent on a range of fermented foods as a source of sustenance and cultural identity. This paper is intended to record the fermented foods in Pakyong and to evaluate the potential probiotic characteristics of lactic acid bacteria (LAB) in the foods.

Methods: A cross-sectional survey was conducted among households in Pakyong district to document fermented food practices. Presumptive LAB were isolated from selected food samples and evaluated for important putative probiotic properties and safety by using standard *in vitro* assays.

Results: Seven conventional fermented foods were documented, with frequent consumption of kinema, gundruk, sinki, and soft chhurpi. Presumptive LAB isolates had putative probiotic-related characteristics, such as resistance to bile and salt, cell surface hydrophobicity, and auto-aggregation. Some isolates showed comparatively stronger probiotic-related characteristics, especially those of gundruk, sinki, and kinema. All isolates were non-hemolytic, exhibited weak biofilm formation, and demonstrated antagonistic activity against *Escherichia coli*.

Conclusions: The study documented seven ethnic fermented foods of Pakyong district (gundruk, sinki, kinema, soft chhurpi, hard chhurpi, masyeura, khalti) prepared by traditional methods. Survey data show frequent consumption of kinema, gundruk, sinki, and soft chhurpi in the region. The presumptive LAB isolates from these foods demonstrated desirable probiotic traits. Three isolates from sinki, gundruk and kinema displayed the strongest overall probiotic profiles (high auto-aggregation and moderate hydrophobicity and bile tolerance). All isolates were safe (no hemolysis, weak biofilm) and showed antagonistic activity against a model pathogen. These findings suggest that traditional fermented foods of Sikkim may harbor presumptive LAB with potential functional relevance.

Keywords: Pakyong Sikkim; fermented foods; lactic acid bacteria; probiotics; traditional diet

1 Introduction

Fermentation is an ancient and widely practised method of preserving and enriching foods. Nutritional quality, flavour and safety can be preserved or enhanced in fermented foods. Vitamins and minerals are usually made more accessible during fermentation. Moreover, fermented foods are generally high sources of living microorganisms (probiotics)

that are useful to the health and immunity of the gut. Lactic acid bacteria (LAB) are common probiotic microorganisms, known for their ability to survive harsh gastrointestinal conditions such as low pH and bile salts (Gizachew et al., 2023). These probiotic qualities make fermented foods viable nutritional products for the various communities.

In India, fermented foods hold strong cultural significance and have gained increasing attention due to their associated health benefits. It can be assumed that the ancient traditions of fermentation among the different ethnic groups of India date back several centuries, and the latter study has proved that fermentation could be applied as a source of nutritional supplement, as a food preservative, or to maintain the health of the gut (Sarma and Gupta, 2022).

Traditional food in the Himalayan region, including Sikkim, is particularly rich in traditional fermented foods. Common examples include kinema (fermented soybeans), gundruk (fermented leafy greens), sinki (fermented radish), chhurpi (cheese), masyeura (fermented black gram), and khalpi (fermented cucumber). These foods are valued not only in the aspects of taste and cultural self-identification but also in the aspects of nutrition and health. An example is the kinema, protein-enriched food, high in antioxidants (Katuwal et al., 2023; Sanjukta et al., 2021), and gundruk, a source of magnesium and calcium (Mahanta, 2023). Sinki is associated with anti-carcinogenic effects (Tamang and Sarkar, 1993), whereas khalpi has anti-inflammatory effects (Tamang et al., 2005). Soft and hard chhurpi are rich in protein and carbohydrates (Chourasia et al., 2022; Panda et al., 2016). Although considered to be important, the traditional preparation techniques are mostly undocumented, and the microbiology of these foods is poorly characterized.

Considering the rich fermentation culture of Pakyong district and the possible health-promoting aspects of probiotics, the proposed study seeks to:

1. Record the varieties and the processes of preparing fermented foods taken by the local families.
2. Assess the probiotic value of presumptive LAB isolated from these foods. The detailed aims are to map the food consumption, maintain the traditions of the kitchen, and identify the beneficial microbial strains that can be studied in the field of food and health.

2 Materials and methods

2.1 Survey and Documentation

The study was conducted in the Pakyong district of Sikkim (India). A non-probability purposive sampling strategy was employed to select households with prior experience in preparing or consuming traditional fermented foods. Within this framework, elements of convenience sampling were applied based on participant availability and willingness to participate. A total of 120 households across five subregions of the district: Rangpo, Pakyong Bazaar, Rhenock, Dikling, and Kartok were selected.

A structured questionnaire was developed by the authors based on relevant literature on traditional fermented foods and dietary assessment, and was refined through pilot testing in a small subset of households before the main survey. The questionnaire included sections on the socio-demographic profile (age, gender, ethnicity, religion, and education) of respondents. Dietary surveys were based on households' food frequency questionnaires (FFQ) that concentrated on the frequency of consumption and preparation of fermented foods. Questions in the survey included the types of fermented products used, how often they were consumed (daily, weekly, rarely), and cooking practices (e.g. with vegetables, meat, etc.).

Data were collected between January and April 2024 via structured household face-to-face interviews and questionnaires. Participation in the survey was voluntary. All respondents were informed about the purpose of the study and the confidentiality of the collected information prior to participation. No personal sensitive data were collected and no medical interventions were involved; therefore, formal ethical approval was not required according to institutional guidelines. In addition, direct observations were conducted in selected households ($n = 120$), where feasible. Interviews were carried out in local languages, namely Nepali, to ensure clarity and accurate responses. Responses were recorded manually and later digitised



Figure 1 Map of Pakyong, Sikkim.
● The symbol indicates the study area. (Source: Google Maps)

for analysis. During interviews, respondents described stepwise preparation methods for each traditional food item. Where possible, processes were observed and photographed. Seven fermented foods (gundruk,

sinki, kinema, soft chhurpi, hard chhurpi, masyeura, khalpi) were documented by mapping each preparation step (washing, slicing, drying, fermentation in air-tight containers or pits, etc.).

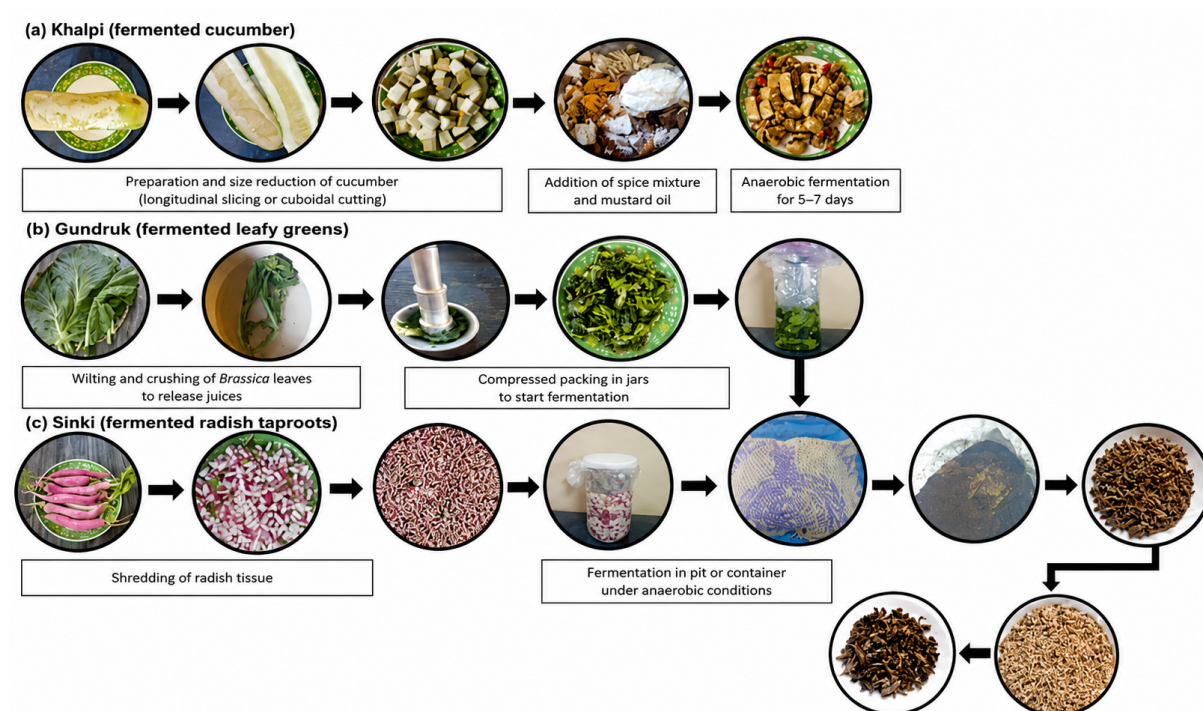


Figure 2 Traditional solid-state fermentation processes of vegetable-based products: (a) Khalpi (fermented cucumber), (b) Gundruk (fermented mustard leaves), and (c) Sinki (fermented radish).

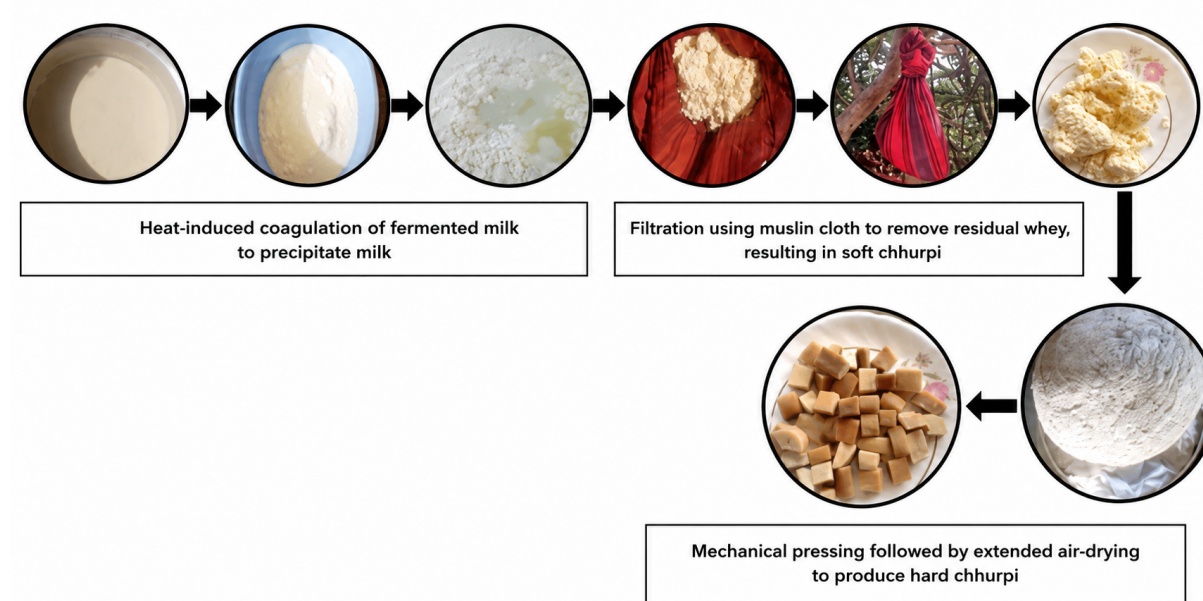


Figure 3 Traditional processing of dairy-based fermented products: soft and hard chhurpi

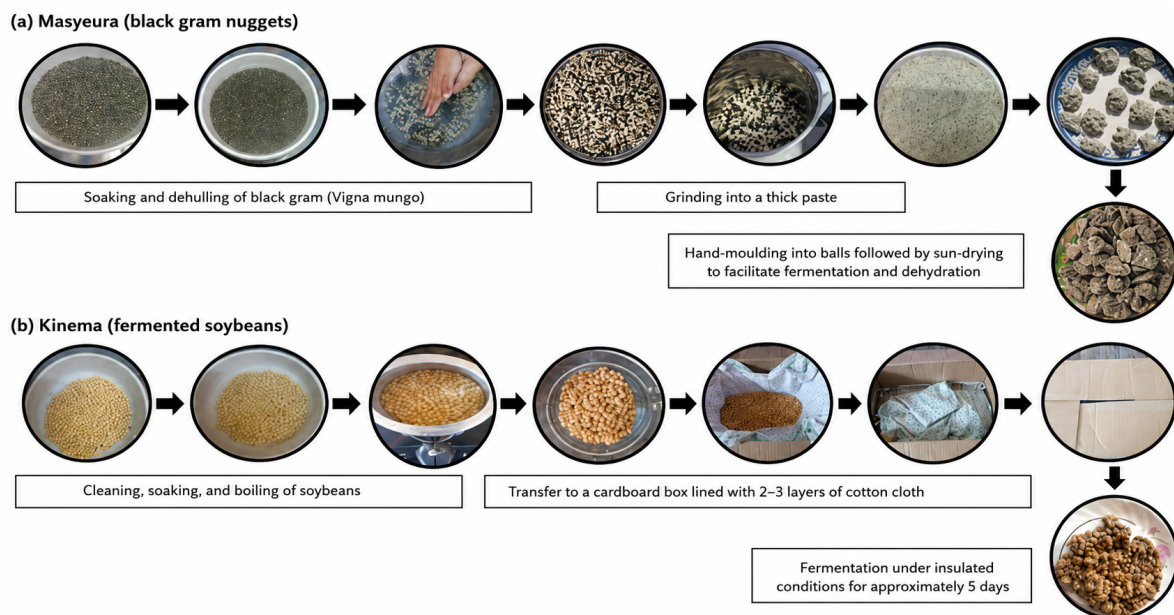


Figure 4 Traditional processing of legume-based fermented products: (a) Masyeura (fermented black gram) and (b) Kinema (fermented soybeans)

2.2 Isolation of Presumptive Lactic Acid Bacteria

The household survey and laboratory sampling were conducted independently. While dietary and preparation practices were documented from households, fermented food samples used for microbiological analysis were purchased separately from local markets in Pakyong Bazaar.

Seven fermented food samples (1 g each) were purchased from markets in Pakyong Bazaar. Samples were stored in sterile containers and transported on ice to the laboratory. Each sample was homogenised in sterile nutrient broth and incubated at 37 °C overnight. One loop of the enriched broth was transferred to nutrient agar and incubated at 37 °C for 24 h. To obtain pure isolates, representative colonies were subcultured on MRS agar selective for *Lactobacillus* and further incubated at 37 °C for 24–48 hours. Pure cultures were stored in nutrient broth with 40% glycerol at -40 °C for further analysis.

The isolates were identified tentatively as lactic acid bacteria (LAB) based on their phenotypic features, such as the colony morphology, Gram staining, and classic biochemical tests (catalase-negative, oxidase-negative, and other appropriate tests) as per established procedures (Obioha et al., 2021; Sirisha et al., 2021; Gebreselassie et al., 2016).

2.3 Presumptive Phenotypic and Biochemical Identification

Isolates were characterized for cultural, microscopic, and biochemical traits. Colony morphology (size, shape, colour) was observed on MRS agar. Gram staining and nigrosin negative staining were performed by standard methods. Catalase and oxidase tests were conducted on fresh cultures. Biochemical tests included indole produc-

tion, methyl red, Voges–Proskauer, and citrate utilisation using standard protocols. A Gram-positive, rod-shaped, catalase-negative profile with specific metabolic tests was used to presumptively identify LAB. No molecular identification (e.g., 16S rRNA gene sequencing) was performed; therefore, the classification of isolates as LAB is considered preliminary and based solely on phenotypic characteristics and does not confirm taxonomic classification at the genus or species level.

2.4 Assessment of Presumptive LAB and Preliminary Screening

Isolates were screened for putative probiotic attributes. All assays were performed under standardized experimental conditions, and the values presented should be interpreted as representative observations obtained under these conditions.

- **Bile tolerance:** Isolates were cultured in MRS broth with 0.3% (w/v) bile salts at 37 °C. Growth was monitored by measuring optical density at 600 nm (OD_{600}) at 0 h and after 4 h. Survival rates (%) were calculated as:

$$\text{Survival (\%)} = (OD_{(\text{bile})} / OD_{(\text{control})}) \times 100$$

- **Cell surface hydrophobicity:** Hydrophobicity was measured by microbial adherence to hydrocarbons (MATH test). Bacterial cells were harvested, washed, and resuspended in PBS (pH 7.2). Initial absorbance (OD_0) at 600 nm was recorded. One millilitre of n-hexadecane was added to 3 mL of cell suspension, vortexed,

and incubated for 1 h for phase separation. The aqueous phase absorbance (OD_i) was measured.

$$\text{Hydrophobicity (\%)} = [(OD_0 - OD_i) / OD_0] \times 100$$

- **Auto-aggregation:** Washed bacterial suspensions in PBS were incubated at 37 °C for 2 h without disturbance. The absorbance of the upper suspension (OD_i) was measured and compared to the initial absorbance (OD_0).

$$\text{Auto-aggregation (\%)} = [(OD_0 - OD_i) / OD_0] \times 100$$

- **NaCl tolerance:** Isolates were streaked on MRS agar plates containing NaCl at 0%, 0.5%, 1%, 5%, 7.5%, 10%, 12.5%, and 15%. Plates were incubated for 24 h at 37 °C. Similarly, isolates were inoculated into MRS broth supplemented with varying NaCl concentrations (0–15%, w/v) and incubated at 37 °C for 24 h. The highest NaCl concentration supporting visible growth was noted for each isolate. Growth in broth with NaCl was also measured by OD_{600} and compared with the control (0% NaCl), but the plate method was primary.

$$\text{Rate of NaCl tolerance (\%)} = (OD_{(\text{NaCl})} / OD_{(\text{control})}) \times 100$$

2.5 Safety and Antimicrobial Assays

- **Antibiotic and Antifungal susceptibility:** The Kirby–Bauer disc diffusion method on Mueller–Hinton agar (MHA) was used. Standard antibiotic discs (cefoxitin 30 µg, ertapenem 10 µg, doripenem 10 µg, meropenem 10 µg, imipenem 10 µg) were placed on inoculated MHA. Antifungal agents like fluconazole and ketoconazole were included for comparative purposes and not interpreted as antibacterial susceptibility. After 24 h at 37 °C, inhibition zones were measured. Susceptibility patterns were interpreted as per the CLSI breakpoints standard for non-fastidious bacteria, for which there are limited standardized breakpoints for LAB.
- **Hemolysis test:** Blood agar (5% blood) plates were streaked with isolates and incubated 24 h at 37 °C. Hemolytic activity was assessed based on zone formation. Absence of hemolysis (γ -hemolysis) indicated non-pathogenic potential.
- **Biofilm assay:** A microtiter plate assay was performed by growing cultures in wells, staining adherent cells with crystal violet, and measuring absorbance at 600 nm. Percent biofilm formation relative to control was calculated.

$$\text{Biofilm (\%)} = (OD_{(\text{test})} / OD_{(\text{control})}) \times 100$$

- **Antimicrobial activity:** Antagonistic activity against *Escherichia coli* ATCC 25922 was evaluated using the agar well diffusion method. Cell-free supernatants of isolates were added to wells, and zones of inhibition were measured after incubation at 37 °C for 24 h.

3 Results and discussion

3.1 Survey Findings

The socio-demographic profile of the 120 respondents showed that the majority were aged 20–30 years (55.8%), followed by 31–40 years (21.7%), 41–50 years (12.5%), and above 50 years (10.0%). Females constituted 60.8% of the respondents. Hindu (45.8%) and Buddhist (30.8%) were the predominant religions. Ethnically, 77.5% of households were Nepali, 15.8% Bhutia, and 6.7% Lepcha.

The outcome of the dietary survey revealed that fermented foods were common among households though the distribution was different among different products. kinema, gundruk, sinki, and soft chhurpi were typically eaten once a week, with a lower percentage stating that they ate it every day. Hard chhurpi, khalti and masheura, on the contrary, were eaten much less often, and in many households were not consumed at all.

Fermented foods were mostly cooked in the form of vegetable preparations. Kinema, gundruk and sinki were made with vegetables (e.g. in curry dishes) by more than 85% of households, but their application with the other categories of food was rare. All these data point to the fact that traditional fermented foods, especially kinema, gundruk, sinki, and soft chhurpi, continue to be part of the local diet in the Pakyong district.

Moreover, some of the food-related perceptions (taboos) related to fermented foods were found during interviews. Some of the beliefs that were reported by a sub-division of respondents included:

- Consumption of fermented food can cause low bone density, thereby leading to joint pain.
- Fermented foods are rotten foods that, when consumed, cause stomachache.
- Consumption of dairy fermented food at night will upset the stomach.
- Frequent consumption of fermented foods can cause cancer.

These beliefs were reported by approximately 29% of total respondents.

Table 1 Survey of Socio-Demographic and Consumption Patterns Among 120 Households in Pakyong District

Parameter	Category	Frequency (n=120)	Percentage (%)
Age of Respondents	20–30 years	67	55.8%
	31–40 years	26	21.7%
	41–50 years	15	12.5%
	Above 50 years	12	10.0%
Gender	Female	73	60.8%
	Male	47	39.2%
Religion	Hindu	55	45.8%
	Buddhist	37	30.8%
	Christian	28	23.3%
Ethnicity	Nepali	93	77.5%
	Bhutia	19	15.8%
	Lepcha	8	6.7%
Weekly Consumption of:	Gundruk	55	45.8%
	Sinki	49	40.8%
	Kinema	39	32.5%
	Soft Chhurpi	59	49.2%
	Hard Chhurpi	7	5.8%
	Khalpi	4	3.3%
	Masyeura	3	2.5%
Rarely/Never Consumed	Khalpi	50 (rarely)	41.7%
	Masyeura	57 (never)	47.5%

3.2 Food Documentation

Seven fermented items were documented in detail. These

processes were consistent with regional practices and were recorded for cultural preservation.

Table 2 Summary of traditional fermented foods of Pakyong district, Sikkim

Name Of Food Product	Raw Materials	Preparation Method (Summary)	Reported Functional Properties	Pictures
1. Gundruk	Mustard leaves	Washing → wilting / sun-drying → pit fermentation → drying Fermentation duration: ~22 days	Antioxidant and antimicrobial properties (Ghatani et al., 2024; Subedi et al., 2024).	
2. Sinki	Radish taproot	Cutting → drying → wrapping → pit fermentation. Fermentation duration: ~22 days	Source of fibre and organic acids; potential health benefits (Swain et al., 2011).	
3. Kinema	Soyabean	Soaking → cooking → wrapping → natural fermentation. Fermentation duration: 4–5 days	May support digestion and metabolic health (do Prado et al., 2022; Katuwal et al., 2023).	

Name Of Food Product	Raw Materials	Preparation Method (Summary)	Reported Functional Properties	Pictures
4. Soft chhurpi	Milk Curd	Boiling → coagulation → draining. Fermentation duration: immediate consumption.	Potential probiotic properties (Ghatani et al., 2022).	
5. Hard chhurpi	Milk Curd	Coagulation → pressing → drying. Fermentation duration: 1–2 months	May aid digestion due to organic acids (Abedin et al., 2022).	
6. Khalpi	Yellow cucumber Dalle chilli Salt Mustard powder	Cutting → salting → fermentation in sealed containers. Fermentation duration: Variable (weeks–months)	Increased phenolic content; possible anti-inflammatory effects (Kao and Lin 2023).	
7. Masyeura	Black lentil	Soaking → grinding → shaping → drying. Fermentation duration: 2 days (drying)	Rich in protein and micronutrients (Bhattarai et al., 2018).	

3.3 Isolation and Presumptive Identification of LAB

From the seven fermented samples, representative colonies were selected based on distinct colony morphology, Gram-positive staining, and catalase-negative characteristics. Due to resource limitations, one presumptive LAB isolate per fermented food sample was selected for preliminary characterisation. All isolates were Gram-positive rod-shaped bacteria, and all were catalase-negative and oxidase-negative. Biochemically, isolates were indole-negative, methyl red-positive, Voges–Proskauer-negative, and citrate-negative, consistent with general characteristics of lactic acid bacteria. Colony morphology on MRS agar was creamy and convex, typical for lactobacilli.

Based on these phenotypic characteristics, the isolates were presumptively identified as lactic acid bacteria (LAB). However, it is important to note that this identification is preliminary and does not confirm classification at the genus or species level, as molecular methods (e.g., 16S rRNA gene sequencing) were not performed.

3.4 Probiotic Properties

The presumptive LAB isolates have probiotic-related properties, which are summarised in Tables 3–6.

Bile Tolerance, Hydrophobicity, and Auto-aggregation: Table 3 shows the survival of the isolates in 0.3% bile, as well as the hydrophobicity and auto-aggregation values. The khalpi sample had the greatest bile tolerance, and soft chhurpi had the least. Hydrophobicity was different among the isolates, with the highest being the sinki isolate and the lowest being masyeura. Likewise, auto-aggregation was lowest in soft chhurpi, moderate in hard chhurpi, and highest in sinki, khalpi, and gundruk isolates.

Values are per cent survival after exposure to 0.3% bile (bile tolerance) and percent adhesion or self-aggregation (hydrophobicity and auto-aggregation). Higher values indicate greater probiotic potential in each assay.

NaCl tolerance: The results of growth of the isolates at different NaCl concentrations are summarized

in Table 4. The gundruk isolate could be grown in the highest salt concentration (up to 10% NaCl), although most of the other isolates were able to grow in 7.5% NaCl. The growth of khalpi and hard chhurpi isolates was up to 5% NaCl.

Maximum salt concentration (w/v) at which the growth of each isolate was observed. Lab assays showed gundruk isolates tolerated up to 10% NaCl, whereas kinema, sinki, soft chhurpi, and masyeura isolates grew up to 7.5% NaCl; khalpi and hard chhurpi isolates tolerated only 5%. No growth was seen at higher concentrations for any isolate.

Antibiotic and Antifungal Susceptibility: Table 5 shows the antibiotic susceptibility profiles. Isolates were all resistant to cefoxitin. Carbapenem antibiotics proved to have variable responses, with some of the isolates being susceptible and the rest being resistant.

Table 3 Bile tolerance, hydrophobicity, and auto-aggregation of presumptive LAB isolates from seven fermented foods

Fermented food	Bile tolerance (% survival)	Hydrophobicity (%)	Auto-aggregation (%)
Gundruk	70.59	23.68	73.42
Sinki	30.44	49.06	83.87
Kinema	50.95	20.55	71.29
Soft chhurpi	25.59	18.00	28.30
Hard chhurpi	64.56	36.59	53.85
Masyeura	51.79	16.00	69.66
Khalpi	90.40	41.11	79.59

Table 4 Maximum NaCl concentration (%) tolerated by presumptive LAB isolates from seven fermented foods

Fermented food	Max tolerated NaCl (%)
Gundruk	10.0
Sinki	7.5
Kinema	7.5
Soft chhurpi	7.5
Hard chhurpi	5.0
Masyeura	7.5
Khalpi	5.0

Table 5 Antibiotic and antifungal susceptibility (S = susceptible; R = resistant) of presumptive LAB isolates to selected drugs

Fermented food	Cefoxitin	Fluconazole	Ketoconazole	Ertapenem	Doripenem	Meropenem	Imipenem
Gundruk	R	R	R	S	S	S	S
Sinki	R	R	R	S	S	S	S
Kinema	R	R	R	S	S	S	S
Soft chhurpi	R	R	R	R	R	R	R
Hard chhurpi	R	R	R	R	R	R	R
Masyeura	R	R	R	R	R	R	R
Khalpi	R	R	R	R	R	R	R

Susceptibility (“S”) or resistance (“R”) to each antibiotic. Gundruk, sinki, and kinema isolates were resistant to cefoxitin (CX), fluconazole (FLC), and ketoconazole (KT) but susceptible to the four carbapenems (ertapenem, doripenem, meropenem, imipenem). All isolates from khalpi, soft chhurpi, hard chhurpi, and masyeura were resistant to every antimicrobial compound tested.

Hemolysis and Biofilm Formation: No hemolytic activity on blood agar (γ -hemolysis) was observed on any of the isolates. Biofilm formation was low in all isolates tested.

Antagonistic Activity: Table 6 shows that all the isolates were inhibitory to *Escherichia coli*. The inhibition zones were different across isolates with the gundruk

Table 6 Antagonistic activity against *Escherichia coli*: Zone of inhibition (mm)

Fermented food	<i>E. coli</i> inhibition zone (mm)
Gundruk	27
Sinki	20
Kinema	23
Khalpi	20
Masyeura	23
Soft chhurpi	20
Hard chhurpi	20

isolate recording the largest inhibition zones.

All seven isolates of LAB showed antagonistic activity

against *E. coli*. The largest inhibition zone was observed for the gundruk isolate (27 mm), while the other isolates showed zones ranging from 20 to 23 mm.

3.5 Discussion

The present study documented traditional fermented foods of Pakyong district, Sikkim, and evaluated the probiotic-related properties of presumptive lactic acid bacteria (LAB) isolated from these foods. According to the survey results, fermented food items like kinema, gundruk, sinki, and soft chhurpi remain part of the local diet. This diet has a strong cultural and dietary background. Other parts of the Himalayas have also reported similar consumption patterns and preparation practices (Rastogi et al., 2022).

The preparation techniques continue to follow traditional methods, such as pit fermentation and natural fermentation at ambient conditions. Such practices are significant in the formation of the microbial composition of fermented foods and the maintenance of indigenous knowledge systems.

The presumptive LAB isolates examined in this study showed several *in vitro* properties associated with probiotic potential, including bile salt tolerance, salt tolerance, cell surface hydrophobicity, and auto-aggregation. These properties were found to differ among isolates, which suggests functional diversity. The same has been observed in LAB isolated from fermented plant-based foods, whereby tolerance to environmental stress conditions is a favourable characteristic (Cai et al., 2023; Kumari et al., 2022).

However, these findings should be interpreted cautiously. The properties observed are based on *in vitro* tests and do not directly validate the probiotic activity *in vivo*. Individually, although bile tolerance and aggregation capacity are believed to be the predictive factors of possible gastrointestinal survival, they do not always correlate to the ability to colonize or to induce functional responses within the human gut.

The antibiotic susceptibility patterns observed in this study revealed variable resistance among the isolates. This resistance can be inherent in some LAB, but it should be assessed cautiously, since it cannot be excluded that they have transferable resistance genes. Thus, interpretations of antibiotic resistance profiles must be viewed with caution regarding the safety of probiotics.

All isolates exhibited γ -hemolysis, indicating a lack of hemolytic activity. Moreover, all isolates showed antagonistic effects against *Escherichia coli*, which could be explained by the synthesis of organic acids or other anti-

microbial agents, as it was previously reported (Chechet et al., 2022). However, more research is necessary to define the particular processes at play.

This study provides baseline data on the variety of fermented foods and related presumptive LAB in the Pakyong district. Nevertheless, there are a number of restrictions that must be considered. First, the sample of isolates studied was low, with just one representative isolate per type of food. Second, LAB was identified using only the phenotypic features, but not through molecular confirmation. In addition, quantitative assays were not statistically replicated, which limits the assessment of experimental variability. Third, the probiotic-related properties were evaluated only through *in vitro* assays, which may not necessarily reflect *in vivo* functionality.

Future research needs to be directed towards molecular identification (e.g., 16S rRNA sequencing), functional and genomic characterisation, and confirmation of probiotic potential by *in vivo* or clinical studies. These methods would reinforce the knowledge of the role of these traditional foods as sources of useful microorganisms. Since only one representative isolate per food type was analysed, the results cannot be generalized to the complete microbial diversity of each fermented food.

The results show the importance of traditional fermented foods as sources of possibly beneficial LAB. They also support their use in food, nutrition, and microbial studies.

4 Conclusion

This study has reported seven Pakyong district fermented foods of Sikkim and has described presumptive lactic acid bacteria (LAB) related to the same. The results affirm that fermented foods like kinema, gundruk, sinki, and soft chhurpi are still significant in the local diet.

The isolates of LAB had various *in vitro* features that are normally linked to probiotic potential, such as bile and salt tolerance, aggregation, and antimicrobial activity. But these are observations made on phenotypic and *in vitro* tests and do not establish the probiotic effectiveness or taxonomic identity at the genus or species level.

On the whole, the research provides preliminary data on the traditional fermented foods and the microbial communities involved in them in the Pakyong district and their significance as potential sources of useful microorganisms. More research is needed to substantiate these results with molecular identification and *in vivo* methods.

Future Prospects

Future studies should focus on:

- Molecular identification of isolates (e.g., 16S rRNA gene sequencing) to confirm taxonomic classification,
- Functional and genomic characterization of chosen strains,
- Assessment of the probiotic potential by *in vivo* or clinical trials, and
- Further surveys to understand regional differences in fermented foods and the microbiota involved.

This will help show the potential of microorganisms in traditional fermented foods, focusing on their practical and functional aspects.

5 Acknowledgement

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